Proceedings

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HydroMediT 2021

4th International Congress on Applied Ichthyology, Oceanography & Aquatic Environment

4-6 November 2021

Virtual

www.hydromedit.gr

Organised by

Department of Ichthyology and Aquatic Environment (DIAE), School of Agricultural Sciences, University of Thessaly (UTh)

Department of Marine Sciences (DMS), School of the Environment University of the Aegean (UoA)

Department of Animal Production, Fisheries & Aquaculture University of Patras

Panhellenic Society of Technologists Ichthyologists (PASTI)

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Trygonis V.
Chatziefstathiou M.

Mytilini, 2021

Organised by

Department
of Marine Sciences (DMS),
School of the Environment
University of the Aegean (UoA)

Department of Ichthyology
and Aquatic Environment (DIAE),
School of Agricultural Sciences,
University of Thessaly (UTh)

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Nutrition Technology, Western Greece
University of Applied Sciences

Panhellenic Society
of Technologists Ichthyologists
(PASTI)

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Chairman's Welcome Message

Dear Colleagues and Friends,

To date "HydroMediT" is being established as one of the major International Congresses on novel scientific and technological trends and aspects on Aquatic Living Resources and Oceanography in our region, covering the Mediterranean and its adjacent Seas.

It is organized biannually by a highly dedicated Committee on behalf of the:

- *Department of Ichthyology and Aquatic Environment* ***(DIAE),*** *University of Thessaly*
- *Department of Marine Sciences* ***(DMS),*** *University of the Aegean*
- *Department of Animal Production, Fisheries and Aquaculture Department* ***(DAPFA),*** *University of Patras*
- *Panhellenic Society of Technologists Ichthyologists* ***(PASTI)***

Our 4th Congress **(HydroMediT 2020)** would have been hosted in Mytilini, Island of Lesvos, Hellas (http://www.mytilene.gr/), in the premises of the University of the Aegean, with a series of National and International stakeholders as Co-Organizers, Supporters and Sponsors. However, the Covid-19 pandemic has led the Congress organizers to postpone the event and res-schedule this virtually this year on 4-6 November 2021.

The general fields and the specific topics of the Congress will attract contributions from all over the Mediterranean and its adjacent seas, and their list can be viewed in full on the Congress website, which is updated regularly.

There is an outstanding set of Keynote Speakers from many fields of the Fisheries, Oceanography, Marine Ecology, Aquaculture and Seafood supply Industry, Aquatic Environment as well as Government Agencies, Environmental Bodies, and Biodiversity Scientists. We have joined our forces and resources in order to host a series of high-profile panel discussions on the encountered thematic and parallel sessions of the Congress. Furthermore, our Congress provides an ideal forum for interactions between Scientists, Regulators, Industry Stakeholders, and the concerned General Public, and has already attracted pivotal support from a range of delegates and interested groups.

The main topic of the 4th Congress **(HydroMediT 2021)** "Aquatic Living Resources, Conservation and Oceanography in an Era of Change", highlights the changes and the impact on the aquatic ecosystems and their resources. Policy Makers, Industry players, Scientists, and Conservationists shall work together as they are all determined to deal with the challenges for the sustainability of the aquatic environment and its living resources in a changing world.

The main Keynote Speaker of the Congress will be the distinguished Professor ALBERTO BASSET of the University of Salento, addressing on ***"Biodiversity, Ecosystem Services and Natural Capital of the Mediterranean Transitional Ecosystems",*** and the different sessions will run from Thursday 4th to Saturday 6th November 2021.

We welcome you to enjoy the Congress sessions!

For the Organizing Committee

Drosos Koutsoubas
Chairman of the Congress

General Information

HydroMediT 2021 focuses on research and innovation technology applied mainly in the Mediterranean and its adjacent waters, but interesting solutions from other parts of the world will also be accepted.

Main topic of the Congress:
Aquatic Living Resources, Conservation and Oceanography in an Era of Change

The official language of the congress is English.

Papers have been peer reviewed by two (2) reviewers and only those accepted by the Scientific Committee were presented in Oral and Poster Sessions. The Scientific Committee reserves the right to re-assign submission to a different type of presentation (from oral to poster, and vice - versa).

The duration of the presentations is **13 minutes** for the Orals and **3 minutes for the Posters**.

Proceedings will be available to delegates upon arrival on site (in digital or web-based format).

For more information:
www.hydromedit.gr
hydromedit@artion.com.gr

Fields of Interest

General fields of interest will include - but not limited to - the following:

Field 1st: Aquaculture

- Innovative Biotechnological Applications and Products
- Physiology, Nutrition and Welfare
- New Species, Farming Methods and Techniques
- Diseases of Aquatic Animals
- Sustainable and Organic Aquaculture

Field 2nd: Fisheries

- Fisheries Policy and Sustainable Management
- Innovative Fisheries Technology
- Marine Fisheries Resources
- Species Biology and Ecology

Field 3rd: Oceanography

Physics and Climate of the Ocean

Marine Biogeochemistry

Marine Geosciences

Marine Microbiology and Biotechnology

Field 4th: Marine Diversity and Conservation

Ecology, Biogeography and Diversity

Invasive Species Biology

Evolutionary Ecology and Genetics of Marine Organisms

Marine Conservation Planning

Marine Protected Areas and Blue Economy

Field 5th: Inland Aquatic Ecology and Resources

Ecology, Hydrology and Limnology

Wetlands and Aquatic Systems Management

Modelling and Monitoring of Aquatic Ecosystems

Field 6th: Processing of Aquatic Products

Processing of Products and Quality

Product Control & Microorganisms Risk Assessment

Hygiene of Products and Consumer's Safety

Product Authentication and Certification

Field 7th: Economics and Marketing of Fisheries and Aquatic Products

Fisheries Socio-Economics

Fisheries and Aquatic Products Marketing

Competitiveness of Fisheries Products

Financial Sustainability of Sector Enterprises

Field 8th: Environmental Management and Education

Integrated Coastal Zone Management

Sustainable water resources management

Monitoring Ecosystem Health

Environmental Management Systems

Citizen Science

Marine Literacy

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Tania Zervoudaki, Hellenic Center of Marine Research, Greece

Keynote Speaker

Professor Alberto Basset

Alberto Basset is full Professor of Ecology and Pro-Rector for Sustainability at the University of Salento and Director of the Lecce seat of the Research Institute on Terrestrial Ecosystems of the National Research Council. He is member of the Executive Board of LifeWatch ERIC, the European eScience Infrastructure for Biodiversity and Ecosystem Research, being also Italian representative in the General Assembly, member of the EMSO ERIC Advisory Committee and of the National Scientific Committee for the Antarctic; he has been member of the Advisory Board of many international initiatives and Projects. His main research interests are in biodiversity organization and ecosystem functioning and services, with a focus on transitional waters and other types of aquatic ecosystems, being author of more than 150 papers in ecological journals on these topics. In the European research community, he is member of the Council and former President of the European Ecological Federation, current President of the Euro-Mediterranean Federation, former President and member of the Board of the Italian Society of Ecology. He is currently serving as Associate Editor of Frontiers in Marine Ecosystem Ecology and Board member of other international scientific journals.

Invited Speakers

Dr Graham John Pierce

Graham John Pierce is a Professor in School of Biological Sciences, University of Aberdeen (presently an honorary position) and Scientific Investigator of Fisheries Instituto de Investigaciones Marinas (Consejo Superor de Investigaciones Cientificas). He is/has been Co-chair in the ICES Working Group on Cephalopod Fisheries and Life History (2017-2022), and in the ICES Working Group on Marine Mammal Ecology (2015-2017), as well as President of the Cephalopod International Advisory Council (CIAC/2009-2012). He has co-ordinated several EU-funded collaborative Projects including "CetAMBICion" (research on Cetacean bycatch, 14 institutions, 2021-2023), "ECOSUMMER" Marie Curie Training Site (research training network for "early stage researchers", comprising 8 EU Institutions, 2006-09); "CEPHSTOCK" Concerted Action (network, 21 institutions, 2002-05, on Cephalopod biology and fisheries); "BIOCET" Research project (7 institutions, on contaminant bioaccumulation in Cetaceans, 2001-05). He has also participated in numerous European collaborative Projects since 1990, including the recent PARASITE (PArasite Risk ASsessment with Integrated Tools in EU Fish production value chains, 2013-2015 and MISTICSEAS III (on the implementation of the Marine Strategy Framework Directive in Macaronesia, 2019-2021). His main research interests focus mainly in marine biology and ecology, conservation and fisheries, including assessment, management and governance options for sustainable use of living marine resources, particularly in relation to Cephalopods and Cetaceans. Recent work includes statistical modelling of life history parameters, distribution and abundance of fish, cephalopods, marine mammals, and nematodes, development of niche models, prediction of climate change effects and applications to indicators for the Marine Strategy Framework Directive. He has been author of more than 290 papers in peer-reviewed scientific journals, co-editor in several books, and has presented or contributed to more than 460 conference talks and posters in these topics.

Dr Paolo Guidetti

Paolo Guidetti has a university degree in Biological Sciences at the University of Genoa (Italy) and a PhD in 'Fundamental Ecology' at the University of Lecce (Italy). Presently is a Research Director at the Stazione Zoologica Anton Dohrn-National Institute of Marine Biology, Ecology and Biotechnology. Formerly he has been full Professor of Ecology at the University Côte d'Azur (UCA, Nice, France; 2012-2020), Director of the ECOSEAS laboratory (UMR 3729) – CNRS-UCA (2016-2020), a Researcher in Zoology at the University of Salento (Italy; 2007-2012) and a visiting scientist in 2003 and 2004 at the Scripps Institution of Oceanography (UC San Diego, USA). His main research interests focus mainly on Marine Conservation, Marine Protected Areas, Fish Ecology and small-scale Fisheries Management. He has been Principal Investigator or WP Leader within more than 50 National and International Projects funded by public and private bodies: EU, Italian Ministries and Agencies, MPAs, Foundations, Private Companies. Professor Guidetti has been author of more than 150 papers in ISI peer-reviewed scientific journals (including 'Nature' and 'Science').

Professor Samuel Martin

Samuel Martin is a Professor in the School of Biological Sciences, University of Aberdeen and director of the Scottish Fish Immunology Research Centre (SFIRC) at the University of Aberdeen. His research group examines aspects of fish health, immunology and nutrition that is directly relevant to aquaculture industry. He is Co-I on the UKRI funded Aquaculture Research Collaborative Hub –UK, that is identifying priority areas for future research for both finfish and shell-fish aquaculture. Professor Martin's lab has been at the forefront of molecular biological approaches for the analysis of health and nutrition in both Atlantic Salmon and Rainbow Trout, with an emphasis on identifying differentially expressed genes related to immune function. The lab group uses a variety of genomics approaches to address key traits relating to improving the performance of fish in culture and has funding from UKRI, EU, SAIC and a number of industrial funded Projects. Professor Martin has been the author of more than 120 papers in peer-reviewed scientific journals and several chapter books in aquaculture topics.

Dr. Joaquim Garrabou

Joaquim Garrabou is a Marine Conservation Ecologist. He holds a PhD in Biology from the University of Barcelona (Spain). He is a Senior Researcher at the Institute of Marine Sciences-CSIC in Barcelona (Spain) and the co-ordinator of the Marine Biodiversity Conservation Group MedRecover (www.medrecover.org). The ultimate objective of his research is to enhance science based management strategies to inform adaptation plans of coastal areas as well as the sustainable use of marine resources. He is particularly interested in promoting the role of Marine Protected Areas as nature based solution face to climate change. In this framework he is the coordinator the T-MEDNet network (www.t-mednet.org) devoted to track climate change impacts in the Mediterranean and the Marine Citizen Science platform Observadores del Mar (www.observadores delmar.es). He has been the author of more than 110 papers in peer review ecological journals on these topics.

Collaborating Journals

- **PeerJ Life & Environment** is a highly respected Open Access journal publishing research in the biological, medical and environmental sciences. Any submission to the HydroMediT 2021 collection would undergo peer-review at the journal (i.e. acceptance by the congress does not guarantee acceptance by the journal) and would be subject to their normal publication fees if accepted (see https://peerj.com/pricing/).

  Instructions to submit an article for peer review:

  1) Head to https://peerj.com/new/
  2) Under "Select Journal" choose "Life, Biological, Environmental and Health Sciences"
  3) Follow the instructions on the submission form
  4) In the Confidential Notes for PeerJ Staff box towards the end of the submission form, please include **"Submitted to the HydroMediT 2021 Collection"**

- **Aquaculture International** (Journal of the European Aquaculture Society) https://www.springer.com/journal/10499

- **MDPI.** Special issue "Selected papers from HydroMediT 2021: Applied Ichthyology, Oceanography and Aquatic Environment". Joint special issue of Conservation (https://www.mdpi.com/journal/conservation) and Sustainability (https://www.mdpi.com/journal/sustainability).

Programme Overview

Thursday, 4 November 2021

HALL 1 - < Mytilene >		HALL 2 - < Volos >		HALL 3 - < Messolonghi >	
10:00 - 12:00	**Oral Session** **1st Field, Aquaculture**	**10:00 - 12:00**	**Oral Session** **4th Field, Marine Diversity & Conservation**	**10:00 - 12:00**	**Oral Session** **5th Field, Inland Aquatic Ecology & Resources**
12:00 - 12:30	**Coffee Break**				
12:30 - 13:15	**Keynote Speech - 1** **Dr Paolo Guidetti - Stazione Zoologica Anton Dohrn** **ITALY**				
13:15 - 14:00	**Oral Session** **1st Field, Aquaculture**	**13:15 - 14:00**	**Oral Session** **4th Field, Marine Diversity & Conservation**	**13:15 - 14:00**	**Poster Session** **5nd Field, Inland Aquatic Ecology and Resources**
14:00 - 15:00	**Lunch Break**				
15:00 - 17:00	**Oral Session** **1st Field, Aquaculture**	**15:00 - 17:00**	**Oral Session** **2nd Field, Fisheries**	**15:00 - 17:00**	**Oral Session** **3rd Field, Oceanography**
17:00 - 17:30	**Coffee Break**				
17:30 - 18:30	**Oral Session** **8th Field, Environmental Management and Education**	**17:30 - 18:30**	**Oral Session** **4th Field, Marine Diversity & Conservation**	**17:30 - 18:30**	**Oral Session** **3rd Field, Oceanography**

HALL 1 - < Mytilene >	
18:30 - 19:30	**Opening Ceremony** **Awards**

Friday, 5 November 2021

HALL 1 - < Mytilene >		HALL 2 - < Volos >		HALL 3 - < Messolonghi >	
10:00 - 12:00	**Oral Session** **1st Field, Aquaculture**	**10:00 - 12:00**	**Oral Session** **6th Field, Processing of Aquatic Products** **7th Field, Economics & Marketing of Fisheries Products**	**10:00 - 12:00**	**Oral Session** **8th Field, Environmental Management and Education**
12:00 - 12:30	**Coffee Break**				
12:30 - 14:00	**Oral Session** **1st Field, Aquaculture**	**12:30 - 13:15**	**Oral Session** **6th Field, Processing of Aquatic Products** **7th Field, Economics & Marketing of Fisheries Products**		
		13:15 - 14:00	**Poster Session** **6th Field, Processing of Aquatic Products** **7th Field, Economics & Marketing of Fisheries Products**	**13:15 - 14:00**	**Poster Session** **8th Field, Environmental Management and Education**
14:00 - 15:00	**Lunch Break**				
15:00 - 15:45	**Keynote Speech - 2** **Dr Graham John Pierce - Institute of Marine Sciences** **SPAIN**				
15:45 - 17:00	**Oral Session** **8th Field, Environmental Management and Education**	**15:45 - 17:00**	**Poster Session** **2nd Field, Fisheries**	**15:45 - 17:00**	**Poster Session** **1st Field, Aquaculture**
17:00 - 17:30	**Coffee Break**				
17:30 - 18:30	**Poster Session** **8th Field, Environmental Management and Education**	**17:30 - 18:30**	**Poster Session** **3rd Field, Oceanography**		

HALL 1 - < Mytilene >	
18:30 - 19:30	**Congress Keynote Speech** **Professor Alberto Basset, University of Salento** **ITALY**

Saturday, 6 November 2021

HALL 1 - < Mytilene >		HALL 2 - < Volos >	
10:00 - 12:00	**Oral Session** **1st Field, Aquaculture**	**10:00 - 12:00**	**Poster Session** **4th Field, Marine Diversity & Conservation**
12:00 - 12:30	Coffee Break		
12:30 - 13:15	**Keynote Speech - 3** **Professor Samuel Martin - University of Aberdeen** **UNITED KINGDOM**		
13:15 - 14:00	**Oral Session** **1st Field, Aquaculture**	**13:15 - 14:00**	**Oral Session** **3rd Field, Oceanography**
14:00 - 15:00	Lunch Break		
15:00 - 15:45	**Keynote Speech - 4** **Dr. Joaquim Garrabou - Institute of Marine Sciences** **SPAIN**		
15:45 - 17:00	**Poster Session** **8th Field, Environmental Management and Education**	**15:45 - 17:00**	**Poster Session** **1st Field, Aquaculture**

HALL 1 - < Mytilene >	
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17:30 - 18:30	**Panhellenic Society of Technologists Ichthyologists (PASTI)** **General Assembly (Statutory)**

Programme

Thursday, 4 November 2021

HALL 1 - < Mytilene >		HALL 2 - < Volos >		HALL 3 - < Messolonghi >	
10:00 - 12:00	**Oral Session** **1st Field, Aquaculture** **Chairs:** *Panagiota Panagiotaki, Vasilios Bakopoulos*	**10:00 - 12:00**	**Oral Session** **4th Field, Marine Diversity & Conservation** **Chairs:** *Stelios Katsanevakis, Georgios Gkafas*	**10:00 - 12:00**	**Oral Session** **5th Field, Inland Aquatic Ecology & Resources** **Chairs:** *Olga Petriki, Ourania Tzoraki*
10:00 - 10:15	GROWTH PERFORMANCE OF SEABASS *(Dicentrarchus labrax)* AND ROCKET PLANT *(Eruca sativa)* IN A BRACKISH AQUAPONICS SYSTEM USING THE NUTRIENT FILM TECHNIQUE (NFT) *Vlahos N., Ioannidis. J., Devetzoglou K., Hotos G., Vidalis K., Mente E.*	**10:00 - 10:15**	HUMAN DISTURBANCE ALTERS TERRITORIAL BEHAVIOR OF THE GHOST CRAB *Ocypode cursor* (Linnaeus, 1758) *Gül M.R.*	**10:00 - 10:15**	NUMERICAL SIMULATION OF HYDRODYNAMIC PARAMETERS OVER RIVER CONTROL STRUCTURES *Farsirotou E., Xafoulis N.*
10:15 - 10:30	EVALUATION OF THREE MICROALGAE SPECIES FOR USE IN AQUAFEED *Pappou S., Papadaki S., Batzakas I., Krokida M.*	**10:15 - 10:30**	MOLECULAR IDENTIFICATION OF A PARASITIC NEMATODE FOUND IN THE DIGESTIVE SYSTEM OF A STRANDED MEDITERRANEAN MONK SEAL (*Monachus monachus*) IN THE AREA OF PAGASITIKOS GULF *Koitsanou E., Sarantopoulou J., Komnenou A., Exadactylos A., Dendrinos P., Gkafas G.A.*	**10:15 - 10:30**	RIVER INTEGRITY ASSESSMENT BASED ON HABITAT QUALITY AND THE MACROINVERTEBRATE COMMUNITY *Atanacković A., Zorić K., Tubić B., Tomović J., Djikanović V.*
10:30 - 10:45	SEASONAL GENE EXPRESSION OF ANTIOXIDANT ENZYMES AND HSP70 IN GILTHEAD SEA BREAM (*Sparus aurata*) *Bellou E., Makri V., Giantsis I.A., Feidantsis K., Michaelidis B., Exadactylos A.*	**10:30 - 10:45**	DENSITY AND RECRUITMENT OF A NEW INVASIVE SPECIES FOR THE TURKISH COAST OF THE BLACK SEA *Aydın M., Gül M.R.*	**10:30 - 10:45**	IMPLEMENTING MULTIPLE MANAGERIAL AND ENVIRONMENTAL SCENARIOS IN A MEDITERRANEAN LAKE *Petriki O., Tsagarakis K., Moutopoulos D.K., Stoumboudi M.*
10:45 - 11:00	SEASONAL CHANGES ON ANTIOXIDANT ENZYMES' GENE EXPRESSION IN RED PORGY (*Pagrus pagrus*). *Makri V., Feidantsis K. , Giantsis I.A., Michaelidis B.*	**10:45 - 11:00**	ASSESSMENT OF INVASIVENESS POTENTIAL OF *Synaptula reciprocans* (ECHINODERMATA: SYNAPTIDAE) BY THE AQUATIC SPECIES INVASIVENESS SCREENING KIT *Çiftçioğlu M., Filiz H.*	**10:45 - 11:00**	HYDROLOGICAL, ENVIRONMENTAL AND TAXONOMICAL HETEROGENEITY DURING THE TRANSITION FROM DRYING TO FLOWING CONDITIONS IN A MEDITERRANEAN INTERMITTENT RIVER *Banegas-Medina A., Montes I.Y., Tzoraki O., Brendonck L., Pinceel T., Diaz G., Arriagada P., Arumi J.L., Figueroa R.*
11:00 - 11:15	HISTOPATHOLOGICAL AND HEMATOLOGICAL EXAMINATION OF FISH AFTER TREATMENT WITH NITROFURAZONE *Andreopoulou, S., Berillis, P., Michail, G., Vlahos, N.*	**11:00 - 11:15**	OCCUPANCY ESTIMATION OF ALIEN, CRYPTOGENIC, AND NEONATIVE SPECIES IN PELOPONNESE (GREECE) *Katsanevakis S.*		
11:15 - 11:30	THE IMPACT OF FEEDING FREQUENCY ON THE GUT MICROBIOTA OF FRESHWATER ADAPTED SEABASS (*Dicentrarchus labrax*) IN A COUPLED AQUAPONICS SYSTEM WITH LETTUCE (*Lactuca sativa*) *Nikouli E., Stathopoulou P., Berillis P., Vlachos N., Mente E., Kormas K.*	**11:15 - 11:30**	THE EFFECTS OF ABIOTIC FACTORS ON THE ECONOMICALLY IMPORTANT BROWN SEAWEED Dictyopteris polypodioides PHYSIOLOGICAL PERFORMANCE *Nakou K., Malea L., Seitidou O., Lachanidou G., Papadimitriou A., Orfanidis S.*		
12:00 - 12:30	**Break**				

12:30 - 13:15	**Keynote Speech - 1** **THE NEED TO HAVE MORE 'STRICTLY & SERIOUSLY' MARINE PROTECTED AREAS IN THE MEDITERRANEAN SEA** *Dr Paolo Guidetti - Stazione Zoologica Anton Dohrn* **ITALY** **Chair:** *Drosos Koutsoubas, Stelios Katsanevakis*

13:15 - 14:00	**Oral Session** **1st Field, Aquaculture** **Chairs:** *Athanasios Exadactylos, Yannis Cladas*	**13:15 - 14:00**	**Oral Session** **4th Field, Marine Diversity & Conservation** **Chairs:** *Vasilios Trygonis, Sotiris Orfanidis*	**13:15 - 14:00**	**Poster Session** **5nd Field, Inland Aquatic Ecology and Resources** **Chairs:** *Dimitrios Moutopoulos, Miranda Tsoumani*
13:15 - 13:30	**HISTORY OF THE INTRODUCTION OF THE NON-NATIVE SPECIES *Pagrus major* (TEMMINCK AND SCHLEGEL, 1843) IN GREEK AQUACULTURE** *Cladas Y., Ketsilis-Rinis V., Moutopoulos D.K., Spala K., Koutsikopoulos C.*	**13:15 - 13:30**	**LENGTH-WEIGHT RELATIONSHIP AND REPRODUCTION OF THE MARBLED ELECTRIC RAY, *Torpedo marmorata* (RISSO, 1810), FROM THE GREEK SEAS** *Karampetsis D., Chatzispyrou A., Gubili C., Touloumis K., Kioulouris S., Anastasopoulou A., Koutsikopoulos C., Batjakas I. E.*	**1**	**USE OF HBV LIGHT MODELTO SIMULATE INTERMITTENT RIVER OF KRASTIC GEOLOGY: THE CASE STUDY OF DRAGONJA RIVER, SLOVENIA** *Papasarafianou S., Tzoraki O., Rusjan S.*
13:30 - 13:45	**ELECTROSTUNNING HAS THE POTENTIAL TO DELAY FILLET DEGRADATION POST-HARVEST IN RED SEABREAM (*Pagrus major*)** *Angelakopoulos R., Dimitroglou A., Papaharisis L., Moutou K.A.*	**13:30 - 13:45**	**SPATIAL VARIATION OF THE VERTICAL DISTRIBUTION OF THE STATUS OF MACROALGAE COMMUNITIES, SEA URCHINS AND FISH POPULATIONS IN THE EASTERN MEDITERRANEAN** *Nikolaou A., Tsirintanis K., Katsanevakis S.*	**2**	**CAROTENOID PROFILE OF A HYPERSALINE *Dunaliella* sp. STRAIN CULTIVATED IN DIFFERENT CONDITIONS** *Ntzouvaras A., Chantzistrountsiou X., Papadaki S., Tzovenis I., Economou-Amilli A.*
13:45 - 14:00	**HETEROTROPHIC BATCH CULTIVATION OF *Chlorella sorokiniana* AND *Chlorella kessleri* USING GLYCEROL: BIOMASS & LIPID PRODUCTIVITIES AND BIOMASS CARBON YIELD COEFFICIENTS** *Metsoviti M. N., Mpesios A., Kasiteropoulou D., Lamprakopoulos S., Gougoulias N., Papapolymerou G.*	**13:45 - 14:00**	***Pinna nobilis*, IN THE BRINK OF EXTINCTION IN GREEK SEAS** *Zotou M., Papadakis O., Ragkousis M., Issaris Y., Gerakaris V., Çinar M.E., Katsanevakis S.*	**3**	**FISHERIES TYPOLOGY OF *Pontastacus leptodactylus* (DANUBE CRAYFISH (ESCHSCHOLTZ, 1823)) IN THE DAM LAKE OF POLYFYTOS (WESTERN MACEDONIA)** *Kafazi N., Katselis G., Moutopoulos D.K.*
				4	**SEX RATIO AND ALLOMETRIC RELATIONSHIPS OF THE WATER FROG (*Pelophylax epeiroticus*) IN LAKE PAMVOTIDA (NORTHWEST GREECE)** *Hatziioannou M., Douska C., Klaoudatos D.*
				5	**VISUALIZATION TYPES OF VARIOUS AQUATIC PHASES OF INTERMITTENT STREAMS** *Stamataki M.D., Tzoraki O., Pnevmatikou Z., Papadopoulos V., Sauquet E.*
				6	**DEVELOPMENT OF PROTOCOLS FOR THE ENVIRONMENTAL MONITORING OF THE COASTAL AND TRANSITIONAL RIVER ECOSYSTEMS** *Stamataki M.D., Koutalakis P., Papadopoulos D., Tzoraki O.*
				7	**FATTY ACIDS QUANTIFICATION AND NUTRITIONAL QUALITY EVALUATION OF FRESHWATER FISH OF LAKE VOLVI** *Kokokiris L., Kyritsi S., Doulgeraki S., Terzidis M.*
14:00 - 15:00	**Break**				

15:00 - 17:00	**Oral Session** **1st Field, Aquaculture** **Chairs:** *Vasilios Bakopoulos, Panagiotis Berillis*	**15:00 - 17:00**	**Oral Session** **2nd Field, Fisheries** **Chairs:** *Dimitris Moutopoulos, Ioannis Batjakas*	**15:00 - 17:00**	**Oral Session** **3rd Field, Oceanography** **Chairs:** *Thomas Hasiotis, Gianis Androulidakis*
15:00 - 15:15	**REPRODUCTION AND BEHAVIOR OF RED HYBRID TILAPIA *Oreochromis mossambicus*** *Kotsarinis S., Siapazis C., Vlachos N., Berillis P., Mente E.*	**15:00 - 15:15**	**LENGTH-WEIGHT RELATIONSHIPS AND NEW FISHERY DATA OF THE THREATENED EUROPEAN SPINY LOBSTER *Palinurus elephas* (J.C. FABRICIUS, 1787) FROM THE REMOTE PSARA-ANTIPSARA ISLANDS, NORTH-EAST AEGEAN SEA, GREECE.** *Kampouris T.E., Batjakas I.E.*	**15:00 - 15:15**	**RESTRICTED LAGOONS SUBJECTED TO DIFFERENTIAL TIDAL FORCING** *Horsch G.M., Fourniotis N.Th., Mastoris-Kourmpanis D.K.*
15:15 - 15:30	**FISHMEAL PROTEIN REPLACEMENT BY *Zophobas morio* LARVAE AFFECTS *Dicentrarchus labrax* GROWTH PERFORMANCE IN AN AQUAPONICS SYSTEM** *Stathopoulou P., Berillis P., Levizou E., Vlahos N., Katsoulas N., Rumbos, C., Mente, E.*	**15:15 - 15:30**	**AGE, GROWTH AND FEEDING HABITS OF THE BLACK GOBY, *Gobius niger*, IN CANDARLI BAY, AEGEAN SEA** *Filiz H., Çiftçioğlu M.*	**15:15 - 15:30**	**METHODOLOGICAL CONSIDERATIONS IN MAPPING A HOSTILE SEACLIFF WITH TERRESTRIAL LASER SCANNER** *Panagou Th., Hasiotis T., Oikonomou E.*
15:30 - 15:45	**THE EFFECT OF TEMPERATURE AND INFECTION ROUTE ON *in vitro* HEMOCYTE PHAGOCYTOSIS ACTIVATION AFTER EXPERIMENTAL INFECTION OF COMMON OCTOPUS, *Octopus vulgaris* (CUVIER, 1797) WITH EITHER *Photobacterium damselae* subsp. *damselae* OR *Vibrio anguillarum* O1** *White D. M., Valsamidis M. A., Kokkoris G., Bakopoulos V.*	**15:30 - 15:45**	**ASSESSMENT OF DEMERSAL FISHERY RESOURCES IN PAGASITIKOS GULF (CENTRAL AEGEAN SEA)** *Klaoudatos D., Vafidis D., Exadactylos A., Neofitou N., Lolas A., Apostologamvrou C.*	**15:30 - 15:45**	**THE ACOUSTIC REPERTOIRE AND THE CHARACTERISTICS OF DOLPHIN WHISTLES IN GREECE** *Tourgeli Provata M., Boisseau O., Frantzis A., Alexiadou V., Trygonis V.*
15:45 - 16:00	**TRANSCRIPTOME COMPARISON OF RESISTANT AND SUSCEPTIBLE SEA BASS (*Dicentrarchus labrax*) CHALLENGED WITH NERVOUS NECROSIS VIRUS REVEALS A MARKED CONTRAST IN GENE EXPRESSION LEVELS IN A TIME-COURSE STUDY** *Toubanaki D.K., Tzortzatos O.P., Efstathiou A., Moulos P., Papaharisis L., Bakopoulos V., Karagouni E.*	**15:45 - 16:00**	**EFFECTS OF ALIEN SPECIES ON THE SUSTAINABILITY OF SMALL-SCALE FISHERIES (A CASE STUDY IN THE ISLAND OF CRETE)** *Klaoudatos D., Samaras G., Exadactylos A., Vafidis D., Apostologamvrou C., Conides A.*	**15:45 - 16:00**	**OCEAN CIRCULATION EFFECTS ON EUTROPHICATION IN THERMAIKOS GULF** *Androulidakis Y., Kolovoyiannis V., Makris C., Krestenitis Y., Baltikas V., Stefanidou N., Chatziantoniou A., Topouzelis K., Mallios Z. Moustaka-Gouni M.*
16:00 - 16:15	**EFFECT OF MICRONUTRIENTS ON THE GROWTH RATES AND BIOMASS PRODUCTIVITIES OF *Microchloropsis gaditana*** *Metsoviti M. N., Papapolymerou G.*	**16:00 - 16:15**	**BATHYMETRIC DISTRIBUTION OF THE FAMILY OCTOPODIDAE IN THE NORTH AEGEAN SEA, EASTERN MEDITERRANEAN** *Gitarakos G., Touloumis K., Koutsoubas D.*	**16:00 - 16:15**	**SEISMIC EVIDENCE OF FLUIDS IN THE SEDIMENTS OF THE SEMI-ENCLOSED GULF OF GERA (LESVOS, GREECE)** *Manoutsoglou E., Hasiotis T., Andreadis O., Velegrakis A.F.*
16:15 - 16:30	**HUMORAL IMMUNE RESPONSE OF SEA BASS (*Dicentrarchus labrax*) WITH NATURAL RESISTANCE TO THE RGNNV GENOTYPE OF B-NODAVIRUS** *Bakopoulos V., Valsamidis M.A., White M.D., Papaharisis L., Toubanaki D.K., Karagouni E.*	**16:15 - 16:30**	**MORPHOMETRIC MEASUREMENTS OF DATA-DEFICIENT ELASMOBRANCH SPECIES FROM THE MEDITERRANEAN SEA** *Pyloridou K., Naasan Aga-Spyridopoulou R., Giovos I., Moutopoulos D. K.*	**16:15 - 16:30**	**INTERANNUAL VARIABILITY OF SEA SURFACE TEMPERATURE OVER THE NE MEDITERRANEAN SEA** *Androulidakis Y., Krestenitis Y.*
17:00 - 17:30	**Break**				

17:30 - 18:30 **Oral Session**
8th Field, Environmental Management and Education
Chairs: *Michael Chatziefstathiou, Georgios Tsirtsis*

17:30 - 17:45 EVALUATION OF POTENTIAL PREVENTIVE MEASURES AGAINST SEAWATER INTRUSION IN NEA MOUDANIA AQUIFER, GREECE
Siarkos I., Katirtzidou M., Sevastas S., Tzoraki O.

17:45 - 18:00 SEASONAL OXIDATIVE AND CELLULAR STRESS RESPONSES OF COMMERCIALLY IMPORTANT INVERTEBRATES AT DIFFERENT HABITATS OF THE NORTH AEGEAN SEA
Feidantsis K., Michaelidis B., Raitsos D.E., Exadactylos A., Gkafas G.A., Staikou A., Hatziioannou M., Vafidis D.

18:00 - 18:15 MODELING SURFACE WATER QUALITY USING ARTIFICIAL NEURAL NETWORKS AND MULTIVARIATE METHODS
Sakaa B., Brahmia N.

18:15 - 18:30 A COMPARATIVE STUDY OF IDENTICATION METHODS FOR BACTERIAL PATHOGENS ISOLATED FROM RAINBOW TROUT (O. mykiss, W.)
Urku C., Secer F.S., Onalan S., Akayli T.

17:30 - 18:30 **Oral Session**
4th Field, Marine Diversity & Conservation
Chairs: *Drosos Koutsoubas, Dimitris Vafidis*

17:30 - 17:45 FUNCTIONAL TRAITS OF POLYCHAETES CHANGE BETWEEN DIFFERENT HABITAT TYPES OF *Posidonia oceanica*
Katsiaras N., Evagelopoulos A., Koutsoubas D.

17:45 - 18:00 PRELIMINARY RESULTS ON THE EFFECT OF GROWTH IN THE SKELETAL ARCHITECTURE OF THE ECHINOID *Diadema setosum.*
Varkoulis A., Voulgaris K., Zaoutsos S., Vafidis D.

18:00 - 18:15 FRIENDLY AQUACULTURE; ARE FISH FARMS UNFAIRLY HELD RESPONSIBLE FOR DEATHS OF MEDITERRANEAN MONK SEAL?
Taskavak E., Ozel F.V.

18:15 - 18:30 THE USE OF THE mtDNA AS A MOLECULAR TOOL TOWARDS AUTHENTICITY OF THE RED PORGY *Pagrus pagrus*
Chatzoglou E., Tsaousi N., Triantaphyllidis G., Malandrakis E., Miliou H.

17:30 - 18:30 **Oral Session**
3rd Field, Oceanography
Ch7airs: *Adonis Velegrakis, Elina Tragou*

17:30 - 17:45 IMPLEMENTING A NUMERICAL MODEL FOR THE INVESTIGATION OF THE HYDRODYNAMICS OF SARONIKOS GULF (EASTERN MEDITERRANEAN)
Kolovoyiannis V., Krasakopoulou E., Zervakis V., Tragou E., Kontoyiannis H.

17:45 - 18:00 PRIORITIZATION OF BEACH VULNERABILITY TO SEA LEVEL RISE: THE CASE OF SANTORINI, GREECE
Monioudi I.N., Markozanes F., Velegrakis A.F., Chatzipavlis A.E., Ćulibrk A., Kontopyrakis K.E.

18:00 - 18:15 AUTOMATED 2-D SHORELINE AND WAVE RUN-UP DETECTIONS FROM HIGH FREQUENCY OPTICAL DATA. EXAMPLE FROM AN URBAN PERCHED BEACH: AMMOUDARA, HERAKLEION - CRETE.
Chatzipavlis A.E., Zamboukas V., Trygonis V., Velegrakis A.F.

HALL 1 - < Mytilene >

18:30 - 19:30 **Opening Ceremony**
Awards

Friday, 5 November 2021

HALL 1 - < Mytilene >

10:00 - 12:00	**Oral Session** **1st Field, Aquaculture** **Chairs:** *Nikos Vlahos, Cosmas Nathanailides*
10:00 - 10:15	MUSCLE CELLULARITY AND PROXIMATE COMPOSITION OF FAST AND SLOW GROWING MARICULTURED MEAGRE, *Argyrosomus regius* *Mitakos I., Kokokiris L., Bitchava, K, Barbouti A., Gouva L., Nathanailides C.*
10:15 - 10:30	SPECIFIC IMMUNE RESPONSE OF SEA BASS (*Dicentrarchus labrax*) IMMUNIZED WITH VARIOUS ANTIGENS OF *Photobacterium damselae* subsp. *piscicida*, *Vibrio anguillarum* O1 and B-NODAVIRUS *Bakopoulos V., Valsamidis M.A., White M.D., Kokkoris G.D.*
10:30 - 10:45	THE PROGRESS OF ECONOMIC PERFORMANCE OF SEABASS & SEABREAM AQUACULTURE PRODUCTION SYSTEMS IN GREECE *Danatskos C., Kokkinakis A.K.*
10:45 - 11:00	HEMOLYMPH ACTIVITIES AFTER EXPERIMENTAL INFECTION OF COMMON OCTOPUS, *Octopus vulgaris* (CUVIER, 1797) WITH *Photobacterium damselae* subsp. *piscicida* *White D. M., Valsamidis M.A., Bakopoulos V.*
11:00 - 11:15	THE EFFECT OF SALINITY ON THE GROWTH PERFORMANCE AND SURVIVAL RATE OF GILTHEAD SEABREAM (*Sparus aurata*) AND ROCK SAMPHIRE (*Crithmum maritimum*) IN AN EXPERIMENTAL BRACKISH AQUAPONIC SYSTEM *Vlahos N., Stefanou Ch., Agapitos A., Berillis P., Levizou E., Krigas N., Kormas K., Mente E.*
11:15 - 11:30	THE EFFECT OF DIFFERENT OZONE CONCENTRATIONS ON THE OXIDATION RATE OF AMMONIA AND NITRITE ION AT A SEAWATER AQUARIUM *Vlahos N., Kapetanios E., Mantzouranis I., Moshopoulos S., Bekiari V., Vidalis, K., Hotos G., Mente E.*

HALL 2 - < Volos >

10:00 - 12:00	**Oral Session** **6th Field, Processing of Aquatic Products** **7th Field, Economics & Marketing of Fisheries Products** **Chairs:** *Ioannis Boziaris, Foteini Parlapani*
10:00 - 10:15	BIOCHEMICAL CHARACTERIZATION OF MARINE INVASIVE SPECIES *Lagocephalus sceleratus (Gmelin, 1789), Pterois miles (Bennett, 1828)* AND *Fistularia commersonii (Rüpp, 1838)* IN THE SOUTHERN AEGEAN *Pappou S., Tsirozoglou F., Papadaki S., Krokida M., Batzakas I.*
10:15 - 10:30	ANTIOXIDANT BIOACTIVE PEPTIDES ENCRYPTED IN MARINE BIVALVE *Mytilus galloprovincialis* PROTEINS: *in silico* ANALYSIS *Vratsistas A., Vafidis D.*
10:30 - 10:45	MICROBIAL SPOILAGE AND SHELF-LIFE OF ICE-STORED MEAGRE (Argyrosomus regius) *Syropoulou F., Parlapani F.F., Mallouchos A., Boziaris I.S.*
10:45 - 11:00	"PROTECTION" UNDER THE SHADE: LACK OF LABELLING LEGISLATION AND UMBRELLA TERMS ALLOW PROTECTED SPECIES IN BATOID WINGS FROM GREECE *Giagkazoglou Z., Griffiths A.M., Imsiridou A., Chatzispyrou A., Touloumis K., Hebb J.L., Mylona D., Malamidou A. K., Apostolidi E.D., Batjakas I.E., Gubili C.*
11:00 - 11:15	AMINO ACID COMPOSITION OF SEAWEEDS FROM ÇANAKKALE, TURKEY *Çankırılıgil E.C., Ak İ.*
11:15 - 11:30	ASSESSING CONSUMER ATTITUDES TOWARDS THE INVASIVE RAYED PEARL OYSTER *Pinctada imbricata radiata* (LEACH 1814) *Ziou A., Douligeri A., Theodorou J.A., Katselis G., Moutopoulos D.K.*

HALL 3 - < Messolonghi >

10:00 - 12:00	**Oral Session** **8th Field, Environmental Management and Education** **Chairs:** *Ourania Tzoraki, Georgios Tsirtsis*
10:00 - 10:15	SPATIAL PRIORITIZATION FOR COASTAL MANAGEMENT BASED ON ERODIBILITY ASSESSMENT PAIRED WITH SOCIO-ECONOMIC ELEMENTS *Chalazas T., Chatzistratis D., Velegrakis A.F.*
10:15 - 10:30	PRELIMINARY STUDY OF HEAVY METALS IN SEDIMENTS OF VOLOS HARBOR (THESSALY, GREECE) *Georgiou K., Gounaris S., Tsamili V., Neofitou N., Kelepertzis E., Kantiranis N., Skordas K., Vafidis D.*
10:30 - 10:45	CLIMATE CHANGE IMPACT ASSESSMENT AND NATURE-BASED SOLUTIONS IN THE KALLONI RIVER BASIN, GREECE *Koutsovili E.I., Tzoraki O., Theodossiou N., Gaganis P.*
10:45 - 11:00	ADAPTING A FRESHWATER FRAMEWORK TO A COASTAL CASE *Ćulibrk A., Tzoraki O.*
11:00 - 11:15	SALMON MATURITY CLASSIFICATION BASED ON BAYESIAN NETWORKS AND SUPPORT VECTOR MACHINES METHODOLOGIES *Kokkinos K., Exadactylos A., Vafidis D.*
11:15 - 11:30	USE OF FUZZY COGNITIVE MAPS AS A DECISION-MAKING TOOL TO FISHERIES MANAGEMENT *Kokkinos K., Exadactylos A., Vafidis D.*

12:00 - 12:30	**Break**

12:30 - 14:00 **Oral Session**
1st Field, Aquaculture
Chairs: *Eleni Golomazou, Constantinos Poulos*

12:30 - 12:45 ASSESSMENT OF GILTHEAD SEA BREAM (*SPARUS AURATA*) BIOMASS IN AQUACULTURE CAGES USING SPLIT-BEAM ECHOSOUNDERS
Trygonis V., Kapelonis Z., Koukourouvli N.

12:45 - 13:00 CAN DIETARY INSECT MEAL AFFECT FISH COLOUR AND BODY SHAPE?
Mastoraki M., Mente E., Chatzifotis S., Antonopoulou E.

13:00 - 13:15 INSECT MEAL DIETS MODULATE HSP EXPRESSION AND MAPK ACTIVATION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND GILTHEAD SEA BREAM (*Sparus aurata*)
Panteli N., Mastoraki M., Chatzifotis S., Mente E., Antonopoulou E.

13:15-13:30 THE EFFECT OF FISH STOCKING DENSITY ON GROWTH PERFORMANCE OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND LETTUCE (*Lactuca sativa* VAR. GREEN TOWERS).
Sakorafas S., Papaefstathiou K., Berillis P., Vlahos N., Levizou E.,Mente E.

12:30 - 13:15 **Oral Session**
6th Field, Processing of Aquatic Products
7th Field, Economics & Marketing of Fisheries Products
Chairs: *Steriani Matsiori, Sofoclis Dritsas*

12:30 - 12:45 SEASONAL VARIATION IN THE PROXY-BIOCHEMICAL COMPOSITION OF THE NON-INDIGENOUS PEARL OYSTER PINCTADA IMBRICATA RADIATA (LEACH, 1814) FROM THE CENTRAL WEST AEGEAN SEA, GREECE
Makri M., Douvi X., Ramfos A., Spinos E., Theodorou J.A.

12:45 - 13:00 DEVELOPMENT AND APPLICATION OF NOVEL METHODS FOR FISH HARVESTING AND PROCESSING FOR QUALITY PRESERVARTION AND SHELFLIFE EXTENSION
Ntzimani A., Angelakopoulos R., Semenoglou I., Stavropoulou N., Dermesonlouoglou E., Tsironi T., Xidia D., Liberis N., Moutou K., Taoukis P.

13:15 - 14:00 **Poster Session**
6th Field, Processing of Aquatic Products
7th Field, Economics & Marketing of Fisheries Products
Chairs: *Steriani Matsiori, Sofoclis Dritsas*

1 EVALUATION AND MODELLING OF SHRIMP MELANOSIS
Georgiadou M., Pliameri I., Stoforos N.G., Tsironi T.

2 AMINO ACID COMPOSITION OF CERAMIUM RUBRUM (RHODOPHYCEAE) FROM NORTH AGEAN SEA, TURKEY
Çankırılıgil E.C., Ak İ.

3 MARINE BIVALVES MICROBIOLOGICAL MONITORING ORIGINATED FROM SEAFOOD MARKETS LOCATED IN THESSALONIKI (NORTH GREECE)
Lattos A., Chaligiannis I., Giantsis I.A., Papadopoulou A., Staikou A., Michaelidis B.

13:15 - 14:00 **Poster Session**
8th Field, Environmental Management and Education
Chairs: *Konstantinos Skordas, Thomas Hasiotis*

1 PARTICIPATORY APPROACHES IN FLOOD RISK MANAGEMENT
Kalli A., Koutsovili E., Tzoraki O.

2 APPLICATION OF THE DPSIR METHODOLOGY FOR SUSTAINABLE MANAGEMENT OF WATER RESOURCES IN THE MYGONIA BASIN, GREECE
Papamichail P., Voudouris K.S.

3 Hg IN SURFACE SEDIMENTS FROM THE HARBOR OF VOLOS, GREECE
Georgiou K., Gounaris S., Tsamili V., Neofitou N., Kelepertzis E., Kantiranis N., Skordas K., Vafidis D.

4 **IDENTIFICATION OF THE PEARL OYSTER (Pinctada imbricate radiata), MISLABELING IN THE GREEK MARKET BY USING INTERNET-BASED TOOLS**
Gkikas M., Koutante E., Theodorou J.A.

4 **PRELIMINARY ACCESSEMENT OF INTERACTIONS BETWEEN DOLPHINS AND FISHERS IN ZAKYNTHOS ISLAND**
Vakouli E., Pardalis S., Komnenou A., Exadactylos A., Gkafas G.A.

5 **Mix infection of cultured African catfish (Clarias gariepinus): Rhizopus sp. AND opportunist bacteria**
Nikolov G., Urku C., Zarpyanova D., Sandeva G.

6 **FLOOD AND HAZARDOUS RISK OF LARNACA CITY UNDER THE CASE OF DIFFERENT SCENARIOS**
Theori N., Ćulibrk A., Tzoraki O.

7 **TOXICITY EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS TOWARDS MARINE MICROALGAE: A SYSTEMATIC REVIEW**
Athanasakou S., Petsas A.S., Vagı M.C.

8 **HYDRAULIC SIMULATION OF THE STREAM OF PLOMARI "SEDOUNTA" LESVOS**
Nousias T., Koutsovili E., Tzoraki O.

9 **MEASURED AND ESTIMATED NITROGEN AND PHOSPHORUS LOADINGS OF FRESHWATER ARCTIC CHAR FISHFARMS**
Mavraganis T., Thorarensen H., Nathanailides C., Tsoumani M.

14:00 - 15:00 Break

15:00 - 15:45 **Keynote Speech - 2**

CEPHALOPOD FISHERIES IN EUROPEAN WATERS: STOCK ASSESSMENT / FORECASTING AND MANAGEMENT
Dr Graham John Pierce - Institute of Marine Sciences
SPAIN

Chair: *Drosos Koutsoubas, Kosmas Vidalis*

15:45 - 17:00 **Oral Session**
8th Field, Environmental Management and Education
Chairs: *Georgios Katselis, Kostantinos Topouzelis*

15:45 - 16:00 **UTILIZING DRONES TO INVESTIGATE DIFFERENT WATER RESOURCES**
Koutalakis P., Tzoraki O., Vousdoukas M., Gkiatas G., Kasapidis I., Zaimes G.N.

15:45 - 17:00 **Poster Session**
2nd Field, Fisheries
Chairs: *Dimitris Moutopoulos, Aikaterini Anastasopoulou*

1 **MORPHOMETRIC CHARACTERISTICS OF *Dentex maroccanus* IN THE AEGEAN SEA**
Stromplou D., Mina A., Pappou G., Anastasopoulou A., Mytilineou Ch.

15:45 - 17:00 **Poster Session**
1st Field, Aquaculture
Chairs: *Elena Mente, Nikos Neofitou*

1 **THE NUTRITIONAL VALUE OF THE CAPRELLID *Caprella scaura* FROM FISH FARM CAGES: A POTENTIAL SOURCE OF MARINE ANIMAL PROTEINS AND LIPIDS IN FISH NUTRITION?**
Lolas A., Psyrra E., Pasintelis K.P., Psofakis P., Neofytou N., Panagiotaki P., Karapanagiotidis I.T.

16:00 - 16:15 **TRACE ELEMENTS, NITRATE, AND SALINITY IN SHALLOW AND DEEP AQUIFERS OF AN AGRICULTURAL BASIN (NORTH-EASTERN TUNISIA)**
Troudi N., Tzoraki O., Hamzaoui-Azaza F., Melki F., Zammouri M.

16:15 - 16:30 **HOW CLOSE TO Posidonia oceanica MEADOWS ARE THE GREEK FISH FARM PARKS?**
Tsolakos K., Karampoula H., Katselis G.

16:30 - 16:45 **MAPPING OF POTENTIAL IMPACTS OF MARINE FISH FARMS IN CENTRAL IONIAN SEA (W. GREECE)**
Karampoula H., Tsolakos K., Katselis G.

2 **AGE AND GROWTH OF *Micromesistius poutassou* IN NORTH AEGEAN USING OTOLITH MORPHOMETRIC OBSERVATIONS**
Zafeiridis I., Touloumis K., Batzakas I.

3 **DIET COMPOSITION OF *Dentex maroccanus* IN THE AEGEAN SEA**
Kaminas A., Mina A., Rekleiti A., Mytilineou Ch., Anastasopoulou A.

4 **OTOLITH MORPHOMETRY RELATIONSHIPS OF *Pagellus erythrinus* (L. 1758) IN THE GREEK SEAS**
Theocharis A., Vlachou M., Anastasopoulou A.

5 **DISTRIBUTION PATTERN of *Dentex maroccanus* IN RELATION TO ENVIRONMENTAL FACTORS IN THE AEGEAN SEA**
Asimakopoulos C., Mytilineou Ch.

6 **MORPHOMETRIC MEASUREMENTS OF *Serranus hepatus* OTOLITHS IN THE GREEK SEAS**
Nikiforidou V., Gkikas E., Mytilineou Ch.[1], Koutsoubas D., Anastasopoulou A.

2 **ANTI-PROLIFERATIVE ACTIVITIES OF THE HOLOTHURIAN *Holothuria tubulosa* (ECHINODERMATA) EXTRACT ON HUMAN CANCER CELLS UNDER HYPOXIA**
Befani C., Apostologamvrou C., Liakos P., Vafidis D.

3 **GROWTH RESPONSES OF F3 GROWTH-SELECTED AND NON-SELECTED BROODSTOCKS OF *SPARUS AURATA* TO FASTING AND RE-FEEDING IN RAS**
Pavlou E., Gayo de Linos P., Martin N, Berbel, C., Manchado M.

4 **EFFECT OF FEED DEPRIVATION ON GROWTH PERFORMANCE AND PROXIMATE COMPOSITION IN GILTHEAD SEABREAM**
Ntantali O., Malandrakis E.E., Golomazou E., Karapanagiotidis I.T., Panagiotaki P.

5 **CULTIVATION OF *Tetraselmis striata* UNDER OPTIMIZED GROWTH CONDITIONS AND BIOMASS QUALITY EVALUATION FOR FISH FEED PRODUCTION**
Patrinou V., Daskalaki A., Kampantais D., Economou C.N., Bokas D., Kanakis D.C., Aggelis G., Vayenas D.V., Kotzamanis I., Tekerlekopoulou A.G.

6 **A NOVEL METHOD FOR DETECTION OF AMOEBA (ENDOLIMAX SP) IN WATER FROM CULTURE TANKS AND APPLICATION TO THE EVALUATION OF METRONIDAZOLE TREATMENTS IN SENEGALESE SOLE**
Carballo C., Miguez C., Berbel C., Zerolo R., Manchado M.

7 **GENE EXPRESSION PATTERNS ASSOCIATED WITH FAST- AND SLOW- GROWING FAMILIES AND GENDER IN SENEGALESE SOLE, *Solea senegalensis***
Gayo P., Zerolo R., Claros M.G., Manchado M.

8 **GENETIC ASSOCIATION STUDY REVEALS NEW SHAPE QUALITY-RELATED MARKERS IN SENEGALESE SOLE (*Solea senegalensis*)**
Guerrero-Cózar I., Claros MG. Zerolo R. and Manchado M.

9 **MULTITROPHIC-AQUAPONIC SYSTEM FOR SUSTAINABLE FOOD PRODUCTION**
Papaefstathiou K., Sakorafas S., Berillis P., Vlahos N., Levizou E. and Mente E.

10 **GIS IN SAMPLING STRATEGY OF AQUACULTURE ENVIRONMENTAL IMPACTS ASSESSMENT**
Dimoudi A., Karampetsou P., Domenikiotis C., Tziantziou L., Neofitou N.

11 **THE EFFECT OF FISHMEAL REPLACEMENT BY *Zophobas morio* LARVAE MEAL ON PROXIMATE COMPOSITION OF GILTHEAD SEABREAM (*Sparus aurata*)**
Asimaki A., Deliopoulos A., Filippakis N., Karaiskou M., Ntalakas I., Psofakis P., Rumbos C.I., Athanassiou C.G., Fountoulaki E., Henry M., Karapanagiotidis I.T.

12 **NATURAL ANTI-FOULING COATINGS ON KNOTTED NYLON FISH FARMING NETS**
Papadaki S., Batzakas I., Krokida M.

13 **DIETARY SUPPLEMENTATION WITH ESSENTIAL OIL NANOEMULSION ON GROWTH PERFORMANCE AND BLOOD PROFILES OF GILTHEAD SEABREAM (*Sparus aurata*)**
Neofytou M.C., Asimaki A., Katouni A., Gkalogianni E., Psofakis P., Michail G., Hatziioannou M., Karapanagiotidis I.T.

17:00 - 17:30 **Break**

17:30 - 18:30 **Poster Session**
8th Field, Environmental Management and Education
Chairs: *Panayiota Koulouri, Magia Chatziioannou*

10 **CAN MARINE SCIENCES STUDENTS SUPPORT THE OCEAN LITERACY FRAMEWORK? A PILOT STUDY FROM GREECE**
Koulouri P., Mogias A., Koutsoubas D., Dounas K., Cheimonopoulou M.

11 **DEVELOPMENT OF SCUBA DIVING TOURISM IN GREECE: THE SHIPWRECK OF PERISTERA IN ALONISSOS**
Douka C., Tourlioti P., Tzoraki O.

12 **MOLECULAR PHYLOGENY OF LAND SNAIL SPECIES OF GREECE, FAMILIES HELICIDAE AND GEOMITRIDAE**
Binia M., Mainou A., Manolouli E., Nikolopoulos A., Sarantopoulou J., Chatziioannou M., Exadactylos A., Gkafas A. G.

13 **OCCURRENCE OF ANTHROPOGENIC MICROPARTICLES IN Boops boops FROM LESVOS COASTAL AREA (NE AEGEAN)**
Vogiatzis G., Marmara D., Krasakopoulou E.

14 **MODELING THE EFFECT OF FUCALEAN COMMUNITIES IN COASTAL HYDRODYNAMICS: FIRST RESULTS**
Papadimitriou E., Papadimitriou A., Orfanidis S.

17:30 - 18:30 **Poster Session**
3rd Field, Oceanography
Chairs: *Evangelia Krasakopoulou, Anastasia Nikolaou*

1 **SPATIOTEMPORAL MONITORING OF SEA SURFACE TEMPERATURE IN A CENTRAL AEGEAN MPA**
Christou P., Domenikiotis C., Neofitou N., Vafidis D.

2 **SUNSCREENS AS EMERGING MARINE POLLUTANTS: ENVIRONMENTAL CONCERNS AND IMPLICATIONS FOR THE MEDITERRANEAN SEA**
Morali O., Nikolaou A.D., Vagi M.C.

3 **PROFILES OF TOXIC METALS IN EASTERN MEDITERRANEAN MARINE ENVIRONMENTS**
Priovolos I., Nikolaou A.D., Kitsiou D.

4 **WIND WAVE ANALYSIS OF COASTAL WATERS USING THE OCEANLYZ TOOLBOX**
Ntintas N., Tzoraki O., Karimpour A.

5 **CONSTRUCTION OF LOW-COST HYDROPHONES USING OFF-THE-SHELF COMPONENTS**
Galanos V., Trygonis V.

15	**MONITORING MARINE LITTER IN PORTS OF THE NORTHERN AEGEAN SEA ACCORDING TO THE MARINE STRATEGY FRAMEWORK DIRECTIVE** *Tegkelidis D., Mylona Z., Doumpas N., Papadimitriou E., Charitou A.*
16	**PRELIMINARY RESULTS OF FISHING FOR LITTER IN GREECE 2020** *Ataktidou M., Kontaxi C., Naasan Aga – Spyridopoulou R.*
18:15 - 18:30	Break

HALL 1 - < Mytilene >

18:30 - 19:30	**Congress Keynote Speech** **BIODIVERSITY, ECOSYSTEM SERVICES, AND NATURAL CAPITAL OF THE MEDITERRANEAN TRANSITIONAL ECOSYSTEMS** *Professor Alberto Basset, University of Salento* **ITALY** **Chair:** *Drosos Koutsoubas, Michael Chatziefstathiou*

Saturday, 6 November 2021

HALL 1 - < Mytilene >

10:00 - 12:00	**Oral Session** **1st Field, Aquaculture** **Chair:** *Eleni Golomazou, Ioannis Karapanagiotidis*
10:00 - 10:15	**PRELIMINARY RESULTS OF NUTRIENT DISTRIBUTION AT AN AQUACULTURE AREAOF PAGASITIKOS GULF USING GIS** *Karampetsou P., Dimoudi A., Domenikiotis C., Tziantziou L.,Klaoudatos D., Skordas K., Neofitou N.*
10:15 - 10:30	**LEAPING GREY MULLET *Liza saliens* AS AN ALTERNATIVE AND CANDITATE FISH SPECIES TO BE USED IN A BRACKISH AQUAPONIC SYSTEM CO-CULTIVATED WITH ROCKET PLANT *Eruca sativa*** *Theodorakaki E., Papadakis G., Kaloussias S., Mente E., Vlahos N.*
10:30 - 10:45	**CELLULAR RESPONSE TO SOYBEAN MEAL REPLACEMENT WITH NETTLE LEAF (*Urtica dioica*) MEAL IN RED TILAPIA (*Oreochromis* sp.), REARED IN AN AQUAPONIC SYSTEM** *Mantzourani S., Panagiotidou P., Panteli N., Demertzioglou M., Tsoumalakou E., Germani R., Mente E., Levizou E., Antonopoulou E.*
10:45 - 11:00	**POTENTIAL ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS AGAINST *Vibrio anguillarum*** *Secer F.S., Urku C., Tunar M.A., Yavuzcan H.Y.*
11:00 - 11:15	**INNOVATIVE APPROACHES TO REDUCE THE USE OF ANTIBIOTICS IN AQUACULTURE: CONTRIBUTION OF MICROALGAE TO THE IMMUNE SYSTEM** *Coşkun Y.M, Bayoğulları B., Tolon M.T.*
11:15 - 11:30	**PRELIMINARY RESULTS OF AN IMTA IN GREECE: CO-CULTURE FISHES, MUSSELS AND OYSTERS** *Chatzivasileiou D., Papageorgiou N., Kalatzi I., Dimitriou P.D., Theodorou J.A., Karakassis I.*

HALL 2 - < Volos >

10:00 - 12:00	**Poster Session** **4th Field, Marine Diversity & Conservation** **Chair:** *Georgios Gkafas, Dimitris Klaoudatos*
1	**CLIMATIC STABILITY OF THE MEDITERRANEAN MARINE PROTECTED NETWORK** *Kyprioti A., Almpanidou V., Katsanevakis S., Mazaris A.D.*
2	**ASSESSING UNCERTAINTY IN CLIMATIC NICHE MODELS FOR JUVENILE SEA TURTLES IN THE MEDITERRANEAN SEA** *Chatzimentor A., Almpanidou V., Doxa A., Dimitriadis C., Mazaris A.D.*
3	**FALSE KILLER WHALES (Pseudorca crassidens) PRESENCE IN GREECE, EAST MEDITERRANEAN: OLD AND NEW DEVELOPMENTS** *Drougas A., Akritopoulou E., Gkafas G., Kofidou E., Komnenou A.*
4	**Paracentrotus lividus AS BIOCONTROLLER OF INVASIVE SPECIES RUGULOPTERYX OKAMURAE. A LABORATORY APPROACH.** *Hachero Cruzado I., Jiménez T., Manchado M.*
5	**ALIEN AND RARELY REPORTED MOBILE SPECIES IN MARINE CAVES OF THE AEGEAN SEA, GREECE** *Digenis M., Ragkousis M., Katsanevakis S., Gerovasileiou V.*
6	**OCCURRENCE OF Trachipterus trachypterus AND Zu cristatus IN THE GREEK SEAS: CONTRIBUTION OF CITIZEN SCIENCE PROJECTS IN RARE SPECIES MONITORING** *Kaminas A., Minasidis V., Doumpas N., Naasan Aga Spyridopoulou R., Tiralongo F.*
7	**ANTI-OXIDANT CAPACITY OF TWO MICROALGAL STRAINS ISOLATED FROM GREEK SALT PANS** *Papadaki S., Chantzistrountsiou X., Ntzouvaras A., Koletti A., Tzovenis I., Flemetakis E., Economou-Amilli A.*

8	**PLANKTONIC MALACOSTRACAN FAUNA OF THE NORTH AEGEAN SEA (NAS) REGION: A PRELIMINARY REPORT** *Gkoulia A., Anastasiadou Ch., Papathanasiou V., Orfanidis S.*
9	**PRELIMINARY STUDY OF THE REPRODUCTIVE BIOLOGY OF SEA CUCUMBER Holothuria poli (Delle Chiaje, 1823) FROM CENTRAL AEGEAN SEA** *Apostologamvrou C., Balatsou A., Vafidis D.*
10	**GROWTH AND MORTALITY OF THE SEA URCHIN Arbacia lixula (LINNAEUS, 1758) IN PAGASITIKOS GULF (CENTRAL AEGEAN SEA)** *Klaoudatos D., Tziantziou L., Neofitou N., Lolas A., Apostologamvrou C., Vafidis D.*
11	**HISTOLOGICAL STUDY OF THE SCALDFISH, Arnoglossus laterna (Walbaum, 1792)** *Ntavaros C., Apostologamvrou C., Vlachou M., Theocharis A., Klaoudatos D.*
12	**PRELIMINARY STUDY ON THE REPRODUCTIVE BIOLOGY OF THE EUROPEAN HAKE Merluccius merluccius (Linnaeus, 1758) IN PAGASITIKOS GULF, GREECE** *Apostologamvrou C., Theocharis A., Vlachou M., Ntavaros C., Klaoudatos D.*
13	**GENETIC DIVERSITY PATTERNS OF MESOPELAGIC FISH IN THE GREEK SEAS** *Sarropoulou X., Tsaparis D., Tsagarakis K., Badouvas N., Tsigenopoulos C.*
14	**FIRST INVENTORY OF SEA CAVES AROUND CYPRUS** *Cai L.L., Savva I., Kleitou P., Alexandrou S., Sini M., Trygonis V., Gerovasileiou V., Ioannou Y., Nicolaou H., Markou M., Koutsoubas D., Kletou D.*

12:00 - 12:30 **Break**

12:30 - 13:15 **Keynote Speech - 3**

MODERN APPROACHES CONSIDERING AQUACULTURE IN EUROPEAN WATERS: MUCOSAL SURFACES, THE FIRST LINE OF DEFENSE IN FISH

Professor Samuel Martin - University of Aberdeen

UNITED KINGDOM

Chair: *Elena Mente, Athanassios Exadaktylos*

13:15 - 14:00 **Oral Session**
1st Field, Aquaculture
Chairs: *Constantinos Poulos, Nikolaos Vlahos*

13:15 - 13:30 AMMONIA AND NITRITE TOLERANCE OF ***Palaemon adspersus*** JUVENILE IN EXPERIMENTAL CONDITIONS
Vlahos N., Giannoulatou K., Nikolopoulos Ch., Patsea E., Mente E.

13:30 - 13:45 MORPHOMETRIC MEASUREMENT AND CA/P LEVELS OF SEA BASS (Dincentrarchus labrax) VERTEBRAE.
Nikiforidou V., Zaoutsos S., Vlahos N., Berillis P.

13:45 - 14:00 EFFECTS OF HEAT HARDENING ON OXIDATIVE DEFENCE CAPACITY IN THE MANTLE OF THERMALLY STRESSES MYTILLUS GALLOPROVINCIALIS
Georgoulis I., Feidantsis K., Giantsis I. A., Kakale A., Bock C., Pörtner H.O., Sokolova I.M., Michaelidis B.

13:15 - 14:00 **Oral Session**
3rd Field, Oceanography
Chairs: *Vassilis Zervakis, Evangelia Krasakopoulou*

13:15 - 13:30 UAS IMAGE QUALITY EVALUATION
Doukari M., Topouzelis K.

13:30 - 13:45 SURFICIAL SEDIMENT DISTRIBUTION IN THE SEMI-ENCLOSED GULF OF KALLONI (LESVOS ISL)
Manoutsoglou E., Oikonomou A., Hasiotis T.

14:00 - 15:00 **Break**

15:00 - 15:45 **Keynote Speech - 4**

MEDITERRANEAN SEA AND CLIMATE CHANGE

Dr. Joaquim Garrabou - Institute of Marine Sciences

SPAIN

Chairs: *Drosos Koutsoubas, Elina Tragou*

15:45 - 17:00 **Poster Session**
8th Field, Environmental Management and Education
Chairs: *Alexis Ramfos, Dimitra Kitsiou*

17 A PRELIMINARY ECOSYSTEM SERVICES ASSESSMENT OF GIOFYROS RIVER, CRETE
Gkaifyllia A., Ćulibrk A., Tzoraki R.

18 GOVERANCE AND EVALUATION OF ECOSYSTEM SERVICES IN THE COASTAL AREA OF SOUTH – SOUTHEAST ATHENS SECTOR
Svolou M.A., Tourlioti P., Tzoraki R.

15:45 - 17:00 **Poster Session**
1st Field, Aquaculture
Chair: *Ioannis Tzovenis, Nikos Neofitou*

14 EFFECTS OF FISHMEAL REPLACEMENT BY Chlorella vulgaris AND FISH OIL REPLACEMENT BY Microchloropsis gaditana AND Schizochytrium sp. BLEND ON WHOLE BODY PROXIMATE COMPOSITION OF EUROPEAN SEABASS (Dicentrarchus labrax)
Gkalogianni E.Z., Psofakis P., Asimaki A., Moustogianni A., Papapolymerou G., Katsoulas N., Karalazos V., Karapanagiotidis I.T.

15 HEPATOSTEATOSIS DERIVED FROM PHYTOGENIC FEED ADDITIVES IN GILTHEAD SEABREAM Sparus autata L.
Antoniadou E., Poulos C., Zolota V., Karapanagiotidis I.T., Panagiotaki P., Golomazou E.

19 **PUMPING COST MINIMIZATION WITH THE USE OF AN ITERATIVE ALGORITHM IN SIMANTRA SETTLEMENT, CHALKIDIKI, GREECE**
Mallios Z., Karagiannopoulos P., Siarkos I.

20 **LIFE CYCLE ASSESSMENT OF SMALL SNAIL FARMING SYSTEMS: THE GREEK CASE**
Hatziioannou M., Doxarioti A., Apostolou K., Exadactylos A., Kokkinos K.

21 **SHORT-TERM SURVIVAL OF FAN MUSSEL (Pinna nobilis L.) DURING AN EXPERIMENTAL TRANSPLANTATION IN MALIAKOS GULF, GREECE: FIRST RESULTS**
Tsamadias I.E., Rizou D., Theodorou J.A., Lattos A., Giantsis I.A., Kyritsi S., Ramfos A., Michaelidis V.

22 **MARINE SPATIAL PLANNING: A GIS TOOLBOX TO ASSESS THE SIGNIFICANCE OF CONFLICTS OF ACTIVITIES IN COASTAL AREAS**
Patera A., Tsoumachidis A., Kitsiou D.

23 **OCCURRENCE OF ORGANOCHLORINATED PESTICIDES IN THE MARINE BIOTA OF THE MEDITERRANEAN SEA: A SYSTEMATIC REVIEW**
Tzourtzoukli H., Petsas A.S., Vagı M.C.

24 **INVESTIGATION OF A MODEL DESIGN IN A LOW DAM FISH PASS HYDRAULIC CONSTRUCTION - THE CASE STUDY OF THE CONSTRUCTION NAMED «ROLLER PATH (RPT)»**
Psilovikos A., Papathanasiou T., Malamataris D.

25 **A SMALL SCALE STUDY ON FISHERIES - DOLPHINS INTERACTIONS IN THE KORINTHIAKOS GULF, EASTERN MEDITERRANEAN SEA**
Spiliopoulou E., Pardalis S., Komnenou A., Exadactylos A., Dendrinos P., Tounta E., Gkafas G.A.

16 **THE EFFECTS OF FISHMEAL REPLACEMENT BY DIETARY Zophobas morio LARVAE MEAL ON LIVER AND INTESTINAL HISTOLOGY OF GILTHEAD SEABREAM (Sparus aurata)**
Asimaki A., Katouni A., Psofakis P., Mente E., Berillis P., Rumbos C.I., Athanassiou C.G., Henry M., Fountoulaki E., Karapanagiotidis I.T.

17 **NERVOUS NECROSIS VIRUS PERSISTENT INFECTION OF SEA BASS (Dicentrarchus labrax) AFFECTS INTERFERON PATHWAY-RELATED GENE TRANSCRIPTION**
Toubanaki D.K., Tzortzatos O.P., Efstathiou A., Palaiologos A., Valsamidis M.A., White D.M., Papaharisis L., Bakopoulos V., Karagouni E.

18 **EFFECT OF STOCKING DENSITY ON THE FEEDING BEHAVIOR OF SEA BREAM (Sparus aurata L.) ADMINISTRATED WITH PHYTOGENIC FEED ADDITIVES**
Pappas C.G., Antoniadou E., Karapanagiotidis I.T., Golomazou E., Panagiotaki P.

19 **CAROTENOID IDENTIFICATION AND QUANTIFICATION IN THE SKIN OF Pagrus pagrus USING DIFFERENT EXTRACTION METHODS**
Kampantais D., Kanakis D.C., Roussos E., Ilia V., Vardali S., Mirtsi E.D., Haroutounian S.A., Miliou H., Kotzamanis Y.

20 **PRELIMINARY RESULTS OF FEEDING BEHAVIOUR OF Holothuria polii: REMAINING TIME IN THE SANDY SUBSTRATE AND AVAILABILITY OF THE ORGANIC MATTER**
Chatzivasileiou D., Sterioti A., Kentouri M.

21 **THE EFFECT OF FEEDING FREQUENCY ON SEABASS DAILY FOOD CONSUMPTION CO-CULTURED WITH LETTUCE IN AN AQUAPONIC SYSTEM**
Ellina M., Niakas K., Gkikas P., Stathopoulou P., Vlahos N., Berillis P., Mente E.

22 **EMPLOYMENT AND SOCIO-DEMOGRAPHICS OF GREEK AQUACULTURE SECTOR**
Danatskos C., Kokkinaki L.A.

23 **OPTIMISATION STUDIES FOR THE COMMERCIAL PRODUCTION OF ARTHROSPIRA PLATENSIS**
Pantazis C., Tzovenis I.

		24	**PRELIMINARY RESULTS OF BRACKISH AQUAPONICS WITH SEA BASS (Dicentrarchus labrax)AND OPPOSITE-LEAVED SALTWORT PLANT OR AGRETTI (Salsola soda)** *Kapetanios E.,Vlahos N., Berillis P., Mente E.*
		25	**PERSPECTIVES OF FRESH WATER AQUACULTURE IN GREECE** *Xenidis A., Kokkinakis A.K., Xenidis V., Apostolidis A.P.*
16:45 - 17:00	Break		

HALL 1 - < Mytilene >

17:00 - 17:30	**Closing Ceremony**
17:30 - 18:30	**Panhellenic Society of Technologists Ichthyologists (PASTI)** **General Assembly (Statutory)**

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1st Field
Aquaculture

MUSCLE CELLULARITY AND PROXIMATE COMPOSITION OF FAST AND SLOW GROWING MARICULTURED MEAGRE, *Argyrosomus regiust*

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Abstract
Muscle cellularity and lipid quality and quantity may change during the growth of cultivated meagre. In this work we investigated differences in the muscle cellularity and proximate composition of fast growing and slow growing meagre reared in marine floating cages in western Greece. The specific aim of this work was to test the hypothesis that different growth rates of similarly aged meagre will be reflected on the proximate composition and muscle cellularity of axial skeletal muscle. The results indicate significant differences in the lipid content and muscle cellulatity between fast and slow growing groups. Fast growing meagre exhibited significantly higher fat content and significantly higher total number of myofibres reflecting the larger cross sectional area of their caudal region. These differences may affect the organoleptic properties of meagre fillets and the rate of lipid oxidation during cold storage.
Keywords: *Muscle cellularity; fish growth; aquaculture*
*Corresponding author: Nathanailides Cosmas (nathan@uoi.gr)

1. Introduction

Meagre (*Argyrosomus regius*) is an excellent new species for diversifying Mediterranean mariculture. This species has already been successfully reared in floating cages with promising growth results. There are numerous reports of excellent performance, including rapid growth, low disease mortality, and high nutritional value of meagre cultivated in Greece (Gregorakis *et al.* 2011; Chatzifotis *et al.* 2012). Nevertheless, as in other cultivated fish, size grading is unavoidable in aquaculture, particularly in a species which can rapidly reach large size and is commercially available in a wide range of sizes and ages, ranging from 400 to well above 1400 g (Fountoulaki *et al.* 2017).

The number and size of fish axial muscle fibers increase with age but changes in size and number of fish muscle can reflect changes in muscle cellularity which occurs due to a range of extrinsic and intrinsic parameters (Weatherley *et al.* 1979; Veggeti *et al.* 1990). Rapid somatic growth is associated with muscle growth with increase in size (hypertrophy) and increased number (hyperplasia) of skeletal muscle fibre. Meagre exhibits a plasticity of muscle cellularity during the aquaculture production cycle (Mittakos *et al.* 2012) and changes in the growth dynamics of muscle fibre affected by age, size, and nutritional parameters (Saavedra *et al.* 2016; 2018). There is some evidence to suggest that muscle cellularity, lipid quality and quantity may change during the growth of cultivated meagre (Mittakos *et al.* 2012; Giogios *et al*. 2013). In the present work we investigated differences in the muscle cellularity and proximate composition of fast growing and slow growing meagre cultivated in marine floating cages in a commercial fish farm of Western Greece.

The specific aim of this work was to test the hypothesis that differences in the growth rate of fish with the same age but different size will be reflected on the proximate composition and muscle cellularity of axial skeletal muscle.

2. Material and Methods

Analysis of proximate composition: samples of fillets (n=10 from each group) were used. Moisture, protein and lipid were determined according to AOAC (2005).Protein and lipid content was estimated using Kjedahl and Soxhlet methods respectively.

Muscle cellularity: Samples of white epaxial muscle tissue (n = 5 fish per group) were obtained from the post-anal caudal region (about 2-3 mm thick) of fish from the two growing groups (fast and slow growing fish). The samples were fixed in 10% phosphate-buffered formol saline and subsequently dehydrated in a graded series of ethanol solutions and embedded in paraffin. Transverse sections (10 μm thick) were obtained and stained with H&E stains. The cross-sectional area (CSA) of individual myofibres (≥ 150 per fish in each group) was traced using a computerised image analysis system. The total number of myofibres contained in the entire caudal whole cross-section of each fish was estimated according to Higgins & Thorpe (1990), by using the ratio of the entire cross sectional area at the caudal region divided by the mean myofibre size.

Statistical Analysis: The results obtained were compared by Student's t-test; Protein and lipid content were arc–sin transformed prior to statistical analysis.

3. Results

Figure 1, presents the growth rate of the two groups which were used in the present work. The two groups were size graded after about one year of growth in floating marine cages. The samples analyzed in this work were obtained on the 30th month, when fast growing fish were double in size of the slow growing fish. Protein content did not vary significantly, but fat content was significantly higher ($P < 0.001$) in fast growing group (Table 1).

The mean myofibre cross sectional area (MCSA) of white muscle did not vary between the two groups. The large group exhibited significantly higher total number of myofibres reflecting the larger cross sectional area of the caudal region of the larger fish group (Table 2). A representative histological section of axial muscle from the fast growing and slow growing meager is shown in Figure 1.

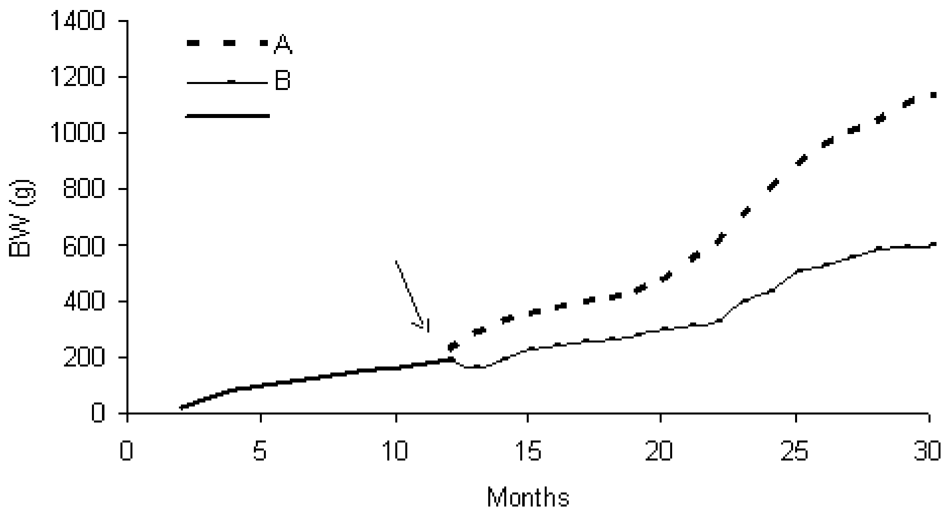

Figure 1. Body weight (BW) of fast (group A, dashed line) and slow (group B, solid line) growing meagre. Arrow indicates the time of size grading (12th month).

Table 1. Body weight (g) and proximate composition of fast (A) and slow (B) growing groups of 2+ yrs farmed meagre.

	Group A	Group B
BW (g)	1147.157 (±265.0)	622,08 (±204.67)
Protein (%)	19.1 (±0.50)	19.0 (±0.12)
Lipids (%)	0.8 (±0.04)	0.14 (±0.01) *

Table 2. Mean myofibre cross sectional area (MCSA) of white muscle, Total cross sectional area (TCSA) of caudal region and total number of myofibres (TNMF) in fast (A) and slow (B) growing groups of farmed meagre, aged 2+ yrs.

An asterisk indicates significant difference at α=0.05, (t-test, P<0.05).

	Group A	Group B
MCSA	435.70 (±39.52)	402.89 (±18.59)
TCSA	227.04(±79.77)	96.94 (±35.88) *
TNMF	568.28 (±211.15)	304.18 (±38.26)*

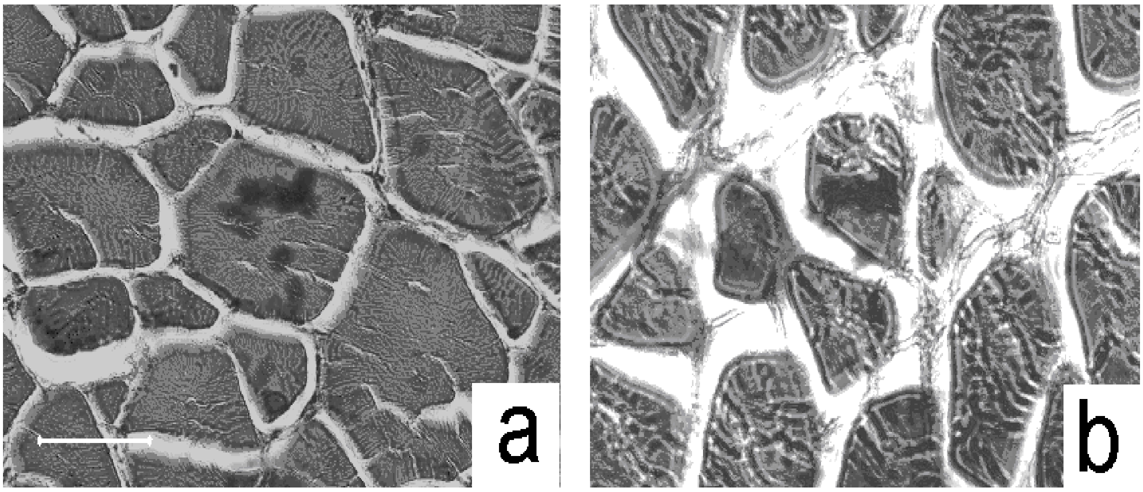

Figure 1. Cross sections of axial muscle tissue from fast (a) and slow (b) growing meagre. H&E stain. Size bar indicates 100 μm.

3. Discussion

The size difference between slow and fast growing meagre increased with time. The results indicate significant differences in the lipid content and muscle cellularity of the two groups. Fast growing meagre exhibited significantly higher fat content compared to the slow growing fish. Myofibre size did not vary between the two groups, but fast growing fish exhibited significantly higher total number of myofibres reflecting the larger cross sectional area of the caudal region and the higher lipid content. These differences may affect the organoleptic properties of fillets (Saavedra *et al.* 2015) and the rate of lipid oxidation during cold storage (Giavasis *et al.* 2014, Mitsagga *et al.* 2016; Secci & Parisi, 2016). Fish quality and preservation are crucial parameters for public safety and the nutritional value of consuming fish (Vatavali *et al.* 2013). Fish is the primary source of polyunsaturated fatty acids, which are unfortunately highly susceptible to degradation processes like oxidation (Sullivan Ritter & Budge 2012).

High lipid content of fish flesh is associated with increased lipid oxidation during cold storage. In the present work, lipid content varied between fast and slow growing fish. Differences in lipid quality and quantity can affect lipid oxidation during cold storage. For this reason, it would be interesting to investigate the differences in fatty acid content and also the rate of lipid oxidation in fillets from the fast and slow growing fish during cold storage.

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References

AOAC (2005). Association of official analytical chemists. Official methods of analysis. 19th edition, suite 500, (AOAC International) 481 north Frederick Avenue, Gaithersburg, Maryland, 20877-2417,USA. : 2005

Chatzifotis S., Panagiotidou M., Divanach, P. (2012). Effect of protein and lipid dietary levels on the growth of juvenile meagre (Argyrosomus regius). Aquaculture international 20(1), 91-98.

Fountoulaki E., Grigorakis K., Kounna C., Rigos G., Papandroulakis N., Diakogeorgakis J., Kokou, F. (2017). Growth performance and product quality of meagre (Argyrosomus regius) fed diets of different protein/lipid levels at industrial scale. Italian Journal of Animal Science 16(4), 685-694.

Giavasis I., Apostolopoulou A., Deirmentzoglou A., Katsanidis E. (2014). Combined hurdle effects of process parameters on biochemical, microbiological and sensory attributes of trout fillets. Journal of Food Processing and Preservation 38(1), 466-476.

Giogios I., Grigorakis K., Kalogeropoulos N. (2013). Organoleptic and chemical quality of farmed meagre (Argyrosomus regius) as affected by size. Food chemistry 141(3), 3153-3159.

Grigorakis K., Fountoulaki E., Vasilaki A., Mittakos I., Nathanailides C. (2011). Lipid quality and filleting yield of reared meagre (Argyrosomus regius). International journal of Food Science & Technology 46(4), 711-716.

Higgins P.J., Thorpe J.E. (1990). Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, Salmo salar L. Journal of Fish Biology 37(4), 505-519.

Mitsagga Ch., Giavasis I., Choremi K., Vidalis K., Vasilaki A., Grigorakis K., Athanasopoulou F. Nathanailides C., 2016. Quality control of wild and farmed gilthead seabream during chilled storage. *2nd* Intern. Congress on Applied Ichthyology & Aquatic Environment, 10-12 Nov. 2016, Messolonghi, Greece, ISBN : 978-618-80242-3-6. ISSN : 2529-1246. pp. 476-480.

Mittakos I., Ayala M. D., López-Albors O., Grigorakis K., Lenas D., Kakali F., Nathanailides C. (2012). Muscle cellularity, enzyme activities, and nucleic acid content in meagre (Argyrosomus regius). Canadian Journal of Zoology 90 (10), 1270-1277.

Saavedra M., Grade A., Candeias-Mendes A., Pereira T.G., Teixeira B., Yúfera M., ... Pousão-Ferreira P. (2016). Different dietary protein levels affect meagre (Argyrosomus regius) larval survival and muscle cellularity. Aquaculture 450, 89-94.

Saavedra M., Pereira T.G., Candeias‐Mendes A., Carvalho L., Pousão‐Ferreira P., Conceição, L.E.C. (2018). Effect of increased dietary protein level in meagre (Argyrosomus regi*us*) juvenile growth and muscle cellularity. Aquaculture Nutrition 24(3), 1153-1159.

Secci G., Parisi G. (2016). From farm to fork: Lipid oxidation in fish products. A review. Italian Journal of Animal Science 15(1), 124-136.

Sullivan Ritter J.C., Budge S.M. (2012). Key lipid oxidation products can be used to predict sensory quality of fish oils with different levels of EPA and DHA. Lipids 47(12), 1169-1179.

Vatavali K., Karakosta L., Nathanailides C., Georgantelis D., Kontominas M.G. (2013). Combined effect of chitosan and oregano essential oil dip on the microbiological, chemical, and sensory attributes of red porgy (Pagrus pagrus) stored in ice. Food and Bioprocess Technology 6(12), 3510-3521.

Veggetti A., Mascarello F., Scapolo P.A., Rowlerson A. (1990). Hyperplastic and hypertrophic growth of lateral muscle in Dicentrarchus labrax (L.): An ultrastructural and morfometric study. Anatomy & Embryology 182, 1-10.

Weatherley A.H., Gill H.S., Rogers S.C. (1979). Growth dynamics of muscle fibres. dry weight and condition in relation to somatic growth rate in yearling rainbow trout (Salmo gairdneri). Canadian Journal of Zoology 57, 2385– 2392.

EVALUATION OF THREE MICROALGAE SPECIES FOR USE IN AQUAFEED

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Abstract
The rapid growth of the aquaculture industry leads to the growing demand for aquafeed specifically designed for the optimal growth and quality of the final products. The improvement of cultivation and quality of farmed species is proposed through the use of bioactive agents from alternative raw materials as aquafeed supplements. In order to establish scientific evidence and to gain a good understanding of how microalgae can best be used in aquafeed, *Haematococcus pluvialis*, *Dunaliella salina* and *Arthrospira platensis* were used in order to investigate the solvents' ability to extract bioactive pigments in order to produce extracts of high antioxidant activity. The *D.salina* extract exhibited the highest antioxidant activity using both solvents, with the ethyl acetate being the optimum solvent to produce extracts of high antioxidant activity for all species. The extracted carotenoids were then identified using HPLC analysis. Microalgae extracts were found to contain astaxanthin, lutein, β-carotene and several of their isomers.
Keywords: *microalgae, HPLC analysis, carotenoids, antioxidant activity*
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Introduction

While aquaculture industry experiences a rapid global expansion the need for the establishment of a more sustainable future is eminent. This has resulted in other sources of feedstuffs being investigated, namely plant origin substitutes or supplements for aquafeed (Shah *et al.* 2017). Microalgae have a potential for use in aquaculture as they are sources of protein, lipid, vitamins, minerals, pigments, etc, in contrast to conventional land-based crops that can result in changes of the nutritional quality of the fish produced (Shah *et al.* 2017). Pigments of microalgae such as carotenoids contribute to their nutritional performance in aquaculture (Shah *et al.* 2017).
Carotenes (β-carotene and lycopene) and the xanthophylls (astaxanthin, canthaxanthin, lutein, and zeaxanthin) are natural pigments which only plants, algae, and some species of fungi and bacteria synthesize but must be provided in the diet of animals, since they are important substances on vertebrates' health and development (Cezare-Gomes *et al.* 2019). They can easily be distributed throughout blood circulation in an organism and protect against free radicals as well as modulate the reproductive performance (Rosas *et al.* 2018). More than 600 carotenoids have been identified. Commercially, the most important carotenoids are astaxanthin, canthaxanthin, lutein, zeaxanthin, β-carotene, and lycopene which present several interesting properties, such as antivirals, antimicrobials, anti-inflammatory, antioxidant, pro-vitamin A, immunomodulatory activity, and anticancer (Cezare-Gomes *et al*. 2019).

D.salina can produce up to 10.0% dry weight of β-carotene while it is considered the largest source of this carotenoid. *H.pluvialis* is recognized as the major source of astaxanthin, which can be accumulated at up to 3% of dry biomass, which represents a quantity higher than those found in plants, bacteria (e.g., *Agrobacterium aurantiacum*), and few fungi (e.g., red yeast *Phaffia rhodozyma*) but at amounts compared to marine animals (salmonids, shrimp, lobsters, and crayfish) (Cezare-Gomes *et al*. 2019). The consumption of carotenoids through the inclusion of *Spirulina* in fish diets causes them to be absorbed and transported through low-density lipids to different organs/cells of the body where they will be accumulated. Their accumulation in the muscle and skin enhances skin colour, which in general, results in an increase in value of the product in the market (Rosas *et al*. 2018).

The role of microalgae in aquaculture has extended further with its use as a potential immunostimulant to commercially important aquaculture species. Microalgae can also help to improve survival rate and immune system in shrimp larvae. There are several studies which highlighted the immunostimulating properties of various microalgae in fish and crustaceans (Madhumathi & Rengasamy 2011; Cerezuela *et al*. 2012; Yeganeh *et al.* 2015). Microalgae have also been proven to be a highly important component of the diet of fish, with various benefits, such as promoting the growth, improving the reproductive performance and enhancing the immune response (Shah *et al.* 2017; Zhang *et al.* 2019).

Microalgae cultivation, when compared to the growth of macroalgae, presents many advantages such as faster growth, higher biomass productivity, and smaller land area requirement for cultivation. For this reason, microalgae are an alternative platform for carotenoid production when compared to the traditional sources (Cezare-Gomes *et al*. 2019). It has also been reported that microalgae can be used for treating wastewater, including effluent from fish culture activities, as it can efficiently metabolize the nutrients and remove the heavy metals contained in aquaculture effluent. Fish aquaculture could apply an integrated strategy of simultaneously treating aquaculture effluent while producing spirulina biomass to supplement fish diets (Shah *et al.* 2017; Zhang *et al.* 2019,).
Aim of the present study is to establish scientific evidence and to gain a good understanding of how microalgae can best be used in aquafeed by identifying the carotenoids contained in the bioactive extracts of three microalgae species, namely *Haematococcus pluvialis, Dunaliella salina* and *Arthrospira platensis* and evaluate their antioxidant activity using different solvent systems.

Materials Methodology

Solvent extractions: In order to obtain the bioactive extracts from the three microalgae species, 1:10 w/v of solvent was added to preweighed microalgal biomass as shown in Table 1 and the conventional extraction was carried out under continuous magnetic stirring for 12 hours at room temperature.

Table 1 Solvent extraction parameters of this study

	Solvents	
Microalgae strains	Hexane	Ethyl Acetate
Haematococcus pluvialis	1:10 w/v	1:10 w/v
Dunaliella salina	1:10 w/v	1:10 w/v
Arthrospira platensis	1:10 w/v	1:10 w/v

HPLC Analysis: HPLC analysis was performed with an HPLC Shimandzu HP 1100 Series (USA) equipped with a diode array detector and an automatic Agilent 1200 Series injector. Carotenoid compounds were analyzed with an YMC C30 (Germany) analytical column (5 m, 250 × 4.6 mm I.D.). The mobile phase consisted of the solvents methanol, t-butylmethylether, and 1% phosphoric acid aqueous solution, and the flow rate was 1 mL min^{-1}. Detection of carotenoids was accomplished using a diode array system at a wavelength of 458 nm. Carotenoids were identified by comparison to external standards of β-carotene, lutein, astaxanthin and certain isomers.

Determination of Antioxidant Activity (IC50): Antioxidant activity was assessed using the DPPH assay (Brand–Williams *et al.* 1995). The IC50 values reported in the present study denote the concentration of sample that is required to scavenge 50% of DPPH free radicals (Sharma and Bhat 2009). All the measurements were performed in triplicate.

Results

The antioxidant activity of each microalgae extract regarding the three different species and two different solvent systems is shown in Figure 1. According to our results *D.salina* exhibited the highest antioxidant activity using both solvents with the ethyl acetate being the optimum extraction solvent. The highest deviation between IC50 values using the two solvents was exhibited by *H.pluvialis*, with ethyl acetate being the optimum solvent. In all caes, hexane exhibited the lower antioxidant activity.

The identified carotenoids using the optimum solvent, namely ethyl acetate, for each microalgae extract are shown in Figures 2,3 and 4. Information about the retention time and concetration of each identified carotenoid is summarized in Tables 2, 3 and 4.

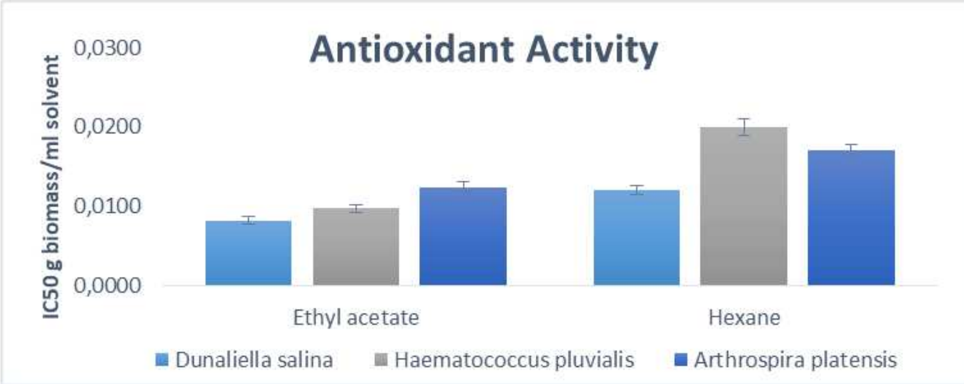

Figure 1. Antioxidant activity of microalgae extract using two different solvent systems

Microalgae extracts were found to contain astaxanthin, lutein, β-carotene and several of their isomers. The highest diversity in carotenoid species was found in *H. pluvialis* extracts and the lowest in *D. salina*.

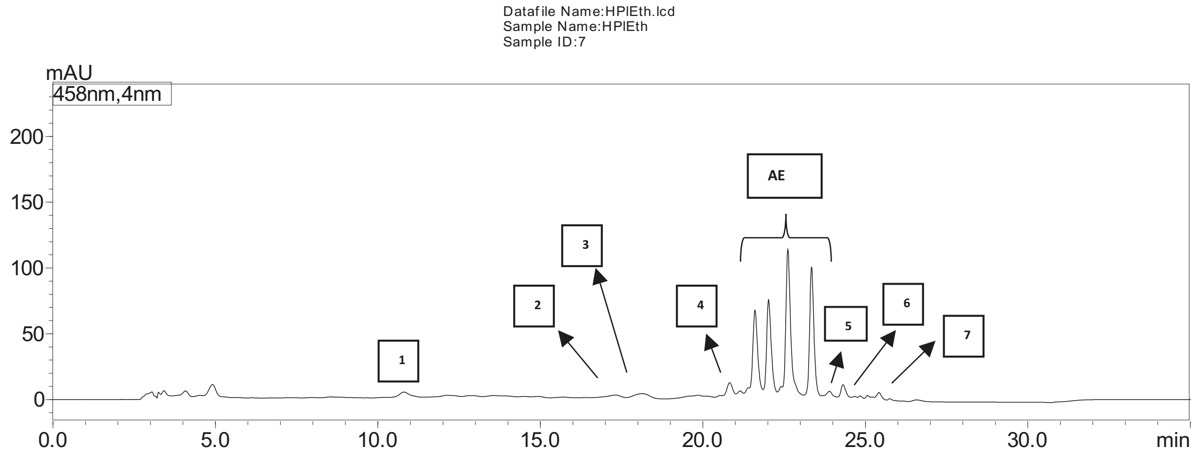

Figure 2. High-performance liquid chromatogram (HPLC) of ethyl acetate extract from *H. pluvialis*.

Table 2. Carotenoids identification of *H. pluvialis* extract using HPLC

Retention Time	Area	Peak No	Identification	%Area
10,804	120789	1	all-trans Astaxanthin	1,83
17,307	104101	2	trans-b-apo-8'-Carotenal	0,835
18,132	134406	3	Unidentified carotenoid	2,367
20,824	182065	4	Unidentified carotenoid	2,535
21,603	845555	AE	astaxanthin esters (AE).	16,44
22,021	820400	AE	astaxanthin esters (AE).	16,88
22,615	1266404	AE	astaxanthin esters (AE).	29,96
23,344	990564	AE	astaxanthin esters (AE).	24,47
23,896	115758	5	Unidentified carotenoid	1
24,311	145255	6	b-carotene	2,52

25,419	63820	7	9-cis b-carotene	1,137

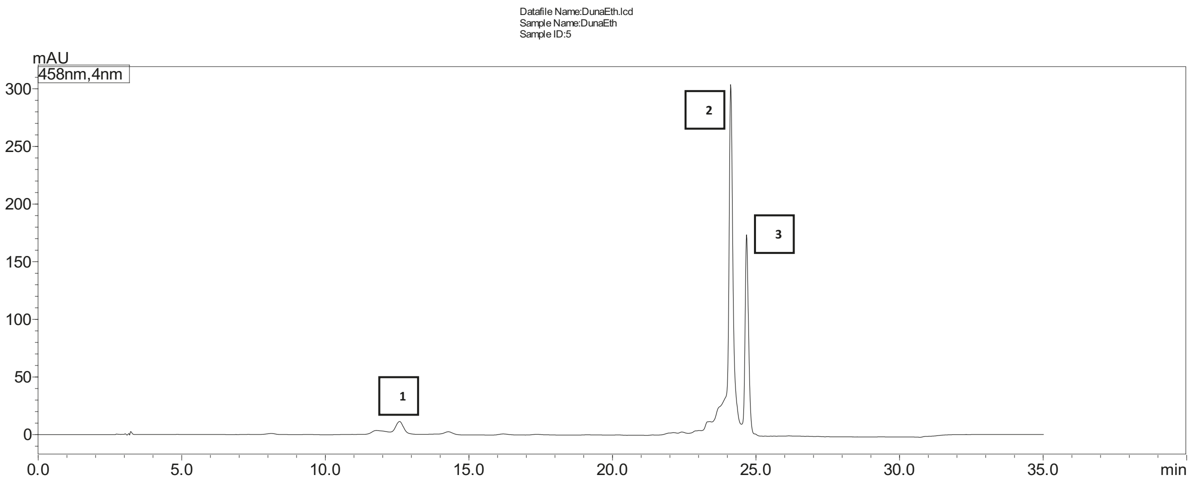

Figure 3. High-performance liquid chromatogram (HPLC) of ethyl acetate extract from *D. salina*.

Table 3. Carotenoids identification of *D. salina* extract using HPLC

Ret.Time	Area	Peak No	Identification	%Area
12,584	232292	1	lutein	3,196
24,119	3731794	2	b-carotene	68,856
24,672	1535705	3	9-cis b-carotene	27,949

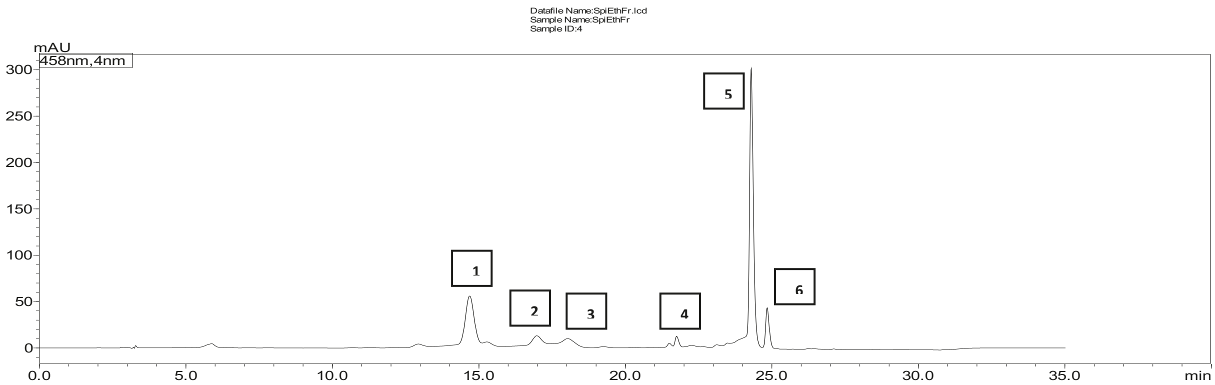

Figure 4 High-performance liquid chromatogram (HPLC) of ethyl acetate extract from *A. platensis*.

Table 4 Detected and identified carotenoids of *A. platensis* extract using HPLC

Ret.Time	Area	Peak No	Identification	%Area
14,684	1397089	1	9-cis Astaxanthin	20,77
16,977	476393	2	trans-b-apo-8'-Carotenal	3,802
18,031	402142	3	Unidentified carotenoid	3,97
21,75	127121	4	Unidentified carotenoid	2,589

24,295	3092871	5	b-carotene	60,97
24,839	399928	6	9-cis b-carotene	7,89

Conclusions

At an industrial scale, the conventional solvent extraction is the most commonly used method for the separation of compounds with biological activity, such as antioxidant, from natural raw materials. The yield of extractables is solvent dependent and different solvents can be used according to the polarity and location of the targeted chemical compounds while phenolics and carotenoids are usually extracted with organic solvents (Srati *et al.* 2011; Jimenez-Lopez *et al.* 2021). Under efficient extraction techniques, microalgae can be regarded as a promising source of bioactive supplements that will ensure sustainability standards in aquaculture through the production of high added value end products.

References

Cerezuela R., Guardiola F., Meseguer J., Esteban M. (2012). Enrichment of gilthead seabream (Sparus aurata L.) diet with microalgae: effects on the immune system. Fish Physiology and Biochemistry 38(6), 1729-1739.

Cezare-Gomes E., Mejia-da-Silva L., Pérez-Mora L., Matsudo M., Ferreira-Camargo L., Singh A., de Carvalho J. (2019). Potential of Microalgae Carotenoids for Industrial Application. Applied Biochemistry and Biotechnology 188(3), 602-634.

Jimenez-Lopez C., Pereira A.G., Lourenço-Lopes C., Garcia-Oliveira P., Cassani L., Fraga-Corral M., Prieto M.A., Simal-Gandara J. (2021). Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits. Food Chemistry 341, 128262.

Madhumathi M., Rengasamy R. (2011). Antioxidant status of Penaeus monodon fed with Dunaliella salina supplemented diet and resistance against WSSV. International Journal of Engineering Science & Technology 3, 7249–7259

Rosas V., Poersch L., Romano L., Tesser M. (2018). Feasibility of the use of Spirulina in aquaculture diets. Reviews in Aquaculture 11(4), 1367-1378.

Strati I.F., Oreopoulou V. (2011). Process optimization for recovery of carotenoids from tomato waste. Food Chemistry 129, 747–752.

Shah M., Lutzu G., Alam A., Sarker P., Kabir Chowdhury M., Parsaeimehr A., Liang Y., Daroch M. (2017). Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology 30(1), 197-213.

Yeganeh S., Teimouri M., Amirkolaie A.K. (2015) Dietary effects of Spirulina platensis on hematological and serum biochemical parameters of rainbow trout (Oncorhynchus mykiss). Research in Veterinary Science 101, 84–88.

Zhang F., Man Y., Mo W., Wong M. (2019). Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. Reviews in Aquaculture 12(2), 582-599.

SEASONAL GENE EXPRESSION OF ANTIOXIDANT ENZYMES AND HSP70 IN GILTHEAD SEA BREAM (*Sparus aurata*)

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Abstract
Seasonal changes of temperature may take organisms to the upper and lower limits of their thermal range, with respective variations in their biochemical and metabolic profile. The present study aims to investigate the seasonal cellular stress response in vital organs, i.e. the liver and the skeletal (red and white) muscles of the gilthead sea bream (*Sparus aurata)* during a 1-year acclimatization period in field conditions. The studied markers included gene expression of heat shock protein and antioxidant enzymatic activity. Specifically, molecular responses were addressed through the gene expression levels of Hsp70, superoxide dismutase (SOD) and glutathione reductase (GR). The increased gene expression levels of Hsp70, SOD and GR in the examined three tissues of the gilthead sea bream indicated a cellular stress response under the prism of a seasonal pattern characterized by distinct tissue specificity. Specifically, Hsp70 induction and the activation of antioxidant enzymes occurred before the increased summer water temperatures, with no further increases in their levels despite the increase of sea water temperature. The expression of these certain proteins can be used as a tool in order to define the extreme thermal limits of the gilthead sea bream.
Keywords: *Antioxidant enzymes, HSP70, gene expression, gilthead sea bream, Sparus aurata*
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Introduction

Global climate change is predicted to result in water temperature increases, but the exact consequences for marine fish species are not completely understood, especially with regard to cellular mechanisms underlying oxidative stress. Warmer temperatures could potentially result in increased oxidative stress, and it is not known whether stenothermal fish can cope with this on cellular and physiological level. Climate change can cause a variety of biological responses in fish, ranging from the biomolecular and biochemical to population and community-level effects. Changes in proteins functioning in energy metabolism, cytoskeleton, oxidative stress, signaling, protein stabilization and turnover are common responses to environmental change (Hofmann & Todgham 2010; Portner & Peck 2010; Somero 2010, 2012; Tomanek 2010, 2012). A common metric used to assess the cellular response to stress in marine organisms, is the expression of heat shock proteins (e.g. Hsp70) (Hofmann 2005; Tomanek 2010). During stress, Hsps are concerned to prevent the induced protein unfolding that might challenge the protein homeostasis of cells (Hofmann 2005; Tomanek 2010). In fish the induction of various Hsp families has been reported in various tissues and their physiological and protective role following exposure to various environmental stressors is of increasing interest (Iwama *et al.* 1998; Iwama 1999; Yamashita *et al.* 2010).

As a ‘‘hotspot’’ for climate change, the Mediterranean Sea has been identified as highly vulnerable to its effects and is expected to alter marine biodiversity and productivity (Nicholls & Hoozemans 1996; Gambaiani *et al.* 2009; Calvo & Marsh 2011).

The gilthead sea bream *Sparus aurata* (Linnaeus, 1758) is one of the most important aquaculture species in the Mediterranean. *S. aurata* is a eurythermal species living in environments with temperatures ranging from 11ºC (winter) to 24ºC (summer) (Arias 1976). Mainly due to its commercial value, gilthead sea bream farming has become a common practice along the Mediterranean coastline in the last three decades. However, little is known about its thermal tolerance and whether it is subjected to seasonal stress in the aquaculture farms.

In this study, we investigated molecular and metabolic responses of farmed *S. aurata*, in the content of the seasonal changes of ambient temperature, by monitoring the changes in the expression Hsp 70 and the enzyme antioxidant activity. To investigate the possible interactions between seasonal responses and oxidative stress, we determined the expression levels of Hsp70 and antioxidant enzymes i.e. superoxide dismutase (SOD) and glutathione redustase (GR) - in the liver, white and red muscle of gilthead sea bream.

The determination of the gene expression levels of these proteins and enzymes in vital organs can contribute in reflecting the extreme thermal limits (Somero 2002; Hofmann 2005) of the specific species to predict more precisely, in combination with other bioindicators, the impact of the climate change on the physiology, the distribution and abundance of *S. aurata*.

Materials and Methods

The experimental procedures and the tissue samplings have been thoroughly described in Feidantsis *et al.* (2013). Briefly, gilthead sea bream specimens with a mean (±SD) body mass of 294.5 ± 4.5 g were placed in the middle of December 2007 in fish cages (2 m h 9 2md 9 2ml) (approximately 300 individuals in each cage) in a sea farm in Chalkidiki (Horozoglou S.A.), North Greece (Fig. 1a). The cages were immersed in the sea water at 0–2 m depth (Fig. 1b) and fish were left to acclimatize in the cages for 2 months **(**Feidantsis *et al.* 2013). After the acclimatization period, fish were collected from the cages monthly and placed in sea water containing MS-222 to a final concentration of 0.15 gl-1. Within 2–3 min, the fish lost balance, became anesthetized and could be removed from the water without struggling. The fish were then dissected, and samples from liver and skeletal (white and red) muscles were removed. The white muscle was removed from the dorsal side of the fish, whereas the red muscle was removed from the sides. Immediately after the dissection, the tissue samples were frozen in liquid nitrogen. Then, tissue samples were transported back to the laboratory and stored at -80 ^{o}C for analysis of Hsp70, SOD and GR gene expression.

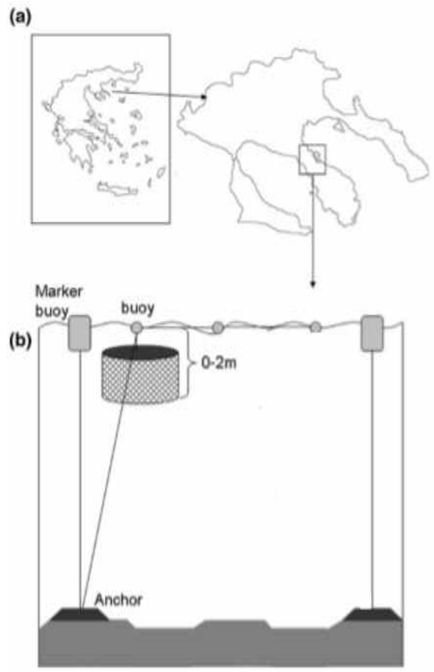

Figure 1: (a) Schematic map of the sampling area in Karagatsi, Chalkidiki where Sparus aurata was cultivated. (b) Schematic presentation of the fish cage suspension at 0-2 m (Feidantsis et al. 2013)

Homogenization of the tissue samples and the isolation of RNA were performed using the NucleoZol kit (Macherey-Nagel). To quantify and assess the purity of RNA, samples were measured in a Thermo Scientific™ NanoDrop, i.e. a microvolume spectrophotometer with patented sample retention technology that measure sample volumes as small as 0.5μL. Depending on the concentration of each sample, the corresponding dilutions were made so that the final concentrations of the samples were modified to approximately 100 ng / µl.

Reverse transcription (RNA to DNA) - cDNA was carried out using the PrimeScript 1st strand cDNA Synthesis Kit (TakaRa, Japan) according to the manufacturer's recommendations. Real-time PCR (RT-PCR) was used for amplification and quantification of the cDNA in a Rotor-Gene Q instrument (Qiagen, Germany). Each reaction comprised 100 ng cDNA, 5 μL 2 X SensiFAST™ SYBR No-ROX mixture (Bioline Meridian Bioscience), 0.3 μL of forward and reverse primer (Table 1), and ultrapure water up to a final volume of 10 μL.

The quantitative PCR reactions steps included denaturation step at 95 °C for 3 min followed by 40 cycles of 15 s at 95 °C (denaturation), 30 s at 55 °C and a fourth step at 72 °C for 30s. The reference genes were L13 protein and Elongase Factor. The data analysis from expression levels carried out with 2-ΔΔCt method (Livak and Schmittgen,2001). Statistical significance was evaluated by an one way anova in SPSS v.14.

Table 1: Primers used for RT-PCR analysis

Gene	Primer	Sequence (5'-3')	Amplicon size (bp)
Hsp 70	Mghsp70-1202-F Mghsp70-1301-R	5´-TGTCCCTTGGTATTGAAACTGC-3´ 5´-TAACTGCTGGTTGGTTGTCGG-3'	119
Cu/Zn superoxide dismutase sod	SodF SodR	5-CAAAGCGCAGTGTGATTGTGG-3 5-CCACTCCGGAGTTTTGCATTTC-3	202
Glutathione reductase	GrF GrR	5-CAAAGCGCAGTGTGATTGTGG-3 5-CCACTCCGGAGTTTTGCATTTC-3	1151

Results

Figure 2 illustrates the seasonal changes in the gene expression levels of Hsp70 and antioxidant enzymes in the three examined tissues of *S. aurata* (liver, white and red muscle). In liver, we observed an upward trend in expression of SOD, a significant decrease from October to April of GR and a general increased expression of Hsp 70 with non-significant decrease from February to April. In white muscle, a significant increase in April of SOD, a significant increase in October and a sharp decrease in December-February of GR expression and a gradual reduction of expression from September to February following by a gradual increase in summer months in Hsp70 expression were observed. In red muscle, we observed a significant decrease in April in SOD, an upward trend of expression in GR and a sharp increase in June-July in Hsp70 expression.

Discussion

The present study investigates the changes in the gene expression levels of the antioxidant enzymes SOD and GR, and Hsp70 in liver, white and red muscle of sea bream. The liver shows a different variation of the seasonal pattern, with the highest SOD values in October. In the summer months (17.4°C- 29°C) a relatively high stability compared to that of February (11.5°C) is observed. As for the red muscle, SOD shows significantly increased gene expression levels in July. Finally, similar to the liver, in February there are high gene expression levels of this enzyme. Similar effects are observed with those of the red muscle in the white muscle. The antioxidant enzyme gene expression levels are similar to the ones in red muscle, with values gradually increasing parallel to the increasing water temperature. The highest levels of GR gene expression in the liver were observed during the summer months, while in many cases, the expression levels in the white muscle were higher than those in the red muscle. Finally, the comparison of the Hsp70 gene expression with that of the two antioxidant enzymes SOD and GR, reveals similarities in changes under the seasonal pattern.

The results revealed distinct tissue specificity in Hsp70 and antioxidant enzymes gene expression. In conclusion, the present results obtained in *S. aurata* during acclimatization in the field indicate a seasonal profile in the stimulation of the cellular stress response in the different examined tissues. Moreover, the results emphasize the fact that under field conditions *S. aurata* exhibits elevated Hsps and antioxidant enzymes gene expression levels at increasing environmental temperature, rather than at the highest ambient temperatures**.** Understanding the physiological responses of farmed organisms to their changing environments, and the aspects of climate change on aquaculture production systems is crucial for conservation strategies, environmental risk assessment and management of marine recourses to be successful (Pineiro *et al.* 2010; Somero 2010; Hofmann & Todgham 2010).

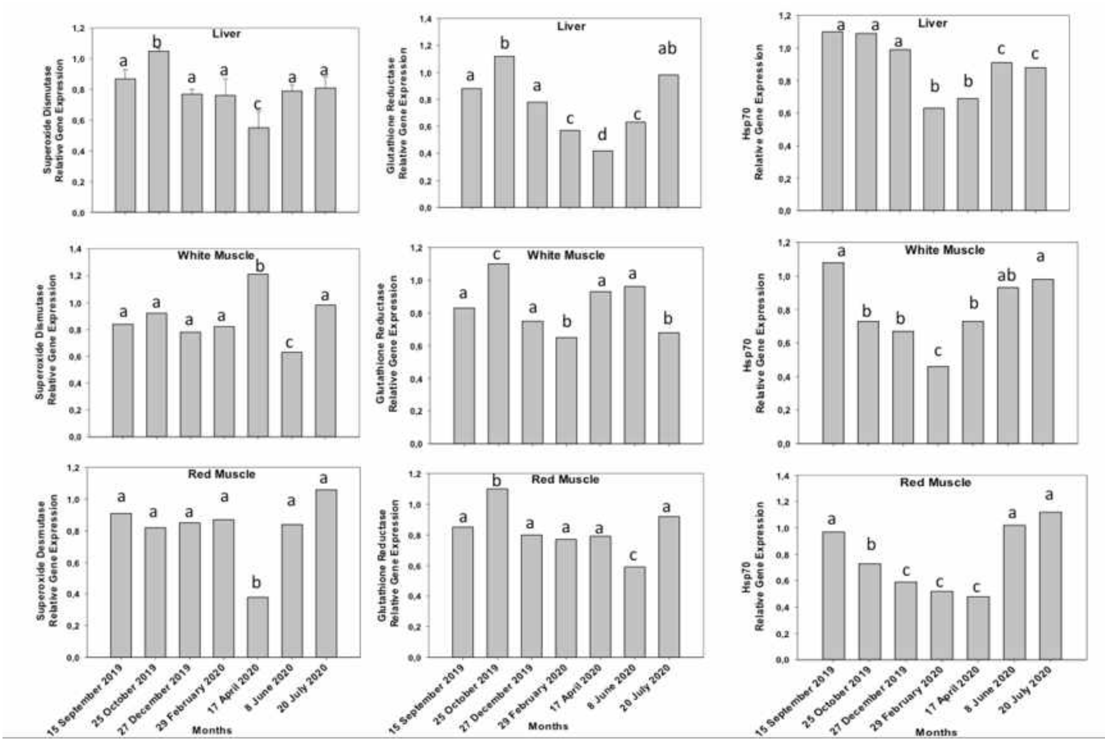

Figure 2. Seasonal Changes of mRNA expression of antioxidant enzymes and Hsp-70 in *Sparus aurata*. The results have been grouped (a, b and c) according to ANOVA test (a<0.05).

References

Arias A. (1976). Sobre la biologı ´a de la dorada, Sparus aurata L., de los esteros de la provincia de Cadiz. Inv Pesq 40, 201–222.

Calvo N., Marsh D.R. (2011). The combined effects of ENSO and the 11-year solar cycle on the Northern Hemisphere polar stratosphere. Journal of Geophysical Research 116, 1–14.

Feidantsis K., Portner H.O., Markou T., Lazou A., Michaelidis B. (2012). Involvement of p38 MAPK in the induction of Hsp70 during acute thermal stress in red blood cells of the gilthead sea bream, *Sparus aurata*. Journal of Experimental Zoology Part A 317, 303–310.

Gambaiani D.D., Mayol P., Isaac S.J., Simmonds M.P. (2009). Potential impacts of climate change and greenhouse gas emissions on Mediterranean marine ecosystems and cetaceans. Journal of The Marine Biological Association UK 89(1), 179–201.

Hofmann G.E. (2005). Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales. Integrated Comparative Biology 45, 247–255.

Hofmann G.E., Todgham A.E. (2010). Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annual Review in Physiology 72, 127–145.

Iwama K.G., Thomas T.P., Forsyth B.R., Vijayan M.M. (1998). Heat shock expression in fish. Reviews in Fish Biology 8, 35–56.

Nicholls R.J., Hoozemans F.M.J. (1996). The Mediterranean: vulnerability to coastal implication of climate change. Ocean Coast Management 31(2–3), 105–132.

Pineiro C., Canas B., Carrera M. (2010). The role of proteomics in the study of the influence of climate change on seafood products. Food Research International 43, 1791–1802.

Portner H.O., Peck M.A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. Journal of Fish Biology 77, 1745–1779.

Somero G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrated Comparative Biology 42, 780–789.

Somero G.N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers. Journal of Experimental Biology 213, 912–920.

Somero G.N. (2012). The physiology of global change: linking patterns to mechanisms. Annual Review on Marine Science 4, 39–61.

Tomanek L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species’ biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology 213, 971–979.

Tomanek L. (2012) Environmental proteomics of the mussel Mytilus: implications for tolerance to stress and change in limits of biogeographical ranges in response to climate change. Integrated Comparative Biology 52(5), 648–664.

Yamashita M., Yabu T., Ojima N. (2010). Stress Protein HSP70 in Fish. ABSM 3(4), 111–141.

SEASONAL CHANGES ON ANTIOXIDANT ENZYMES' GENE EXPRESSION IN RED PORGY (*Pagrus pagrus*).

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Abstract
Recent studies have shown that aquaculture, a major sector of economic activity, is threatened by climate change. At the same time, the growth of global population requires the increase of food production which promotes the development of aquaculture. The present research focuses on the correlation of the seasonal changes of gene expression of glutathione reductase, superoxide dismutase and catalase of the red porgy (*Pagrus pagrus*) with ambient sea water seasonal temperatures. The samplings were carried out on a monthly basis in a fish farm in Larymna which is located in North Euboean Gulf, Greece. The samplings were based on previous years' seasonal temperature profiles of the above marine area. From each fish, liver, heart, white and red muscle samples were removed. In these samples a quantitative real time PCR was employed: a) RNA isolation from tissues, b) mRNA transcription to cDNA and c) use of modified gene-specific PCR primers, and estimation of each gene expression from the exponential phase of the reaction with 2-ΔΔCt statistical method. The results have shown various changes in gene expression, with their highest values observed in June in most tissues. These results can assist in the elucidation of how antioxidant capacity's seasonal changes may contribute in the thermal tolerance of farmed fish, and of the possible relationship among seasonality and oxidative stress of *Pagrus pagrus*.
Keywords: *Glutathione reductase, Superoxide dismutase, catalase, oxidative phosphorylation*, *Pagrus pagrus*
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1.Introduction

Energy plays a central role in tolerance to various environmental conditions, among which temperature is considered a main stressor, affecting physiological processes that range from the denaturation of proteins to the fluidity of the membrane that affects organ function (Hochachka & Somero 2002). Energy charge is a measure of the metabolic energy which is available for the organism (Atkinson 1977) and has been shown to vary in response to environmental changes (Ivanovici 1979). However, mitochondria also constitute an important source of reactive oxygen species (ROS) (Andreyev *et al.* 2005). ROS are a mixture of molecules and free radicals derived from molecular oxygen (Turrens 2007). Molecular oxygen is the final acceptor of electrons in the oxidative phosphorylation process. The cellular defense strategies against the oxidative damage include the activation of the peroxide-binding enzyme which collects superoxide free radicals. The hydrogen superoxide, formed by the superoxide dismutase (SOD), is collected by catalase which catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen (Turrens 2007; Berg *et al.* 2002). The definition of 'oxidative stress' is closely related to the production of ROS. There must be a balance between ROS production and oxidative stress. Changes in this balance will lead to adaptation due to response to environmental conditions and in most cases will lead to cell death (Burton *et al.* 2011). Although during recent years research has been developed in this scientific field, the responses of marine ecosystems to climate change are still poorly understood. This study focuses on the changes in expression of genes of antioxidant enzymes in relationship with temperature in cultured *Pagrus pagrus* (red porgy) (Linnaeus, 1758).

2.Materials and Methods

Samplings were performed on a monthly basis in a fish farm (Prometheus Ltd) located in Larymna in the North Euboean Gulf, Greece. Samplings were based on previous years' seasonal temperature profiles of the above marine area. From each fish, liver, heart, white and red muscle samples were removed. The tissues were transferred to the Laboratory of Animal Physiology, School of Biology, Aristotle University of Thessaloniki, and stored in -80 ° C.

Isolation of the total RNA was performed using the Nucleozol kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The quality and quantity of the isolated RNA was spectrophotometrically evaluated by 260/280 ratio in a Nanodrop (Biospec-Nano, SHIMADZU) and was reverse transcribed to cDNA using the PrimeScript™ 1st strand cDNA Synthesis kit (TaKaRa, Japan), applying the manufacturer's recommended protocol and the Oligo dT Primer. Expression of different genes was estimated in a real-time quantitative PCR (qPCR), by measuring the amplified product at each cycle. Each reaction comprised of 0.4 μL of cDNA, 5 μL 2 X SensiFAST™ SYBR No-ROX mixture (Bioline Meridian Bioscience), 0.3 μL of forward and reverse primer (Table 1), and ultrapure water up to a final volume of 10 μL. All reactions were run in triplicates. The quantitative PCR reactions steps included a denaturation step at 95°C for 3 min followed by 40 cycles of 15 s at 95°C (denaturation), 30 s at 55°C and a fourth step at 72°C for 30s. L13 protein and Elongation

Factor were used as housekeeping (reference) genes for quantification purposes. The data analysis from expression levels was carried out with the 2-ΔΔCt method (Livak and Schmittgen, 2001). The 2–ΔΔCt method for relative gene expression analysis is widely used and easy to perform.

Table 1. The list of genes and sequence of primers.

Gene	Forward	Reverse
Glutathione reductase	5-CAAAGCGCAGTGTGATTGTGG-3 ´	5-CCACTCCGGAGTTTTGCATTTC-3
Superoxide dismutase	5-CAAAGCGCAGTGTGATTGTGG-3	5-CCACTCCGGAGTTTTGCATTTC-3
Catalase	5- TTCCCGTCCTTCATTCACTC-3	5- CTCCAGAAGTCCCACACCAT-3

3. Results

Figure 1 depicts the seasonal changes of antioxidant enzymes (SOD, Gr and catalase) in cultured *Pagrus pagrus*. The most significant increases in GR gene expression were observed in heart in June, and red muscle in June and July. Concerning SOD, low levels of expression were exhibited in general, with significant increases in heart and liver in June, while in the red muscle increases of SOD gene expression were mostly observed in April and June. Additionally, the gene expression of Catalase exhibited a gradual increase in the heart until July when the highest levels were observed, low levels of expression in white muscle and liver, and a significant increase in red muscle in June.

4. Discussion

The present study investigates the changes in the gene expression levels of significant enzymes of the antioxidant machinery such as SOD and GR, and catalase in liver, white and red muscle in the red porgy. In the summer months when the highest sea water temperatures were recorded (29ºC), the highest levels of antioxidant gene expression were observed. Specifically, in heart appeared sharp increase and general an upward trend in all three genes. Contrary to the white muscle where no significant changes were observed, the heart, red muscle and liver exhibited their most significant increases in their antioxidant summer months.

The investigation of the physiological and biochemical responses of *Pagrus pagrus* in relation to seasonal sea water temperature changes is of great importance in order to elucidate how this species responds to changes in temperature and how antioxidant capacity's seasonal changes may contribute in the thermal tolerance of farmed fish, and of the possible relationship among seasonality and oxidative stress of *Pagrus pagrus*.

Temperature is the most important abiotic factor as it affects the physiological functions of an organism. These changes in physiological functions are directly related to climate change. In the last decade, the latter has been globally escalated to an intense area of investigation as climate change effects on fish and generally on marine organisms significantly impacts all levels of biological organization.

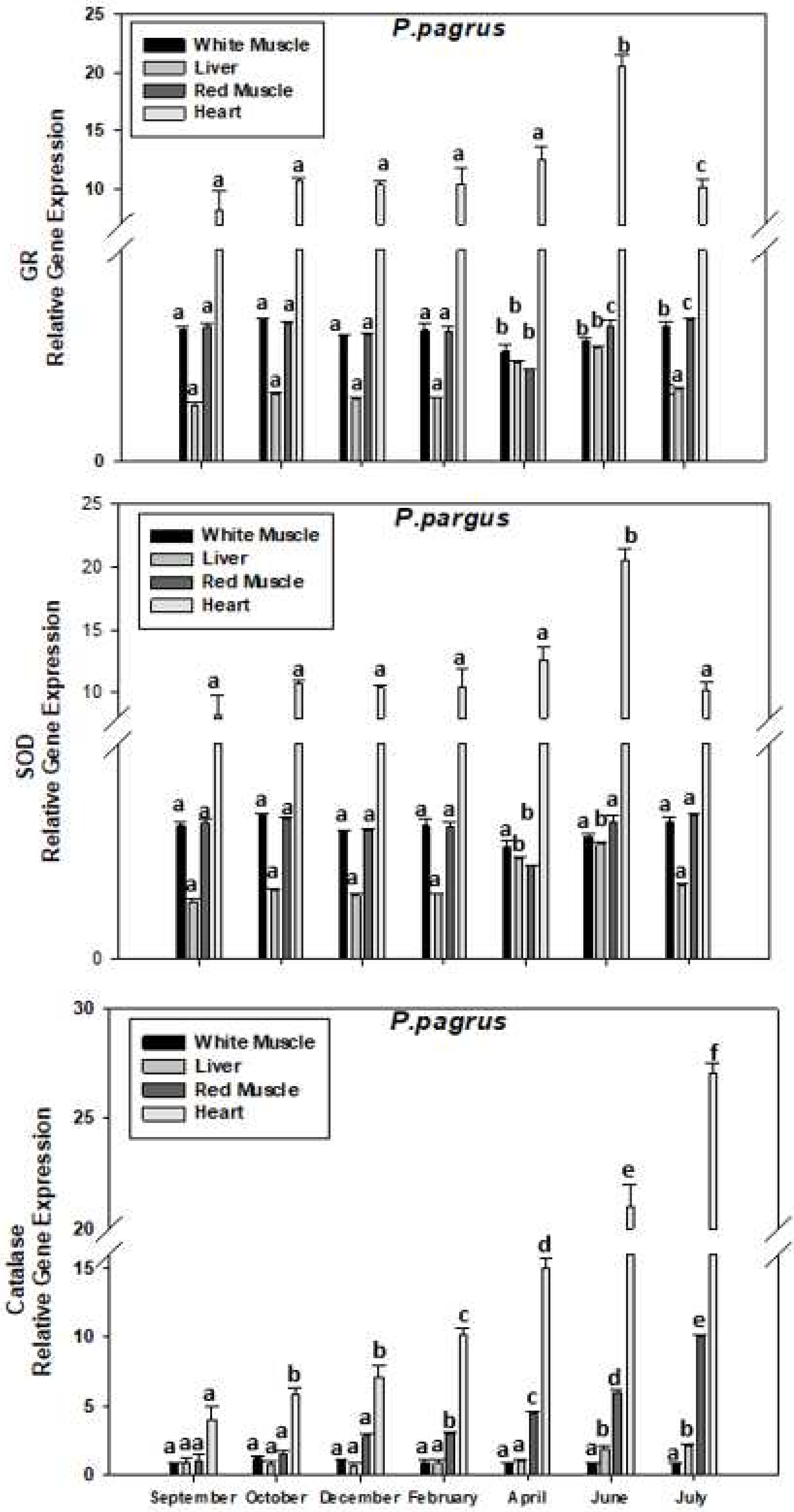

Figure 1. Seasonal Changes of mRNA expression of antioxidant enzymes in the white and red muscle, liver and heart of *Pagrus pagrus*. The results have been grouped according to anova test. Lower case letters indicate statistically significant changes between samplings ($p<0.05$).

References

Atkinson D.E. (1977). Cellular Energy Metabolism and its Regulation, Academic Press, London.

Ivanovici A.M. (1979). Adenylate energy charge. Potential value as a tool for rapid determination of toxicity effects. p 241–255 In Proc 5th Ann Aquatic Toxicity Workshop, Hamilton Ontario, November 7–9, 1978. Fisheries and Marine Service Technical report, p 862.

Livak & Schmittgen, (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25, 402-408.

Berg J.M., Tymoczko J.L., Stryer L. (2002). Biochemistry, 5th edition. New York:W. H. Freeman; 2002.

Hochachka G.E., Somero G.N. (2002). Biochemical adaptation: mechanism and process in physiological evolution, Oxford University Press, New York, p. 1-466.

Turrens J.F. (2007). Formation of Reactive Oxygen Species in Mitochondria. Mitochondria. In: Schaffer S.W., Suleiman MS. (eds) Mitochondria. Advances in Biochemistry in Health and Disease, vol 2. Springer, New York, NY.

Burton J.L., Xiong Y., Solomon M.J. (2011). Mechanisms of pseudosubstrate inhibition of the anaphase promoting complex by Acm1. EMBO Journal 30(9), 1818-1829.

HISTOPATHOLOGICAL AND HEMATOLOGICAL EXAMINATION OF FISH AFTER TREATMENT WITH NITROFURAZONE

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Abstract
The purpose of this study is to examine hematological parameters and histopathological alterations on gilthead sea bream (*Sparus aurata*) after administration of nitrofurazone. Peripheral blood sample was collected from nine individuals with no external lesions and from nine individuals with visible abrasions on the skin, for hematocrit measurement and blood smears. Necropsy was also performed. In each smear, white blood cells (WBC) were differentiated into lymphocytes, monocytes, neutrophils, eosinophils and basophils. Eighteen sea bream individuals with visible abrasions on the skin were treated with broad spectrum antibiotic (nitrofuran) via food and baths for a period of nine days. Blood samples were collected for hematocrit and blood smears. Nitrofuran treatment seemed to increased hematocrit value.
Key words: *hematocrit, leucocytes, gilthead sea bream*, *nitrofurazone*
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1. Introduction

According to the World Wide Fund for Nature (WWF), aquaculture is the most sustainable solution to the food problem. Gilthead sea bream (*Sparus aurata*) along with European sea bass (*Dicentrarchus labrax*) are the most important species cultured in Greece. According to Federation of Greek Maricultures (FGM), the production of these two species amounted 117,000 tons in 2018, showing an increase of 7 % compared to the previous year. Gilthead sea bream accounted 57 % of the total production volume. Gilthead sea bream (*S. aurata*) can be found widely in the waters of the entire Mediterranean but also along the coasts of the East Atlantic Ocean, from the United Kingdom to Senegal, and rarely in the Black Sea (FAO 2018). It is found on rocky or sandy bottoms, but can also be found in underwater meadows. It is a poikilothermic species but has been shown to have difficulty adapting to low temperatures (Tort et al. 2004). Young sea bream individuals are found at shallow depths (over 30 m), while adults can reach deeper waters (maximum depth 150 m) (FAO 2018). In the spring, sea bream is found in brackish waters of coastal lagoons (FishBase 2010). In its early stages of its life prefers brackish and warmer waters (Craig *et al.* 2008).

Gilthead sea bream is affected by various diseases, especially in closed breeding systems, with bacterial, viral or parasite origin (FAO 2018). These diseases mainly affect fish its young age. Some of the main diseases are pasteurellosis (*Photobacterium damselae*), vibriosis (*Vibrio alginolyticus, Photobacterium damselae, Vibrio anguilarum)*, winter syndrome (*Pseudomonas anguilliseptica*), viral encephalopathy (*Nodavirus*), lymphocystis (*Iridoviridae*) and parasitic enteritis (*Myxidium leei).*

Antibiotics belonging to the broader category of antimicrobials (European Food Safety Authority) are used to treat bacterial diseases. The families of antibiotics that are used are: oxytetracycline, sulphonamides such as the combination of trimethoprim with sulphadiazine, oxolinic acid flumequine belonging to fluoroquinolones, and florfenicol and nitrofuran belonging to furans (Lulijwa 2020). Antibiotics are chemicals produced by microorganisms that either destroy (bactericidal) or inhibit the growth of other microorganisms (bacteriostatic) (Cañada Cañada *et al.* 2009). Antibiotics can be either broad-spectrum, which means that they are active against a wide range of microorganisms. Narrow-spectrum antibiotics target a specific group of microorganisms and are able to interfere with metabolic processes specifically for these organisms. Antibiotics generally act: (i) by blocking the synthesis of bacterial cell wall components (e.g. penicillins); (ii) by destroying the cytoplasmic membrane of bacteria; (iii) by interfering with synthesis of proteins or nucleic acids (e.g. tetracyclines) (Samanidou & Evaggelopoulou 2007). Nitrofurazone is an antimicrobial organic compound that belongs to the class of nitrofurans and is active against gram-positive bacteria and gram-negative bacteria (Colorni & Paperna

1983). The mechanism of action of nitrofuran remains unclear. Nitrofuran inhibits many systems of microbial enzymes, including those involved in carbohydrate metabolism (Vardanyan & Hruby 2016).

The hematological profile of a fish population shows the physiology and health of the species and thus hematology in combination with routine diagnostic methods, could be used to identify and assess conditions that cause stress in fish, and then diseases. (Pavlidis *et al.* 2007).

2. Materials & Methods

The experiment was performed on sea bream individuals *(S. aurata*) in aquariums in the facilities of the Aquaculture laboratory of the Department of Ichthyology and Aquatic Environment of the University of Thessaly (Fig. 2). The salinity was adjusted to 30 ‰. Initially, a sample of nine individuals with no visible abrasions was taken to be used as a control group and nine individuals with obvious wounds to analyze hematocrit value and differentiate white blood cells. A necropsy was also performed on these fish. Eighteen young sea bream were used for treatment. Initially, before the treatment quantity of blood was taken in order blood smears to be prepared and hematocrit to be measured. The fish were fed twice daily, once in the morning and once in the afternoon with conventional pellet feed. The fish were fed at a rate of 2% of their body weight (3.4 g at each meal). Fish were given an oral antibiotic with the active ingredient nitrofurazone for five days, which was mixed with the food, adding a very small amount of olive oil. The dose of nitrofuran was 170 mg per 5.2 g of food. On the fifth day, nitrofuran baths were performed for best results over a period of five days, in combination with oral antibiotics. Nitrofuran treatment started three days after the fish entered the aquarium to fully acclimatize and reduce transport stress. Control group, sea breams without visible abrasions, did not receive treatment. However, a quantity of blood was taken to measure hematocrit and differentiate white blood cells. Three samplings took place after the end of treatment. On the first day, the eighth day and the fifteenth day after the end of the treatment. Length and weight were measured in all fish, a macroscopic examination was performed and blood samples were taken for smear and hematocrit measurement. Six sea breams were used in each sampling. The fish were killed according to the recommended dose of anesthetic, 0.2 ml / L (phenoxyethanol) and immersion in ice water (hypothermia) (Tsantilas *et al.* 2006).

3. Results

All fish in the aquarium were at the same level of maturity (Table 1).

Table 1: Mean weights ± standard error and mean lengths ± standard error during the experiment.

Fish	Mean weights ± standard error (g)	Mean lengths ± standard error (cm)
Fish without external abrasions (n=9)	21,39±1,60	11,53±0,25
Fish with external abrasions-Day 0 (n=9)	14,98±1,45	9,91±0,30
Day 1 after the end of treatment (n=6)	19,69±0,88	10,88±0,11
Day 8 after the end of treatment (n=6)	20,22±2,29	11,23±0,33
Day 15 after the end of treatment (n=6)	21,01±1,51	11,37±0,23

3.1 Blood smears and hematocrit values

Comparing the results from hematocrit values, an increase in the hematocrit value was observed on day 15, after treatment with nitrofuran compared to the initial sample of sea bream individuals with skin abrasions (table 2). Blood smears examination revealed that lymphocytes constitute the majority of white blood cells, neutrophils and monocytes to follow. No eosinophilic and basophilic cells were observed (table 2).

Table 2: Hematocrit values and white blood cell differentiation. Data expressed as mean ± standard error. The means of each parameter between different treatments with the same superscript do not show statistically significant differences (P> 0.05)

Percentage (%)	Fish without external abrasions (n=9)	Fish with external abrasions- Day 0 (n=9)	Day 1 after the end of treatment (n=6)	Day 8 after the end of treatment (n=6)	Day 15 after the end of treatment (n=6)
Hematocrit	19,61±0,62^{a}	15,44±1,62^{b}	17,92±0,20^{a}	25,75±0,65^{c}	31,85±0,16^{d}
Lymphocytes	95,80±0,97^{a}	93,50±2,26^{a}	-	96±1,45^{a}	98,33±0,56^{a}
Neutrophils	3,80±1,15^{a}	4,63±1,25^{a}	-	1,60±0,51^{a}	1±0,45^{a}
Monocytes	0,4±0,4^{a}	1,75±0,84^{a}	-	1,60±0,75 ^{a}	0,67±0,33^{a}
Eosinophils/Basophils	0	0	-	0	0

3.2 Macroscopic anatomy

During the 3 samplings after treatment, macroscopic examination and necropsy were performed at fish. Fish on day 0 carried external wounds mainly on the sides of their body, as well as very often along their caudal peduncle. No macroscopic lesions were found in the gills. Intestine and liver showed inflammation signs (swollen intestine and discoloration of the liver).

No external lesions were found in any fish, suggesting that nitrofuran baths had a direct effect on treating skin lesions that initially occurred on sea bream in tanks.

4. Discussion

The study of hematological parameters and morphological characteristics of fish blood cells are useful tools for the aquaculture industry. Erythrocytes are the predominant cell type in fish blood. In a comparative study between different species of fish of the family Sparidae, which are bred in the Mediterranean, it was found that in the blood of the sea bream are found the smallest erythrocytes in size (Pavlidis *et al.* 2007). The white blood cells of fish are less in number compared to the red ones, which constitute 96.5% of the total blood cells and work in various ways in order to rid the blood of various harmful factors (Verillis & Mende 2017; Tzironis 2010). It is widely accepted that fish, like most vertebrates, have a common leukocyte pattern consisting of neutrophils, basophils, eosinophils, monocytes, and lymphocytes (Rey Vazquez & Guerrero 2007). Blood constitutes 1.3 - 7% of the body weight of fish and is one of the most active components that contribute to metabolic processes, ensuring ion exchange between the body and the environment (Fazio *et al.* 2012).

Hematocrit is a reliable indicator in aquaculture and fisheries management for the control of anemic status, as well as for the control of fish health related to diet, disease and stress level (Brill *et al.* 2008). Hematocrit value determines the ratio of the volume of the morphological components of the blood to the volume of the plasma.

The reference range of many hematological parameters has not yet been determined for a large number of fish species, and recent studies conducted to estimate blood values for some important species highlighted the importance of this knowledge as a prerequisite for assessing the health status of wild and farmed fish (Fanouraki *et al.* 2007; Tavares Dias & Moraes 2007). Hematological parameters are used as an early indicator of changes in fish health and have proven to be a valuable approach to controlling the effects of habitat changes, as well as conditions, on fish biology (Sheikh & Ahmed 2016). In order to use blood parameters as biomarkers it is necessary to know their standard values as well as the reference period for the species of fish we study (Parrino *et al.* 2018). However, the normal range of blood parameters is still undetermined for many species of fish and requires additional research to have a library of normal blood values.

In the present study it was shown that the hematocrit values improved and this shows a good clinical picture, while the blood smears show an increased number of lymphocytes, which may be associated with some bacterial infection. Lymphocytes play an important role in the chemical and cellular immunity of fish (Clauss *et al.* 2008).

5. References

Cañada-Cañada F., Muñoz De La Peña A., Espinosa-Mansilla A. (2009). Analysis of antibiotics in fish samples. Analytical and Bioanalytical Chemistry 395(4), 987–1008.

Clauss T.M., Dove A.D.M., Arnold J. E. (2008). Hematologic Disorders of Fish. Veterinary Clinics of North America - Exotic Animal Practice 11(3), 445–462.

Fanouraki E, Divanach P, Pavlidis M. (2007). Baseline values for acute and chronic stress indicators in sexually indicators in sexually immature red porgy (*Pagrus pagrus*). Aquaculture 265, 294–304.

Fazio F., Ferrantelli V., Fortino G., Arfuso F., Giangrosso,G., Faggio C. (2015). The influence of acute handling stress on some blood parameters in cultured sea bream (*Sparus aurata* Linnaeus, 1758). Italian Journal of Food Safety 4(1), 4–6.

Lulijwa R., Rupia E.M., Alfaro A.C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review on the top 15 major producers, Reviews in Aquaculture 12, 640-663.

Pavlidis M., Futter W.C., Katharios P., Divanach P. (2007) Blood cell profile of six Mediterranean mariculture fish species. Journal of Applied Ichthyology 23, 70–73

Tavares-Dias M., Moraes F. R. (2007). Haematological and biochemical reference intervals for farmed channel catfish. Journal of Fish Biology 71(2), 383–388.

Tort L., Rotllant J., Liarte C., Acerete L., Hernández A., Ceulemans S., Coutteau P., Padros, F. (2004). Effects of temperature decrease on feeding rates, immune indicators and histopathological changes of gilthead sea bream *Sparus aurata* fed with an experimental diet. Aquaculture 229(1–4), 55–65.

Tsantilas H., Galatos A.D., Athanassopoulou F., Prassinos N.N., Kousoulaki K. (2006). Efficacy of 2-phenoxyethanol as an anaesthetic for two size classes of white sea bream, *Diplodus sargus* L., and sharp snout sea bream, *Diplodus puntazzo* C. Aquaculture 253(1–4), 64–70.

http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en

https://www.fgm.com.gr/uploads/file/FGM_19_GR_WEB_Spreads(4).pdf

THE EFFECT OF FISH STOCKING DENSITY ON GROWTH PERFORMANCE OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND LETTUCE (*Lactuca sativa* VAR. GREEN TOWERS).

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Abstract
The aim of this study was to investigate the effect of three different fish stocking densities, twelve, eighteen and twenty-four fish (LD with four fish per tank, MD with six fish per tank, and HD with eight fish per tank, respectively) in three indoor small scale aquaponic systems on growth performance of sea bass (*Dicentrarchus labrax*) and lettuce plants (*Lactuca sativa* var. Green Towers). Over a period of 45 days, 54 individual sea bass with an average body weight of 41.44 ± 0.84 g and 24 lettuce plants with initial height of 6.33 ± 0.02 cm were used. LD, MD and HD, did not show significant difference either in their final weight or in FCR, SGR and weight gain. The most effective fish stocking density for lettuce growth was the MD treatment which produced biomass of 4.09 kg/m^2, followed by HD treatment with 4.02 kg/m^2, while LD had the lowest performance with 1.27 kg/m^2 produced biomass. This, therefore, showed that MD and HD densities contributes to the more efficient plant growth.
Keywords: *Aquaponics, Dicentrarchus labrax, Lactuca sativa, fish density*
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1. Introduction

Aquaponics combines fish and plant production in a closed water recirculating system (Rakocy *et al.* 2004). Recirculating aquaculture systems (RAS) are designed to culture large quantities of fish using small volumes of water by recirculation and by removal of the toxic fish waste products (Rakocy *et al.* 2017). Plants in aquaponic systems grow from dissolved nutrients that come directly by fish. The production of dissolved nitrogen in these systems can occur at very high levels (Rakocy *et al.* 2017). This combination of fish-plant culture has gained attention and popularity as a bio-integrated model for sustainable food production (Savidov *et al.* 2005; Kloas *et al*. 2015; Konig *et al.* 2016). Several fish and plant species can be optimally cultured in aquaponic systems, and their overall growth performance varies, depending on the treatment (Ani *et al.* 2021).

Fish stocking density is an essential factor having a great impact on fish production in the aquaponic system. It affects fish growth, survival, behavior, water quality and production of fish (Oke & Goosen 2019; Maucieri *et al.* 2019). The present study aimed to investigate the effect of fish stocking density on growth performance of European sea bass (*Dicentrarchus labrax)* and lettuce (*Lactuca sativa* var. Green Towers).

2. Materials and Methods

The experiment took place at the aquaponics laboratory at the University of Thessaly, School of Agricultural Sciences over a period of forty-five days. Three separated indoor small scale aquaponic systems of 500 L of water were used for this experiment. These systems were consisted by three fish tanks, one grow-bed (1 m^2) filled with clay pebble (8–16 mm) substrate and one filter separated in three parts (mechanical filter, bio-filter, and a pump, which suppled the systems with water through the filter, Q=6.16 L/min). These systems also had thermostats to keep temperature at 20.8°C, air pumps to constantly oxygenate the water (8.45 mg/L), three fans and three HPS lamps 400 W (one over each grow-bed). The photoperiod was 12L:12D. pH values ranged from 6.3 to 6.9 ($p<0.050$) during the whole experiment.

Fifty-four individual European sea bass (*Dicentrarchus labrax)* and twenty-four lettuce plants (*Lactuca sativa* var. Green Towers) were used for the experiment. Three different stocking densities were used, twelve *D. labrax* (four per tank), eighteen (six per tank) and twenty-four (8 per tank), and assigned as LD (Low Density), MD (Medium Density) and HD (High Density), respectively. A 10-day acclimatization period was used. The initial fish weights were recorded. Eight plants were placed in each grow-bed. The seedlings were stayed over the grow-beds and treated with Ca (spraying form), 0.5 ml m^{-2} and irrigated with fertilizer such as potassium sulfate (K_2SO_4) 11 mmol L^{-1} and chelated iron Fe DTPA 11% 40 µmol L^{-1} (Sonneveld & Straver 1994), in order to boost them before planting. Food was offered to the fish three times daily (8 am, 3 pm, 1 am), at 5% of their body weight and food consumption was measured daily. Water's physiochemical parameters (i.e., pH, temperature, and O_2, HQ40d, HACH) were measured on a daily basis. TAN (total ammonia nitrogen), nitrite (NO_2^-) and nitrate (NO_3^-) were measured twice a week with API test kit, while once a week TAN was measured photometrically with HACH LANGE DR 3900.

Fish and plant growth performance measurements were recorded every fifteen days. In addition, for plants the number of leaves, temperature and PAR was measured every fifteen days. The harvest day, measurements of stem height, leaves weight (wet and dry) and roots dry weight, were also recorded. The final weight and length of *D. labrax* per system was taken and growth parameters were calculated, such as feed conversion ratio (FCR), specific growth rate (SGR%/day) and weight gain. An additional analysis for the plants was conducted for proteins and nitrogen (N) that was absorbed by the plants during the experiment (Kjedhal digestion).
The data were analyzed using analysis of variance (ANOVA) in the SPSS Statistical Package (SPSS version 26).

Data were tested for normality and homogeneity with Kolmogorov–Smirnov and Shapiro-Wilk test. To determine any significant difference between treatments, one-way ANOVA was used, followed by Tukey's post-hoc test. Statistical analyses were carried out on a significance level of p-value >0.05. Values are presented as means ± standard error (S.E.M). Mean values ± (S.E.M) with a different letter in the same row are significantly different ($p<0.05$).

3. Results

The physicochemical water parameters were monitored (Table 1). The differences among treatments were statistically significant different, both in the concentration of TAN and NO_3^-. TAN concentrations in the grow bed input (GBin) were statistically significant different ($p<0.05$) between all treatments, with higher value in HD and lower in LD. NO_3^- concentrations, in GBin were not significantly different ($p>0.05$) between MD and HD while LD had statistically significantly different values ($p<0.05$) compared with the other two. In contrast, NO_3^- concentrations in the output of the grow bed (GBout) had no significant difference ($p>0.05$) between LD, MD while HD there was a statistically significant higher value for the HD treatment.

Table 1. Physicochemical water parameters

mg L^{-1}	*LD*	*MD*	*HD*
TAN (GBin)	0.19 ± 0.018^c	0.24 ± 0.01^b	0.28 ± 0.099^a
NO_3^- (GBin)	235.71 ± 11.03^b	275 ± 11.42^a	317.86 ± 18.61^a
NO_3^- (GBout)	225 ± 10.15^b	246.43 ± 9.76^b	303.57 ± 19.23^a

Data are expressed as mean ± S.E.M. Means in a row followed by the same superscript are not significantly different ($p>0.05$).

Tables 2 shows the growth performance of *D. labrax*. The results of the statistical analysis using one-way ANOVA showed initial and final weights, weight gain, feed conversion ratio (FCR) and specific growth rate (SGR%) were non-statistically significant ($p>0.05$) different between all treatments. The mean final weight was higher in LD treatment as well as the weight gain.

Table 2. Growth performance of *D. labrax*

	LD	*MD*	*HD*
Survival	91.67	100.00	95.83
Fish number per tank	4	6	8
Fish number per system	12	18	24
Initial weight (g)	42.45 ± 2.33	42.1 ± 1.00	39.78 ± 1.8
Final weight (g)	81.64 ± 4.92	78.11 ± 2.61	69.72 ± 3.47
Weight gain	38.48 ± 2.59	36.01 ± 2.05	30.36 ± 1.81
FCR	1.75 ± 0.13	1.95 ± 0.24	2.03 ± 0.17

SGR (%/d)	1.40 ± 0.06	1.36 ± 0.06	1.26 ± 0.04

Data are expressed as mean ± S.E.M. Means in a row followed by the same superscript are not significantly different (p>0.05).

Table 3 shows lettuce performance during the experiment. The MD treatment with 6 fish per tank, provided the best lettuce growth. The stem height at MD was statistically significant higher ($p<0.05$) than LD and HD treatments. The number of leaves and roots dry weight were statistically significant different in the LD treatment ($p<0.05$) compared with the MD and HD treatments, respectively. Total produced biomass showed that MD and HD were higher than LD. No statistically significant differences were observed in the proteins and nitrogen values of the plants between the three different treatments ($p>0.05$).

Table 3. Lettuce growth performance

	LD	*MD*	*HD*
Survival %	100	100	100
Stem height (cm)	8.98 ± 0.45^b	10.68 ± 0.18^a	8.6 ± 0.49^b
Number of leaves per plant	36.38 ± 0.94^b	47.13 ± 1.72^a	42.75 ± 1.60^a
Leaf fresh weight (g)	137.58 ± 7.8^b	424.94 ± 31.28^a	423.81 ± 47.66^a
Leaf dry weight (g)	5.78 ± 0.33^b	19.7 ± 1.69^a	17.62 ± 1.74^a
Root dry weight (g)	0.56 ± 0.06^b	1.84 ± 0.2^a	2.11 ± 0.47^a
Total produced biomass (kg/m2)	1.27	4.09	4.02

Data are expressed as mean ± S.E.M. Means in a row followed by the same superscript are not significantly different (p>0.05).

4. Discussion

Water quality parameters in aquaponic systems are essential for the fish, as they will directly affect the growth and the weight gain of the fish (Al-Tawaha & Wahab 2020; Harmon 2009). In this study the nitrate concentration was higher in the treatment with the higher fish number. Al-Tawaha *et al.* (2021), report that this can related to the feed input, which is also related to this study, as long as HD had the higher feed input due to the higher number of fish. The NO_3 levels in this study were higher in HD even though MD had the best plant growth. Goddek *et al.* (2015) such as Kloas *et al.* (2015), mention that increasing stocking density of fish causes pH reduction. In order to keep the stability of pH, which was decreased faster in HD treatment, than the other two treatments due to the higher number of fish, we had to add more frequently freshwater to this aquaponic system. Thus, the freshwater changes in HD system, led to a constant NO_3^- change and as a result lettuce plant could not absorb all the produced NO_3^-.

Fish stocking density is an important factor for plant growth in an aquaponics system. Mauceri *et al.* (2019) showed that lower stocking densities affect lettuce production positively. In addition, Al Tawaha *et al.* (2021) showed that the lowest fish stocking density gave the highest lettuce production. These results contradict this research, as MD followed by HD, gave the highest yield of lettuce. Moreover, the NO_3^- concentrations values in the three treatments were satisfying the nutritional requirements of the lettuce in aquaponic systems and there were within the range of 116.42 mg L^{-1} to 185.93 mg L^{-1} (Rakocy *et al.* 2006)

Maucieri *et al.* (2019) and Hayat *et al.* (2018), did not find any statistically significant differences in FCR when they use different fish stocking densities in aquaponics. In the present study, there are no significant differences in fish growth parameters such as length, weight, weight gain, FCR and SGR, in contrast with Tran *et al.* (2019). The results of Ani *et al.* (2021), Palm *et al.* (2014) and Greenfeld *et al.* (2018) correspond to this study, regarding SGR and fish stocking density. HD leads to lower SGR and higher FCR (Al-Harbi & Siddiqui 2000). The MD treatment with 6 fish per tank, provided the best lettuce growth.

References

Al-Harbi A.H., Siddiqui A.Q. (2000). Effects of tilapia stocking densities on fish growth and water quality in tanks. Asian Fisheries Science 13(4), 391–396.

Al-Tawaha A.R.M., Wahab P.E.M, Jaafar H.B., Zuan A.T.K., Hassan M.Z (2021). Effects of Fish Stocking Density on Water Quality, Growth Performance of Tilapia and Yield of Butterhead Lettuce Grown in

Decoupled Recirculation Aquaponic Systems. Journal of Ecological Engineering 22(1), 8–19 https://doi.org/10.12911/22998993/128692
Ani J.S., Manyala J.O., Masese F.O., Fitzsimmons K. (2021) Effect of stocking density on growth performance of monosex Nile Tilapia (Oreochromis niloticus) in the aquaponic system integrated with lettuce (Lactuca sativa). Aquaculture and Fisheries. https://doi.org/10.1016/j.aaf.2021.03.002
Goddek S., Delaide B., Mankasingh U., Ragnarsdottir K.V., Jijakli H., Thorarinsdottir R. (2015). Challenges of sustainable and commercial aquaponics. Sustainability 7(4), 4199–4224.
Greenfeld A., Becker N., Mcilwain J., Fotedar R., Bornman J.F. (2018). Economically viable aquaponics? Identifying the gap between potential and current uncertainties. Reviews in Aquaculture 11(3), 1–15. https://doi.org/10.1111/raq.12269
Harmon T.S. (2009). Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture 1(1), 58–66. https://doi.org/10.1111/j.1753–5131.2008. 01003.x
Kloas W., Groß R., Baganz D., Graupner J., Monsees H., Schmidt U., Staaks G., Suhl J., Tschirner M., Wittstock B. (2015). A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. Aquaculture Environment Interactions 7(2), 179–192. https://doi.org/10.3354/ aei00146
Konig B., Junge R., Bittsanszky A., Villarroel M., Ko míves T. (2016). On the sustainability of aquaponics. Ecocycles 2, 26–32.
Maucieri C., Nicoletto C., Zanin G., Birolo M., Trocino A., Sambo P. (2019). Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. PloS One 14(5), Article e0217561. https://doi.org/10.1371/journal.pone.0217561
Oke V., Goosen N.J. (2019). The effect of stocking density on the profitability of African catfish (Clarias gariepinus) culture in extensive pond systems. Aquaculture 507, 385–392. https://doi.org/10.1016/j.aquaculture.2019.04.043. Elsevier.
Palm H.W., Bissa K., Knaus U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part II: Fish and plant growth. Aquaculture, Aquarium, Conservation & Legislation 7(3), 162–175.
Rakocy J.E, Masser M.P., Losordo T.M., Beem M. (2017). Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture. Oklahoma State University
Rakocy J.E., Shultz R.C., Bailey D.S., Thoman E.S. (2004) Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. University of the Virgin Islands. DOI: 10.17660/ActaHortic.2004.648.8
Savidov N., Hutchings E., Rakocy J. (2005). Fish and plant production in a recirculating aquaponic system: A new approach to sustainable agriculture in Canada. International Conference and Exhibition on Soilless Culture: ICESC 2005 742, 209–221.
Sonneveld C., Straver N. (1994). Nutrient solutions for vegetables and flowers grown in water or substrates. Voedingspolossingen glastijnbouw. 21pp.
Suhl J., Tschirner M., Wittstock B. (2015). A new concept for aquaponic systems
Thorarinsdottir R. (2015). Challenges of sustainable and commercial aquaponics. Sustainability 7(4), 4199–4224.
Tran N., Chu L., Chan C.Y., Genschick S., Phillips M.J., Kefi A.S. (2019). Fish supply and demand for food security in Sub-Saharan Africa: An analysis of the Zambian fish sector. Marine Policy 99, 343–350. https://doi.org/10.1016/j. marpol.2018.11.009

THE IMPACT OF FEEDING FREQUENCY ON THE GUT MICROBIOTA OF FRESHWATER ADAPTED SEABASS (*Dicentrarchus labrax*) IN A COUPLED AQUAPONICS SYSTEM WITH LETTUCE (*Lactuca sativa*)

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Abstract
The composition of the gut microbiota is modified by multiple internal and external factors, such as diet. To evaluate whether different feeding strategies influence the diversity and composition of host-associated microbiota, we analyzed the gut microbiome of freshwater adapted (0.60 PPT) seabass (*Dicentrarchus labrax*) reared in a coupled aquaponics system with lettuce. In a 45-days feeding trial, fish were fed daily every two, four and eight times (FF2, FF4, and FF8, respectively) 5% of their body weight, with a commercial pellet diet (54.8% protein). For the microbiota analysis, midguts of *D. labrax* were collected at days 0, 15 and 45 and were analyzed using 16S rRNA gene amplicon sequencing. The findings of this study showed that the three feeding frequencies (FF2, FF4 and FF5) affect the composition and structure of *D. labrax* gut microbiota. Despite that no significant changes reported regarding the OTUs relative abundance between sampling points and feeding frequency, every feeding treatment was characterized by different dominant bacteria, with the majority of them affiliated within Actinobacteria. The most abundant bacterial phyla in the midgut samples of the fresh water adapted *D. labrax* was the same that have been reported previously in populations reared in seawater systems, suggesting stability of the intestinal microenvironment that favors specific bacterial groups.
Keywords: *gut microbiota, aquaponic, European seabass, 16S rRNA*
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1. Introduction

In intensive aquaculture, feed accounts >50% of the production cost (Rana *et al.* 2009). In this context feeding strategies (e.g. frequency and rate) for aquatic animals are necessary for optimization of feed conversion ratio, but also for improving the rearing conditions, like the water quality (Abdelghany & Ahmad 2002; Biswas *et al.* 2006). Previous studies, have shown that very high or low frequencies deteriorate growth performance because the animals either receive a new meal before finishing the digestion of the previous, or starving until receive the next one (Booth *et al.* 2008; Riche *et al.* 2004). In *D. labrax*, according to the available literature data, the optimal feeding frequencies vary from once a day to 8 times/day, depending on the rearing system and age/size (Tsevis *et al.* 1992; Güroy *et al.* 2006; Stathopoulou *et al.* 2021).

Despite that feeding regimes can alter the structure and composition of fish gut microbiota and consequently their activity (digestion and absorption of nutrients) (Nayak 2010), only Stathopoulou *et al.* (2021) included a gut microbiome analysis in their study. Here, we analyze further whether the different feeding strategies influence the diversity and composition of host-associated microbiota of fresh water adapted seabass in a coupled aquaponic system with lettuce

2. Material and Methods

Experiment was conducted at the registered experimental facility (EL-43BIO/exp-01) of the Laboratory of Aquaculture, Department of Ichthyology and Aquatic Environment (University of Thessaly, Greece) as described previously in Stathopoulou *et al.* (2021). Briefly, after the successful adaptation of the fish to fresh water (0.60 PPT), 171 juvenile sea bass (*D. labrax*) individuals, with an average body weight of 6.80 ± 0.10 g and an average body length of 8.62 ± 0.05 cm, were placed in the aquaponic fish tanks (9 fish tanks in total, 19 individual/tank) into three autonomous closed loop aquaponics systems, with eight (8) lettuce plants evenly placed in each hydroponic bed. In a 45-days fish/feeding frequency treatment were fed daily every two, four and eight times (FF2, FF4, and FF8) respectively, 5% of their body weight, with a commercial pellet diet (55% protein and 15% crude fat).

For the microbiota analysis, three fish per feeding frequency treatment at days 0, 15 and 45 were sacrificed and their midguts were collected. All experimental procedures were conducted according to the guidelines of the EU Directive 2010/63/EU regarding the protection of animals used for scientific purposes and were applied by

FELASA accredited scientists (functions A–D). The experimental protocol was approved by the Ethics Committee of the Region of Thessaly, Veterinary Directorate, Department of animal protection-Medicines-Veterinary applications (n. 18402/05-09-2019).

Microbial DNA from the midgut samples was extracted with the QIAGEN QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol "DNA Purification from Tissues" according to manufacturer's instructions. Bacterial diversity was assessed by amplification of the V3-V4 region of the bacterial 16S rDNA gene on the MiSeq Illumina platform 2 × 300 bp (MRDNA Ltd., Shallowater, city, TX, USA, sequencing facilities) using the primer pair S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth *et al.* 2013). Polymerase Chain Reaction (PCR) was performed with HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, country) for 30 cycles of the following conditions: 94 °C (3 min), 94 °C (30 s), 53 °C (40 s), 72 °C (1 min) and a final extension at 72 °C (5 min).

Sequencing raw data were processed with the MOTHUR software (v. 1.39.5) (Schloss et al. 2009, Schloss *et al.* 2011) and the operational taxonomic units (OTUs) were classified with the silva release 132 (Quast *et al.* 2013, Yilmaz *et al.* 2014) following the methodology described in Nikouli *et al.* (2018). Identification of closest relative of the Most abundant OTUs was performed with Nucleotide Blast (https://blast.ncbi.nlm.nih.gov). Statistical analysis and graphical illustrations were performed in the Palaeontological STudies (PAST) software (Hammer *et al.* 2001).

3. Results

A total of 2,506 bacterial operational taxonomic units (OTUs) were found in all samplings, ranged between 40 ± 8.5 to 106 ± 36.0 per time point and/or treatment. In feeding frequencies 2FF and 8FF, the highest number of observed and estimated OTUs reported in D45 (Table 1). On the contrary the treatment 4FF, presented the higher number of OTUs in D15, while the number of reads was always higher in D15 (Table 1). Permutational Analysis of Variance (PERMANOVA) of the OTUs relative abundance indicated that there were no statistically significant differences between sampling points and feeding frequency.

Proteobacteria (34.82 %), Actinobacteria (32.39 %), Firmicutes (18.30 %) and Bacteroidota were the dominant bacterial phyla in the midgut samples (Figure 1). At the beginning of the trial (D0), Proteobacteria and Bacteroidota were the predominant phyla with 36.07 % and 35.81 % relative abundance, respectively. However, Bacteroidota detected in ~4 - 138x lower relative abundance (≤ 8.39%) in the rest time points (D15 and D45) in all three (2FF, 4FF, 8FF) feeding frequencies, where the bacterial phyla Proteobacteria, Actinobacteria and Firmicutes constituted the majority of the gut microbiota (82.50 - 99.14% of the total reads). Moreover, every feeding treatment and time points, was characterized by different dominant bacteria (Table 1).

Table 1. Overview of the bacterial sequence reads operational taxonomic units (OTUs) and diversity metrics (values are presented as means ± standard deviation of the mean) in the midgut of *D. labrax*. fed every two (FF2), four (FF4) and eight times (FF8)/daily at D0, D15 and D45. (D=day)

Sample	Reads	Chao-1	Observed OTUs	Simpson 1−D ± SD	No. of dominant OTUs*	Most Abundant OUT (% of total reads) &Closest Relative (≥97%)
D0	7418 ± 4651.3	158 ± 38.0	106 ± 36.0	0.90 ± 0.032	17	OTU069 (11.1) -
D15_FF2	4826 ± 5155.0	70 ± 33.4	46 ± 14.8	0.84 ± 0.027	12	OTU6 (24.6) - *Aurantimicrobium minutum*
D15_FF4	8161 ± 7862.5	66 ± 23.4	48 ± 15.0	0.40 ± 0.480	2	OTU14 (66.8) - *Kocuria marina*
D15_FF8	1480 ± 1722.2	59 ± 32.9	43 ± 24.6	0.83 ± 0.039	7	OTU48 (25.7) - *Corynebacterium bovis*
D45_FF2	1537 ± 1075.3	92 ± 21.0	65 ± 28.8	0.91 ± 0.083	38	OTU7 (10.3) - *Acinetobacter johnsonii*
D45_FF4	1101 ± 1208.1	50 ± 9.3	40 ± 8.5	0.87 ± 0.060	13	OTU221 (11.2) - *Pseudonocardia carboxydivorans*
D45_FF8	689 ± 466.6	62 ± 27.2	52 ± 23.5	0.87 ± 0.086	31	OTU292 (15.2) - *Mycoplasmataceae* sp.

* Cumulative relative abundance ≥ 80%.

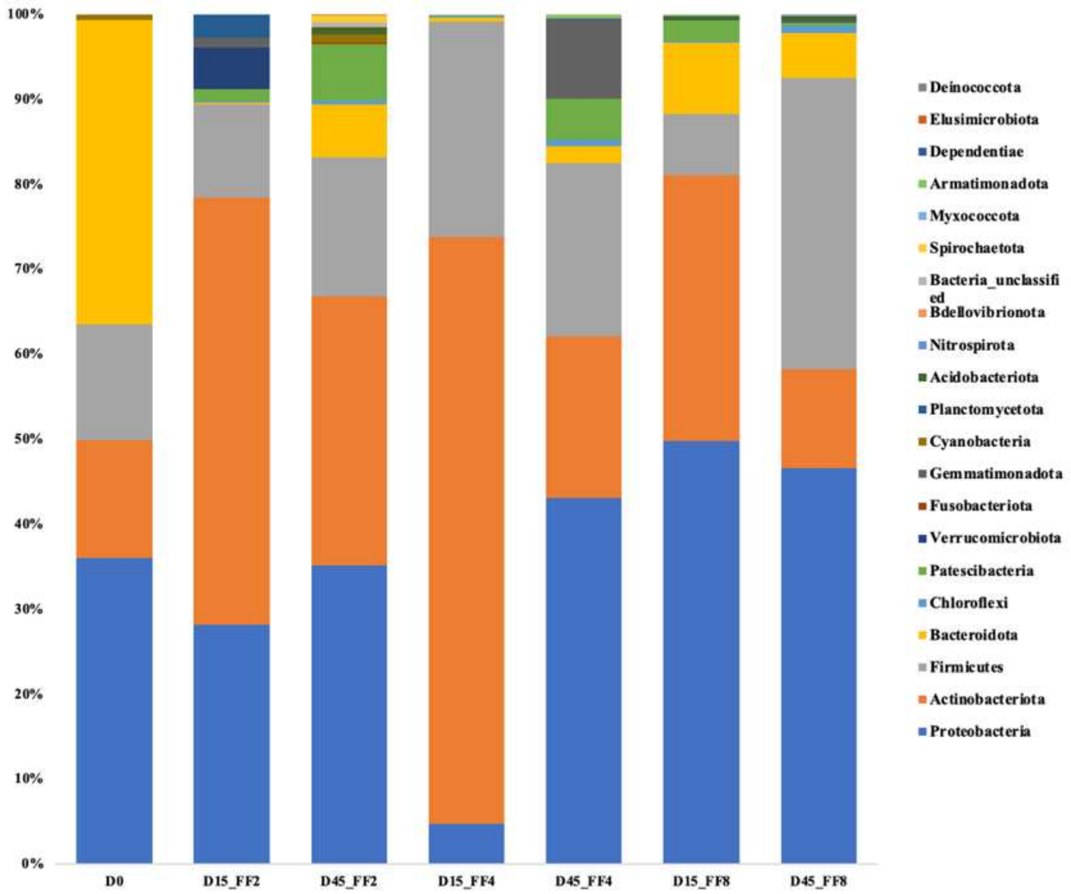

Figure 1. Relative abundance of bacterial phyla in the midgut samples of the *D. labrax* fed every two (FF2), four (FF4) and eight times (FF8)/daily at D0, D15 and D45. (D=day)

4.Discussion

This study demonstrated that the feeding frequency (FF2, FF4, FF8) affect the composition and structure of *D. labrax* gut microbiota. Despite that Stathopoulou *et al.* (2021), reported that the FF4 and FF8 feeding frequency contributes to more efficient utilization of nutrients by *D. labrax*, through the microbiota analysis presented here, the OTUs relative abundance was not significantly changed between sampling points and feeding frequency. However, the dominant bacterial OTUs differ not only between the feeding strategies but also across the feeding trial (D0 – D45). The majority of the dominant OTUs affiliated within the Actinobacteria phyla, while close characterized relative with ≥97% similarity was not detected for the dominant OTU at D0 (OTU069). Finally, the most abundant bacterial phyla in the midgut samples of the fresh water adapted *D. labrax* (Proteobacteria, Actinobacteria, Firmicutes and Bacteroidota) was the same that have been reported previously in populations reared in seawater systems (Nikouli *et al.* 2018), suggesting stability of the intestinal microenvironment that favors specific bacterial groups.

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References

Abdelghany A.E., Ahmad M.H. (2002). Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquaculture Research 33, 415–423.

Biswas G., Jena J.K., Singh S.K., Patmajhi P., Muduli H.K. (2006). Effect of feeding frequency on growth, survival and feed utilization in mrigal, *Cirrhinus mrigala*, and rohu, *Labeo rohita*, during nursery rearing. Aquaculture 254, 211–218.

Booth M.A., Tucker B.J., Allan G.L., Fielder D.S. (2008). Effect of feeding regime and fish size on weight gain, feed intake and gastric evacuation in juvenile Australian snapper *Pagrus auratus*. Aquaculture 282, 104–110.

Güroy D., Onsekiz Mart Deveciler E., Güroy B.K., Tekinay A.A. (2006). Influence of feeding frequency on feed intake, growth performance and nutrient utilization in European Sea Bass (*Dicentrarchus labrax*) fed pelleted or extruded diets. Turkish Journal of Veterinary and Animal Sciences 30, 171-177.

Hammer O., Harper D.A.T., Ryan P.D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1), 9.

Klindworth A., Pruesse E., Schweer T., Peplies J., Quast C., Horn M,. Glöckner F.O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research 41(1), e1.

Nayak SK. (2010). Role of gastrointestinal microbiota in fish. Aquaculture Research 41(11), 1553–73.

Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E. and Kormas, K.A. (2018). Gut Bacterial Communities in Geographically Distant Populations of Farmed Sea Bream (*Sparus aurata*) and Sea Bass (*Dicentrarchus labrax*). Microorganisms. 6(3), 92.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glockner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590-D596

Rana K.J., Siriwardena S., Hasan M.R. (2009). Impact of rising feed ingredient prices on aquafeeds and aquaculture production.FAO Fisheries and Aquaculture Technical Paper. No. 541. Rome, FAO.

Riche, M., Haley, D. I., Oetker, M., Garbrecht, S., Garling, D. L. (2004). Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). Aquaculture 234, 657–673.

Schloss P.D., Gevers D., Westcott S.L. (2011) Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. PLOS ONE 6(12), e27310

Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J., Sahl J.W., Stres B., Thallinger G.G., Van Horn D.J., Weber C.F. (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology 75(23), 7537.

Stathopoulou P., Berillis P., Vlahos N., Nikouli E., Kormas K. A., Levizou E., Katsoulas N., Mente E. (2021). Freshwater-adapted sea bass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. PeerJ 9, e11522. https://doi.org/10.7717/peerj.11522

Tsevis N., Klaoudatos S., Conides A. (1992) Food conversion budget in sea bass, Dicentrarchus labrax, fingerlings under two different feeding frequency patterns. Aquaculture 101, 293-304.

Yilmaz P., Parfrey L.W., Yarza P., Gerken J., Pruesse E., Quast C., Schweer T., Peplies J., Ludwig W., Glockner F.O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Research 42, D643-D648.

REPRODUCTION AND BEHAVIOR OF RED HYBRID TILAPIA *Oreochromis mossambicus*.

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Abstract
Species of the Oreochromis genus have an important role in the world market. Both in food production and in ornamental usage. They are characterized by high resistance to extreme environmental conditions and their ability to survive and reproduce in captivity. In the present study, the optimal breeding condition and behavior of the red hybrid of Oreochromis mossambicus were investigated. Individuals were tested for reproduction in different combinations of environmental and biological parameters in glass aquarium systems of 180L. The temperatures of the experiments were 25 °C and 28 °C, photoperiods were 6:18 and 12:12 (light: dark) and the sex ratios of fish were 1:1 and 1:3 (males: females). Each combination was performed for a period of 20 days until the gradual change to the next combination. The same behavioral pattern was found during reproduction in every experiment with more intense behaviors during temperature and photoperiod increase. The optimal reproduction condition with the highest number of incidences was at 28 °C with a photoperiod of 12:12 (light: dark) and a sex ratio of 1: 3 (males: females).
Keywords. *Oreochromis mossambicus, reproduction, behavior, temperature, photoperiod, sex ratio*
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1. Introduction

Tilapias are fish of the order Perciformes and belong to the family Cichlidae. The second includes about 220 genera and over 1000 species according to Mayers (2021). The genus *Oreochromis* is a subgene of an older genus of tilapia. According to El Sayed (2019) the genus of *Tilapia* was divided into 3 subgenes in dependance of reproduction and parental care. During reproduction, the female lays her eggs on the bottom in a clean and protected area. After the fertilization of the eggs by the male, the special parental behavior of the genus follows. The female, after reproduction, collects the fertilized eggs in her oral cavity and performs the oral incubation known as the mouthbrooding (Russell et al. 2012). This process takes place until the young can be fed independently (Balshine - Earn & Earn 1998). An important characteristic of the genus and especially of the species *Oreochromis mossambicus* is its tolerance to a wide range of environmental parameters such as salinity, temperature, nitrogen derivatives and the concentration of oxygen and carbon dioxide in water. The genus *Oreochromis* is quite important from a financial point of view for humans as many species and hybrids of it are cultured around the world for food production and other applications (Blessing 2018). The aim of the present study was to investigate the reproductive activity and behavior of the red hybrid of *Oreochromis mossambicus*. In addition to the other results, the optimal reproduction conditions in terms of reproductive events for aquarium systems during the differentiation of certain physicochemical and biological parameters was examined.

2. Material and Methods

The reproduction experiments were carried out at the aquaculture laboratory of the Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly. The study was divided into 2 parts lasting 80 days each, depending on the prevailing temperature in the systems (80 days at 25 °C and 80 days at 28 °C). The same procedures and fish management were performed at both temperatures. At intervals of 40 days, the photoperiod varied between 6L:12D (Light: Dark) and 12L:12D (Light: Dark). At intervals of 20 days, fish were added or removed to form the sex ratio of 1:1 male : female and 1:3 male : female. The aquarium systems which were used had a useful volume of 180L of freshwater with 0‰ salinity. All systems had a thermostat to regulate and maintain the temperature at the desired value. There were also mechanical and biological filters for the best possible maintenance of low levels of nitrogen derivatives. Finally, there was an oxygen pump to ensure high oxygen concentration in the water. 150 red hybrids of the species *Oreochromis mossambicus* were used. The individuals used in both temperature experiments were the same. At the beginning of the first part the average weight was 45 ± 10g while at the beginning of the second part the initial weight was 89.66 ± 10g. The systems were managed daily with great care to avoid stress on fish. Processes performed twice daily were the followings: Cleaning of the systems, temperature measurements, feeding and observation of the fish. Weekly processes were the measuring of oxygen, pH, salinity, and nitrogen derivatives. The feeding ratio of the broodstock was carried out with a diet of 2.5% (El Sayed 2019). The basic diet of fish consisted of commercial concentrates of medium diameter 1.5mm (Biomar ABEE, Greece) while in addition were given compacted Tetra bits complete (Tetra, Germany), frozen food Discus frozen formula, Cyclops and Daphnia (Ocean nutrition, USA). For each movement in and out of the experimental systems the individuals were subjected to anesthesia and disinfection. The anesthesia was performed in dilute phenoxyethanol solution (0.15 mL anesthetic / 1L water) and was intended not to stress the fish. Disinfection was performed in dilute aqueous formaldehyde solution 4/1000 mL/mL and aimed to avoid contamination of the systems and other individuals.

3. Results and Discussion

Physicochemical parameters were kept within the desired limits during the experiments. Temperature according to the requirements of each experiment cept at 25 ± 0.5 ºC and 28 ± 0.5 ºC. These values according to El Sayed & Kawanna, (2008) belong among the desired ones for the genus *Oreochromis*. During the weekly measurements in the systems pH ranged from 7.6 to 8.4 and the dissolved oxygen (D.O.) concentration was always above 70%, and in agreement with the optimal values given by Ross (2000) and Abdel-Tawwab et al. (2015). Despite the high levels of oxygen, tilapias rose to the surface for receiving atmospheric air. This is in contrast to Ross's 2000 study, which states that this process takes place under low oxygen conditions, leading to the conclusion that this movement is simply a habit of the species in some cases. Concentration of non-ionized ammonia (NH_3) was at values close to 0 and without any incident of difficulty in feeding or even mortality. NO^{-3} ranged from 0 to 50 mg/L while NO^{-2} from 0 to 2 mg/L.

Species of the genus *Oreochromis* are characterized by their unique way of reproduction and incubation and have triggered the interest of several researchers to understand their reproductive behavior (Fridman et al. 2012; El Sayed, 2019; Freitas et al. 2019). Reproductive behavior for males and females of the species were observed and recorded during the experiments and different tests. All habits and manners of individuals during that time, categorized in three stages. First the pre-reproduction behavior, then the behavior during reproduction, and then the post-reproduction or parental behavior. Tilapia habits before reproduction were divided into two phases. In the first, the fish did not show aggressive behavior, while the desire for food and exploration was evident. The beginning of the second phase was perceived with the intensity and aggression between the individuals. Aggressive behavior became visible in four ways (figure 1). The first was the intense competition in obtaining food despite its satisfactory supply from the authors. The second was threatening moves towards the other fish while maintaining a stable position in the water column. Such were the openings of the pectoral, pelvic and dorsal fins as well as the mouth and the gills. The third was the dispute with other members of the aquarium system over the imposition of dominance. Attacks on other fish, of both sexes, were carried out either head-on with a collision of the mouths, or with the attacking fish damaging the opponent's side.

Figure 1. Images showing conflicts between individuals in systems. 1. Static demonstration in the water column, 2. frontal collision, 3. pectoral collision, 4. Moments from chase, 5. Hierarchy within the system.

Finally, the last aggression observation was the hunt inside the aquarium between fish, with swimming speed accelerations much higher than those defined by Ross (2000) and El Sayed (2019). These researchers stated the slow speed of the species as a grazing one. The order in which the conflicts took place was not always the same. Some disputes could be detrimental or fatal to the losers and even to the winner. They ranged from simple scale losses up to bleeding mortality. However, there were times when the controversy stopped without injuries, that is, only with the display of the fins. This fact was also observed by Arnott & Elwood (2009a) for the species *Oreochromis niloticus*.

After the conflicts end, the hierarchy of the system was formed with the strongest person choosing his place and protecting it. The winning individual was usually the largest (Freitas et al. 2019). However, there were cases where the winning person was either smaller or female, which deviates from the rule of Medeiros et al. (2007). It was common for younger individuals to recognize their disadvantage and retreat, which was also observed by Barreto et al. (2010).

The male broodstock created a nest, delimiting the breeding area and continuously protecting it from other individuals. In case of substrate existence, the person dug to create the nest, constantly cleaning the selected area. In systems where no substrate was used, males simply protected and cleaned the boundary area without leaving the circular shape of the nest at the bottom. The period of finding the right partner followed. This consisted of many failed attempts to mate with other females. When the female was not ready the male caused from simple persecution to heavy injuries to the second fish. This characteristic was attributed to the observation of faster reproductive maturity of males compared to females. When a female was ready to mate, she followed the male to his marked area.

From this moment until the fertilization of the eggs, the behavior of reproduction followed. The duration of the cooperation between the two parents was not stable and no correlation was observed with any environmental factor. Lowe (1956) in an earlier study notes that the partnership of the two fish lasts a few minutes. However, there were cases in our study where the couple was in the nest for a few hours, or even a few days. Initially, the couple protected and cleaned their nest while feeding normally. The male usually pushed the female to start spawning without her being ready yet. Shortly before spawning took place, while observing the two individuals externally, it was easy to notice the pointed protrusion of the urogenital papilla in the male and the oval protrusion in the female (figure 2). When the female was ready to lay eggs, she approached the bottom, followed by spasmodic movements, and almost touching the bottom, the eggs were partially deposited in the clean nest (figure 2). The male was standing next to her ready to start the fertilization of the eggs. With successive passes over the eggs he carried out the fertilization. The female, without leaving much time for the male, placed the eggs in his oral cavity to initiate the third and final part of the behavior, that of parental care (figure 2).

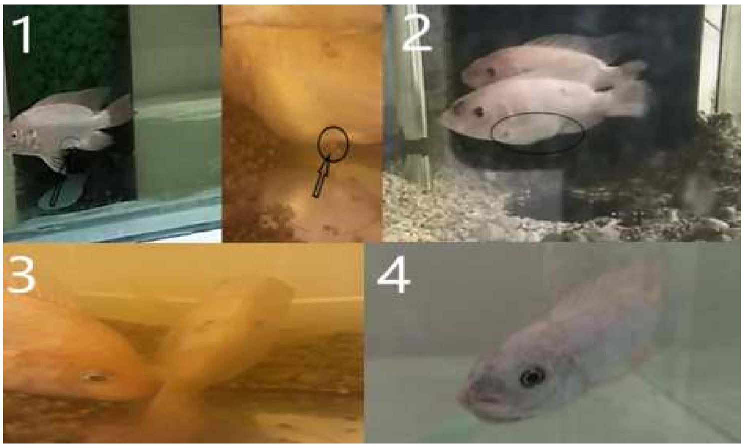

Figure 2. Images showing the reproductive and parental behavior. 1. Protrusions of the urogenital tract in the male (left) and female (right). 2. The pair has been created. 3. A female lays eggs on the bottom while the male is next to her. 4. A female incubates eggs right after fertilization of eggs.

After the fertilization of eggs the couple separated usually immediately. The male continued to claim his space and begun his search for the next female to reproduce. Females formed a cavity in the lower part of their mouth a few days before spawning which they used for the oral incubation of eggs (Weber 2010). The placement of the eggs inside this cavity served to protect the eggs from predators or other individuals and their constant stirring in adequate ventilation and prevention so that the eggs do not stick together. Females did not feed during the incubation period even though they approached the food possibly because of its smell, a habit which was also observed by Nguyen et al. (2010).

The intentional divarication of environmental parameters and the sex ratio of the fish did not cause different kinds of behaviors. However, the intensity of the behaviors and conflicts became greater with the existence of a higher daylight (photoperiod 12L:12D) and with the increase of the temperature to 28 ºC. At lower temperatures in combination with a shorter photoperiod (6L:18D) the lowest mobility in the systems was observed. Sex ratio was the most important parameter for the determination of behavior of individuals according to the authors. Most injuries and deaths occurred at intervals when the sex ratio in the systems was 1:1 males: females. This is due to the fact that there are less females for each male in that ratio than on 1:3 males:females. So male broodstock had all of their aggression divided in fewer number of females, causing much more damage to the female population. The same ratio (1:3 males:females) for the species is suggested by El Sayed (2019), however there are studies that suggest different values such as Hughes & Behrends (1983) the ratio 1: 2 males: females and Mires (1982) the ratio 2: 1 males : females.

During the intervals in which the changes of temperature, photoperiod and sex ratio took place, the incidents of reproduction in the systems were noticed. A reproduction event was successful if the whole ritual of mating happened, up until the start of mouthbreeding. As can be seen below (Table 1), the 28ºC temperature condition with a photoperiod of 12L:12D (light: dark) and a sex ratio of 1:3 males:females had the most cases of reproduction. The different photoperiod seemed to affect the mobility and general activity of the fish. In the first case 6L:18D (light: dark), the fish showed less activity and movement than in the photoperiod 12L:12D (light: dark). This observation is confirmed by previous studies (Biswas et al., 2002; Biswas & Takeuchi, 2002). The results of the present study showed that during the photoperiod with longer light duration, more incidents occurred. There were 7 incidents at 25 ºC with the best condition for this temperature to be 12 hours a day and a sex ratio of 1:3 males: females. At 28 ºC there were a total of 16 reproductive events with most occurring in 12 hours of light in the day and with a sex ratio of 1:3 males:females. Webers (2010) and El Sayed (2019) report that the temperature range for reproduction in the genus *Oreochromis* is between 23 ºC and 35ºC. In the present study, 25 ºC and 28 ºC were used to perform the experiments as these temperatures are easily achievable with small and inexpensive temperature maintenance equipment as opposed to higher values. Thus, it is possible to achieve a satisfactory number of reproductive events without the cost being high enough.

Table 1. Number of reproductive events under different environmental/biological conditions.

Temperature (per 80 days)	25ºC				28 ºC			
Photoperiod (Light:Dark) (per 40 days)	6 : 18		12: 12		6 : 18		12: 12	
Sex ratio (males:females) (per 20 days)	1:1	1:3	1:1	1:3	1:1	1:3	1:1	1:3
Number of reproductive incidents	0	2	2	3	3	4	4	5

In conclusion, we observed the exceptional behavior of the species during its reproductive cycle. This fact, in addition to the information of easy reproduction in captivity that promises high production yields makes even more interest in its use for food production and for ornamental aquaculture. The optimal reproduction conditions in aquarium systems were found to be 28 ºC with a photoperiod of 12L:12D and a ratio of the two sexes of 1:3 males: females. However, further research is needed on the *Oreochromis* species-specific reproduction for both aquaculture and ornamental aquaculture industry.

References

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. A. M. & Monier, M. N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity. *Aquaculture International,* 23**,** 1261-1274.

Balshine-Earn, S. and Earn, D.J., (1998). On the evolutionary pathway of parental care in mouth–brooding cichlid fishes *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1411), pp.2217-2222.

Barreto, R.E., Carvalho, G.G.A. and Volpato, G.L., (2011). The aggressive behavior of Nile tilapia introduced into novel environments with variation in enrichment. Zoology, 114(1), pp.53-57.

Biswas, A. K., Endo, M. & Takeuchi, T. (2002). Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia Oreochromis niloticus: Part I. Fisheries science, 68, 465-477.

Biswas, A. K. & Takeuchi, T. (2002). Effect of different photoperiod cycles on metabolic rate and energy loss of fed and unfed adult tilapia Oreochromis niloticus: Part II. Fisheries science, 68, 543-553.

Blessing Mapfumo, Tilapia Trade Global and Regional Trends, FAO/ASTF GCP/RAF/510/MUL: Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture, 2018

EL-Sayed, A.-F. M. (2019). *Tilapia culture*, Academic Press.

EL-Sayed, A.-F. & Kawanna, M. (2008). Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. *Aquaculture Research,* 39**,** 670.

Fridman, S. Bron, J.E. Rana, K.J. (2012). Ontogenic changes in the osmoregulatory capacity of the Nile tilapia Oreochromis niloticus and implications for aquaculture. Aquaculture 356-357(2012) 243-249

Gareth Arnott, Robert W. Elwood, Assessment of fighting ability in animal contests, Animal Behaviour, Volume 77, Issue 5, 2009, Pages 991-1004, ISSN 0003-3472

Gonçalves-de-Freitas, E., Bolognesi, M.C., Gauy, A.C.D.S., Brandão, M.L., Giaquinto, P.C. and Fernandes-Castilho, M., (2019). Social Behavior and Welfare in Nile Tilapia. *Fishes*, *4*(2), p.23.

Lowe, R.H. (1956). The Breeding Behaviour of Tilapia Species (Pisces; Cichlidae) in Natural Waters: Observations On T. Karomo Poll and T. Variabilis Boulenger. Behavior, Volume 9: Issue 1, pages: 140–162

Mires, D. (1982) Study of the problems of the mass production of hybrid tilapia fry. In: Pullin, R.S.V. and Lowe-McConnell, R.H. (eds) The Biology and Culture of Tilapias. ICLARM Conference Proceedings No. 7, ICLARM, Manila, Philippines, pp. 83–113

Myers, P., R. Espinosa, C. S. Parr, T. Jones, G. S. Hammond, and T. A. Dewey. 2021. The Animal Diversity Web (online). Accessed at https://animaldiversity.org.

Nguyen, N.H., Ponzoni, R.W., Abu-Bakar, K.R., Hamzah, A., Khaw, H.L. and Yee, H.Y., (2010). Correlated response in fillet weight and yield to selection for increased harvest weight in genetically improved farmed tilapia (GIFT strain), Oreochromis niloticus. Aquaculture, 305(1-4), pp.1-5

Ross, L. 2000. Environmental physiology and energetics. *Tilapias: biology and exploitation.* Springer.

Russell, D., Thuesen, P. & Thomson, F. (2012). A review of the biology, ecology, distribution and control of Mozambique tilapia, Oreochromis mossambicus (Peters 1852)(Pisces: Cichlidae) with particular emphasis on invasive Australian populations. Reviews in Fish Biology and Fisheries, 22, 533-554.

Weber, R. M. (2010) Behavioral, Reproductive and Growth studies on *Oreochromis mossambicus* (Peters 1852). Submitted in fulfillment of the requirements for the degree of Master of Science in the School of Biochemistry, Genetics, Microbiology and Plant Pathology, University of KwaZulu-Natal.

FISHMEAL PROTEIN REPLACEMENT BY *Zophobas morio* LARVAE AFFECTS *Dicentrarchus labrax* GROWTH PERFORMANCE IN AN AQUAPONICS SYSTEM

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Abstract
Aquaponics combines the culture of aquatic animals and the cultivation of plants in fully recirculating or decoupled systems, integrating aquaculture and hydroponics in a soil-less system. Consequently, fish feed formulation can affect plant growth. Recently, insect meal has introduced to aquafeeds due to its high nutritional value. A combined cultivation of 90 *Dicentrarchus labrax* juveniles fish (21.55±0.28g initial mean weight) and 24 *Lactuca sativa* var. Romana plants (2.10±0.077g initial fresh weight) was performed for 45 days in three aquaponics systems with a total water volume of 500L/system. Fish were fed daily 5% of their mean body weight, diets of different fishmeal replacement with defatted *Zophobas morio* larvae meal (0%, 10% and 20% replacement respectively). Each dietary treatment represented in all aquaponics systems. Insect meal did not affect fish survival. Higher weight gain (WG) resulted fish fed control diet (FM) and 10% *Z. morio* replacement diet (ZM10) compared to 20% (ZM20) (27.86±0.98g, 26.17±0.96g and 24.46±0.89g respectively). Sea bass fed FM presented statistically significant higher specific growth rate (SGR) (1.84±0.03%/day) compared to ZM10 (1.75±0.02%/day) and ZM20 (1.68±0.03%/day). Feed Conversion Ratio (FCR) did not differ significantly between diets (1.24±0.05, 1.32±0.45 and 1.38±0.06 respectively for FM, ZM10, ZM20 diets). Lettuce grown in aquaponics system 2 (AP2) and APS3 had statistically higher leaf fresh weight (184.47±18.63g and 245.32±19.66g respectively) compared to AP1 (141.70±9.09g). Increased total biomass production was shown for APS3 (2.2kg/m^2) compared to APS2 (1.72 kg/m^2) and APS1 (1.3 kg/m^2). In conclusion, 10% fishmeal replacement with *Z.morio* larvae meal can achieve satisfying sea bass growth. Lettuce growth was not adversely affected by the introduction of insects into aquafeed.
Keywords: *aquaponics, fish meal replacement, zophobas morio, sea bass, lettuce*
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1. Introduction

The contribution of aquaculture to world fish production is growing, accounting for 46% in 2018 compared to 25.7% in 2000 (FAO 2020). However, the increased production and use of aquafeeds has negative environmental impacts such as the reduction of marine fish stocks (Hua *et al.* 2019) and greenhouse gas emissions. Aquaponics is a sustainable farming system with small environmental footprint in relation to aquaculture and soil monoculture. Aquaponics combines the farming of aquatic organisms or the culture of aquatic plants and the cultivation of plants in a soil-less system where over 50% of nutrients needed for plants growth are derived from the aquatic organism's wastes. (Goddek *et al.* 2019). Systems can be fully recirculated or decoupled.

The quality of the feed is vital for both fish and plants optimal growth. Fish meal is rich in protein (56-76%) providing the fish with essential amino acids and fish oil is rich in fatty acids. Nutrients such as nitrogen and phosphorus needed for plants growth, are released by fish through their metabolism. Supplementation of nutrients such as Ca, K and Fe may be needed to achieve optimal plant growth.

The high cost of fish meal and fish oil has led to the introduction of alternative sources of protein in aquafeeds such as plant-based products (soybean, barley, corn), algae (macroalgea), animal by- products (poultry meal, blood meal) and insects. Recently, the replacement of fish meal with insects (Sánchez-Muros *et al.* 2014; Wang *et al.* 2019) has been of great interest due to their high nutritional value (Nogales-Mérida *et al.* 2019; Rumbos *et al.* 2020) and low rearing costs. Abdel-Tawwab *et al.* (2020) state that up to 50% replacement of fish meal with *Hermetia illucens* can reduce sea bass feeding cost by 15.5%. The larvae of *Zophobas morio* have high nutritional value and are rich in protein (431.3 - 516.2 g/kg dry matter) and fatty acids (328.0 - 435.4 g/kg dry matter) (Bednářová *et al.* 2013; van Broekhoven *et al.* 2015).

Sea bass (*D. labrax*) is an important euryhaline species in European marine aquaculture. According to several studies sea bass can successfully be adapted to freshwater (Nebel *et al.* 2005) which makes it suitable for aquaponics with the combined cultivation of leafy greens (Waller *et al.* 2015; Nozzi *et al.* 2016; Stathopoulou *et al.* 2021).

The aim of the study was to evaluate the effect of three different fish feeds (0%, 10% & 20% replacement of fishmeal with larvae *Z. morio*) on water quality, survival and growth of sea bass (*D. labrax*) and to determine the most efficient dietary treatment of sea bass in aquaponics ensuring maximum sea bass and lettuce growth.

2. Material and Methods

A combined cultivation of sea bass (*Dicentrarchus labrax*) and lettuce (*Lactuca sativa* var. Romana) was performed for 45 days in three autonomous aquaponics systems (fish tank, hydroponic unit filled with clay, mechanical filter, biological filter, sump filter) with a total water volume of 500L/system. Fish were gradually acclimatized to lower salinities 1ppt during a 60-day period. 90 sea bass juveniles of 21.55±0.28g initial mean weight and 12.97±0.06cm initial length were distributed in triplicates (30/system). Each aquaponic system consisted of three fish tanks represented by all three dietary treatments. 8 lettuce plants were placed in each hydroponic bed. Artificial light was supplied with controlled photoperiod at 10 h light: 14 h dark.

Three isonitrogenous (520 g/kg crude protein) and isoenergetic (21 MJ/Kg) diets were formulated. Fish meal protein was replaced by defatted *Zophobas morio* larvae meal at 10% (ZM10) and 20% (ZM20). Lysine and methionine were supplemented to ZM10 and ZM20 diets due to lack of insect meal to these amino acids (da Cunha Pedrosa 2016). Fish were fed daily 5% of their body weight, four times per day throughout the day (24 hours). Each day uneaten food was removed and measured. Every 15 days, fish growth was measured (weight and length) and feeding amount was adjusted according to fish weight.

Water temperature and pH of fish tanks were recorded daily, while oxygen concentration and electrical conductivity were recorded every three days (Hach multimeter, HQ40d; Crison multimeter, CM35). Ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions were monitored once a week (Hach photometer, DR3900). Water samples were taken at the water outlet point (FTout) of the fish tanks, at the inlet point (GBin) and at the exit point (GBout) of the hydroponic cultivation tank. At the end of the experiment, fish final body weight and length were measured and growth performance was calculated. In addition, plants final weight and total biomass were measured.

Data were tested for normality and homogeneity with Kolmogorov–Smirnov and Levene's tests, respectively. To determine any significant differences in fish growth between diets and in water quality and plants growth between systems, one-way ANOVA was used, followed by Tukey's post-hoc test. Statistical analyses were carried out using the software package IBM SPSS Statistics V22. Values are presented as means ± standard error (S.E.M).

3. Results

Water temperature, electrical conductivity and dissolved oxygen did not differ statistically ($p > 0.05$) between the three aquaponics systems. Mean water temperature was kept constant at 20.5ºC. In all aquaponic systems the dissolved oxygen level was 7.38±0.04mg/L while electrical conductivity was 1.59±0.01 mS/cm. pH mean value was 7.28±0.01, 7.17±0.02 and 7.04±0.02 for the systems APS1, APS2 and APS3 respectively.

The mean NH_4^+, NO_3^- and PO_4^{3-} concentrations in all aquaponics systems did not differ statistically ($p > 0.05$) between the tree sampling points (water outlet of fish tank (FTout), inlet point (GBin) and exit point (GBout) of the hydroponic cultivation tank). However, PO_4^{3-} concentrations found statistically lower ($p < 0.05$) at APS1 compared to APS3.

At the end of the experiment, final fish weight and length did not differ statistically ($p > 0.05$) between the three diets. Statistically significant differences ($p < 0.05$) were found in fish weight gain and specific growth rate (Table 1). Higher growth recorded for fish fed the control diet (FM) compared to diets where fish meal was replaced with *Z. morio* larvae by 10% (ZM10) and by 20% (ZM20). Fish fed with 20% replacement of fish meal with insect meal showed the lowest growth. However, FCR values for diet ZM20 were not statistically significant different compared to diets ZM10 and FM.

The final plant growth parameters are presented in Table 2. Statistically significant differences ($p < 0.05$) were found in steam height, leaf fresh weight and leaf number. Plants grown in aquaponic systems 2 (APS2) and 3 (APS3) had statistically higher leaf fresh weight compared to aquaponic system 1 (APS1). However, plants in APS2 resulted statistically higher stem and more leaves compared to APS1 and APS3. At the end of the experiment, aquaponic system 3 (APS3) had the maximum total produced biomass.

Table 1. Sea bass growth performance

	FM	ZM10	ZM20
Survival (%)	96.67±3.33	100±0.00	96.67±3.33
Final weight (g)	49.27±1.37	47.73±1.43	45.86±1.34
Final length (cm)	16.20±0.15	16.17±0.14	15.98±0.14
Weight Gain (g)	27.86±0.98^{a}	26.17±0.96^{ab}	24.46±0.89^{b}
Specific Growth Rate (SGR, %/day)	1.84±0.03^{a}	1.75±0.02^{b}	1.68±0.03^{b}
Feed Conversion Ratio (FCR)	1.24±0.05	1.32±0.45	1.38±0.06

Note: Data are expressed as means ± S.E.M (n=29 for FM and ZM20, n=30 for ZM10). Means in a row followed by different superscript letter are significantly different ($p < 0.05$). Where no letters exist, no significant differences were detected ($p > 0.05$).

Table 2. Lettuce growth performance

	APS1	APS2	APS3
Steam height (cm)	11.45±1.08^{ab}	13.7±1.04^{a}	8.36±0.43^{b}
Leaf fresh weight (g)	141.70±9.10^{b}	184.47±18.63^{a}	245.32±19.66^{a}
Leaf number	35.13±1.72^{b}	43.38±3.63^{a}	34.88±0.72^{b}
Total produced biomass (TPB, g/m^2)	1.30	1.72	2.20

Note: Data are expressed as means ± S.E.M (n=8). Means in a row followed by different superscript letter are significantly different ($p < 0.05$). Aquaponics systems (APS) 1, 2 and 3.

4. Discussion

In the present study, water temperature was kept at 20.5±0.05°C, meeting the requirements of both sea bass and lettuce plants. Oxygen levels of > 5 mg/L ensured the adequate ventilation for sea bass respiration and strengthen the plant's root system. pH mean values were 7.28±0.01, 7.17±0.02 and 7.04±0.02 for the systems APS1, APS2 and APS3 respectively. This slight difference between the systems can be explained due to different nitrification rate by the biological filters (Tyson *et al.* 2004). The higher concentrations of NH_4^+, NO_3^- and PO_4^{3-} at the inlet point (GBin) compared to the exit point (GBout) of the hydroponic cultivation tank indicate the nutrient absorption by lettuce plants.

In our study, insect diets ZM10 and ZM20 did not affect sea bass survival. Similar results were reported by Henry *et al.* (2018) with *Tenebrio molitor* meal. *Dicentrarchus labrax* final weight and FCR did not differ ($p > 0.05$) between the three diets. Nevertheless, fish fed control (FM) and 10% fish meal replacement by *Z. morio* (ZM10) diets, resulted in statistically higher weight gain comparing to ZM20. FM dietary treatment showed statistically significant ($p < 0.05$) higher SGR. According to Jabir *et al.* (2012) 25% replacement of *Z. morio* can be considered as the optimal level for *Oreochromis niloticus* (5.57 ± 0.15g initial weight) growth compared to 50%, resulting in higher WG, SGR and lower FCR (4.39±0.11g, 1.02±0.41%/day και 1.25±0.02 respectively for 25% replacement). On the contrary, no significant differences in WG, SGR and FCR were found by Prachom *et al.* (2021) when Asian sea bass (*Lates calcarifer*) was fed diets with different FM replacements by deffated *Z. morio* larvae meal. Gasco *et al.* (2016) state that, sea bass (5.22 ± 0.82g) fed 0-25% fish meal replacement with *T. molitor* larvae meal can result higher WG, SGR and FCR compared to 50%. Compared to this study, sea bass fed with 25% replacement with *T. molitor* had higher SGR (1.89%/day) and lower FCR (0.91). This may be due to the different fish size and the different type of insect meal. According to Magalhães *et al.* (2017) *H. illucens* larvae can replace up to 19.5% of FM protein without adverse effects on growth performance, feed utilization and digestibility of European sea bass while Piccolo *et al*. (2017) reported that *T. molitor* larvae meal can replace fish meal up to 25% in *Sparus aurat*a diet without negative effects on weight gain.

In this study, the only plants' nutrient source originated from fish feed and no external fertilization was performed. Lettuce plants had morphological differences between the three systems. Higher growth was noted for plants grown in APS2 and APS3, with statistically ($p < 0.05$) higher leaf fresh weight compared to AP1.

Nevertheless, the highest biomass production was observed for lettuce grown in APS3. Higher pH values at APS1 compared to the other two systems may have affected plant growth, inhibiting the absorption of specific micronutrients (Bugbee 2004) leading to lower produced biomass (Schwarz 2012). To our knowledge, there are no similar studies examining lettuce growth under different fish meal replacements with insect meal in an aquaponic system. However, Medina *et al.* (2016) state that a lower-protein (32%), plant-based aquafeed can achieve higher red amaranth (Amaranthus tricolor) height, number of leaves and yield, compared to a higher protein (40%) fishmeal-based aquafeed. When lettuce was grown in a non-fertilized aquaponic system, Nozzi *et al.* (2018) reported similar results to the findings of the present study, e.g. an average shoot weight of 222.0g when Nile tilapia was fed with 37.5% protein for 57 days. On the contrary, higher steam height (23.81cm) and final aerial biomass (445.44g) were reported for lettuce co-cultivated with Nile tilapia and catfish fed with 31-33% protein for 49 days (Andriani *et al.* 2017).

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References

Abdel-Tawwab M., Khalil R.H., Metwally A.A., Shakweer M.S., Khallaf M.A., Abdel-Latif H.M. (2020). Effects of black soldier fly (Hermetia illucens L.) larvae meal on growth performance, organs-somatic indices, body composition, and hemato-biochemical variables of European sea bass, Dicentrarchus labrax. Aquaculture 522, 735-736.

Andriani Y., Dhahiyat Y., Zahidah Z., Zidni, I. (2017). The effect of stocking density ratio of fish on water plant productivity in aquaponics culture system. Nusantara Bioscience 9(1), 31-35.

Bednářová M., Borkovcová M., Mlček J., Rop O., Zeman L. (2013). Edible insects-species suitable for entomophagy under condition of Czech Republic. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 61(3), 587-593.

Bugbee B. (2004). Nutrient management in recirculating hydroponic culture. In South Pacific Soilless Culture Conference-SPSCC 648, pp. 99-112.

da Cunha Pedrosa R. (2016). Potential of prepupae meal of Black Soldier Fly (Hermetia illucens) as fish meal substitute in European sea bass juveniles (Dicentrarchus labrax): Implication in flesh quality. Master thesis, Department of Biology, University of Porto, Portugal. pp. 48.

FAO (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en.

Gasco L., Henry M., Piccolo G., Marono S., Gai F., Renna M., Lussiana C., Antonopoulou E., Mola P., Chatzifotis, S. (2016). Tenebrio molitor meal in diets for European sea bass (Dicentrarchus labrax L.) juveniles: growth performance, whole body composition and in vivo apparent digestibility. Animal Feed Science and Technology 220, 34-45.

Goddek S., Joyce A., Kotzen B., Burnell G.M. (2019). Aquaponics Food Production Systems. Springer. pp. 619.

Henry M.A., Gasco L., Chatzifotis S., Piccolo G. (2018). Does dietary insect meal affect the fish immune system? The case of mealworm, Tenebrio molitor on European sea bass, Dicentrarchus labrax. Developmental & Comparative Immunology 81, 204-209.

Hua K., Cobcroft J.M., Cole A., Condon K., Jerry D. R., Mangott A., Praeger C., Vucko M.J., Zeng C., Zenger K., Strugnell J.M. (2019). The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. One Earth 1(3), 316-329.

Jabir M.A.R., Jabir S.A.R., Vikineswary S. (2012). Nutritive potential and utilization of super worm (Zophobas morio) meal in the diet of Nile tilapia (Oreochromis niloticus) juvenile. African Journal of Biotechnology 11(24), 6592-6598.

Magalhães R., Sánchez-López A., Leal R.S., Martínez-Llorens S., Oliva-Teles A., Peres, H. (2017). Black soldier fly (Hermetia illucens) pre-pupae meal as a fish meal replacement in diets for European seabass (Dicentrarchus labrax). Aquaculture 476, 79-85.

Medina M., Jayachandran K., Bhat M.G., Deoraj A. (2016). Assessing plant growth, water quality and economic effects from application of a plant-based aquafeed in a recirculating aquaponic system. Aquaculture international 24(1), 415-427.

Nebel C., Romestand B., Nègre-Sadargues G., Grousset E., Aujoulat F., Bacal J., Charmantier G. (2005). Differential freshwater adaptation in juvenile sea-bass Dicentrarchus labrax: involvement of gills and urinary system. Journal of Experimental Biology 208(20), 3859-3871

Nogales-Mérida S., Gobbi P., Józefiak D., Mazurkiewicz J., Dudek K., Rawski M., Kierończyk B., Józefiak A. (2019). Insect meals in fish nutrition. Reviews in Aquaculture 11(4), 1080-1103.

Nozzi V., Parisi G., Di Crescenzo D., Giordano M., Carnevali O. (2016). Evaluation of Dicentrarchus labrax meats and the vegetable quality of Beta vulgaris var. cicla farmed in freshwater and saltwater aquaponic systems. Water 8(10), 423.

Nozzi V., Graber A., Schmautz Z., Mathis A., Junge, R. (2018). Nutrient management in aquaponics: comparison of three approaches for cultivating lettuce, mint and mushroom herb. Agronomy 8(3), 27.

Piccolo G., Iaconisi V., Marono S., Gasco L., Loponte R., Nizza S., Bovera F., Parisi, G. (2017). Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (Sparus aurata). Animal Feed Science and Technology 226, 12-20.

Prachom N., Boonyoung S., Hassaan M.S., El-Haroun E., Davies S.J. (2021). Preliminary evaluation of Superworm (Zophobas morio) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, Lates calcarifer. Aquaculture Nutrition.

Rumbos C.I., Karapanagiotidis I. T., Mente E., Psofakis P., Athanassiou C.G. (2020). Evaluation of various commodities for the development of the yellow mealworm, Tenebrio molitor. Scientific Reports 10(1), 1-10.

Sánchez-Muros M. J., Barroso F. G., Manzano-Agugliaro F. (2014). Insect meal as renewable source of food for animal feeding: a review. Journal of Cleaner Production 65, 16-27.

Schwarz M. (2012). Soilless culture management (Vol. 24). Springer Science & Business Media. pp.208.

Stathopoulou P., Berillis P., Vlahos N., Nikouli E., Kormas K.A., Levizou E., Katsoulas N., Mente, E. (2021). Freshwater-adapted sea bass Dicentrarchus labrax feeding frequency impact in a lettuce Lactuca sativa aquaponics system. PeerJ 9, e11522.

Tyson R., Simonne E., White J., Lamb E. (2004). Reconciling water quality parameters impacting nitrification in aquaponics: the ph levels, Proceedings of The Florida State Horticultural Society 117, 79-83.

Van Broekhoven S., Oonincx D.G., Van Huis A., Van Loon, J.J. (2015). Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. Journal of Insect Physiology 73, 1-10.

Waller U., Buhmann A. K., Ernst A., Hanke V., Kulakowski A., Wecker B., Orellana J., Papenbrock J. (2015). Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquaculture international 23(6), 1473-1489.

Wang G., Peng K., Hu J., Yi C., Chen X., Wu H., Huang Y. (2019). Evaluation of defatted black soldier fly (*Hermetia illucens L.*) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (*Lateolabrax japonicus*) diets. Aquaculture 507, 144-154.

HETEROTROPHIC BATCH CULTIVATION OF *Chlorella sorokiniana* AND *Chlorella kessleri* USING GLYCEROL: BIOMASS & LIPID PRODUCTIVITIES AND BIOMASS CARBON YIELD COEFFICIENTS

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Abstract

The heterotrophic growth of two microalgal species namely, *Chlorella sorokiniana* (CS) and *Chlorella kessleri* (CK) was examined at five different initial carbon concentrations. The initial nitrogen concentration were held constant in order to examine the effect of the initial carbon concentration and the initial ratio of carbon to nitrogen concentrations (C_o/N_o) on the carbon uptake rate, the biomass, lipid and protein productivities. Glycerol was used as the sole source of organic carbon and the potential of using glycerol in heterotrophic cultivations for biodiesel production is discussed. Co/No ratios varied from 9.4 to 146.4 for CS and from 7 to 130.6 for CK. The cultivations were carried out simultaneously in five 5L cylindrical glass bioreactors. It was found that as the initial ratio of Co/No increases, the carbon uptake rate (g C/(l-d)) initially increases sharply but, above about 60 and for both species it becomes nearly independent of the Co/No ratio. The protein content decreases, while the lipid content increases as the Co/No ratio is increased. Lipid content increased up to 42.9% and 32.7% for CS and CK respectively. In general, biomass and lipid productivities were found to be higher for CS compared to CK. From the biomass produced, the organic carbon consumed and the estimated carbon content of the biomass, the carbon biomass yield coefficients were calculated. They were found to range from 0.18 to 0.34, indicating that the majority of carbon is not utilized for biomass production.

Keywords: *C. sorokiniana, C. kessleri, biomass-lipid productivities, carbon biomass yield coefficient*

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1. Introduction

Microalgae are unicellular photosynthetic organisms that use light and carbon dioxide, with higher photosynthetic efficiency than plants, for the production of biomass. Some microalgae species can also grow and multiply heterotrophically in the absence of light if an organic carbon source becomes available (Mata *et al*. 2010). The main advantage of heterotrophic growth is higher biomass growth rates and biomass production because, unlike autotrophic growth, heterotrophic growth is not limited by light transmission through the growth medium. Another advantage of heterotrophic growth is the potential of achieving higher lipid content and, as a result, higher lipid productivities. This is needed if microalgal cultivation is to be used for biodiesel production. Disadvantages of heterotrophic growth are the susceptibility to contamination which requires that all parts of the bioreactors as well as all growth media must be carefully sterilized in order to minimize the risk of contamination and the cost of organic carbon which must be provided to the growth medium (Huang *et al*. 2010).

A number of review papers focus on the heterotrophic growth of several microalgal species and the trend is that heterotrophic growth enhances both the biomass and lipid productivity (Bumbak *et al.* 2011; Perez-Garcia & Bashan 2015). Different sources of carbon have been used for microalgae growth, such as glucose, sucrose, fructose, mannose, acetate, lactose or galactose (Perez-Garcia *et al*. 2011), but studies using glycerol are limited. In the heterotrophic cultivation of *Chlorella sorokiniana*, the growth rate was higher using glucose and sodium acetate as the carbon source compared to cultivation with fructose (Qiao &Wang 2009). In the cultivation of *Chlorella vulgaris*, optimal cell growth and lipid productivity were attained using glucose at 1% (w/v). Growth of *C. vulgaris* on glycerol had a similar dose effect as those from glucose (Liang *et al.* 2009). Ogbonna *et al.* (1998) report that the heterotrophic cultivation of *Euglena gracilis* was enhanced when cultivated with glucose in comparison with cultivation with ethanol.

In *Chlorella protothecoides*, corn powder hydrolysate instead of glucose was used as organic carbon source in heterotrophic culture medium in fermenters in order to increase the biomass and reduce the cost of cultivation (Xu *et al.* 2006). O'Grady & Morgan (2011) studied the heterotrophic growth of *Chlorella protothecoides* with different carbon sources and specifically glycerol, glucose and a glucose/glycerol mixture. They found that the specific growth rate and lipid yields when using glycerol and a glucose/glycerol mixture were higher in comparison when using only glucose. Similarly, Kong *et al*. (2013) found that the growth rate of *C.*

vulgaris as well as the biomass production of the species was enhanced when cultivated with a mixture of glycerol and glucose.

In the present study, the heterotrophic growth of two *Chlorella* species namely *Chlorella sorokiniana* (CS) *and Chlorella kessleri* (CK) was investigated using glycerol as the only carbon source. The purpose of these experiments was: a) to determine how the carbon concentration affects the growth rate, the carbon uptake rate and the biomass productivities, b) to determine the lipid and protein productivities, c) to calculate the biomass carbon yield coefficients and d) to compare the two microalgae species.

2. Materials and Methods

C. sorokiniana (SAG strain 211-31) and *C. kessleri* (SAG strain 211-11h) were obtained from the University of Goettingen, Germany (EPSAG). They were cultivated (SAG, 2007) in 5 L glass circular flasks that were filled up to 4.5 L. Air was continuously passed through the solution at 300 L/h through a 2 mm glass tubing positioned at the tip of a magnetic bar and the air bubbles were dispersed with a magnetic bar at the bottom of the glass flasks at a rotational speed of 500 rpm. Temperature, as well as the pH were kept constant to 30 ±1 ^oC and 7 ± 0.3 respectively. Crude glycerol was obtained from a local biodiesel manufacturing plant. Its composition is approximately 86% glycerine, 0.5% methanol, 4% free fatty acids and 7.5% H_2O. From this analysis, the initial carbon content was calculated so that the approximate Co/No ratios in each bioreactor would be estimated. After the growth media (from inorganic salts) in each bioreactor were prepared, the initial carbon content in each bioreactor was measured analytically and the ratio of the initial carbon and nitrogen concentrations (Co/No) was determined.

The bioreactors, the glass tubing and the culture medium were sterilized before use. Analyses of samples were according to AOAC (AOAC, 1995) methods. Total nitrogen content in samples was measured with digestion using the Kjeldhal method (Jones 1998). Total protein content of samples was calculated using a conversion factor of 6.25. For the determination of organic carbon, the method of Walkley-Black was used. The total lipid content was determined with extraction using co-solvents of n-hexane/isopropanol in the microalgal biomass according with the method of Bian *et al.* (2018). Biomass yields were calculated by centrifugation of the culture media at the end of the experiments and drying the algae at 45^{o}C. The biomass carbon yield coefficient was defined as the ratio of the amount in g of organic carbon contained in the biomass of the algae to the amount of in g of organic carbon consumed by the algae during the cultivation. Therefore, it was defined by the following equation: $Y_{Cb/\Delta C} = C_b/\Delta C$ (1)

Where, $Y_{Cb/\Delta C}$ is the biomass carbon yield coefficient, C_b is the amount in g of elemental carbon contained in the biomass and ΔC is the total amount in g of organic carbon consumed in the cultivation. Therefore, C_b is given by:

$C_b = M_b f_C$ (2)

Where M_b is the biomass produced during the cultivation and f_C is the elemental carbon fraction of the biomass.

3. Results and Discussion

Figures 3.1 and 3.2 show the experimental data for the decrease in organic carbon concentration (utilization of carbon by the algal cells) in the media as a function of cultivation time for the microalgal species *C. sorokiniana* and *C. kessleri* respectively. It is noted that carbon decreases with time, but at a different rate, in all growth media. This is because the carbon in organic form found in the growth media is utilized by the microalgal cells to make proteins, lipids and carbohydrates (macronutrients), as well as other organic compounds such as secondary metabolites and finally to divide. The initial concentrations of nitrogen are equal in all five bioreactors. It is also noted that the time needed for the organic carbon to be fully utilized increases with increasing Co/No ratio. It is also noted for both species, that while the lag phase is very short, in the order of one day or less at low initial carbon concentrations, it increases substantially, up to 3 days, at high carbon concentrations. This may be due to the time the cells need in order to adjust to such a high carbon environment. Also, oxygen solubility may be affected by glycerin concentration in the growth medium.

Figure 3.3 shows a comparison of the rate of carbon (C) utilization (uptake) as a function of the ratio Co/No for the two microalgal species. The rate of C uptake was determined from the average slope of the curves during the exponential phase growth which, is characterized by the sharp drop in organic carbon concentration in the curves in Fig. 3.1 and 3.2. As it is noted in Fig. 3.3, the rate of C utilization or bio-absorption, for both species increases with increasing Co/No ratio. The rate of C uptake varies by almost a factor of 5 as the Co/No ratio is increased from below 10 to above 60. When Co is varied and No is held constant and at low carbon concentrations, the combination of excess N and low C concentrations lead to low rates of C uptake but as the C concentration is increased and for a ratio Co/No above about 60 the rate of carbon uptake becomes relatively constant for both species. This is probably due to the fact that in the exponential growth phase the microalgal cells utilize both C and nitrogen for their growth so, at higher Co/No ratios the rate of C uptake becomes progressively independent of the C concentration as nitrogen becomes the limiting nutrient during the exponential growth phase. Average C content in microalgal lipids is 75% while in protein and carbohydrates it is 53% and 44% respectively.

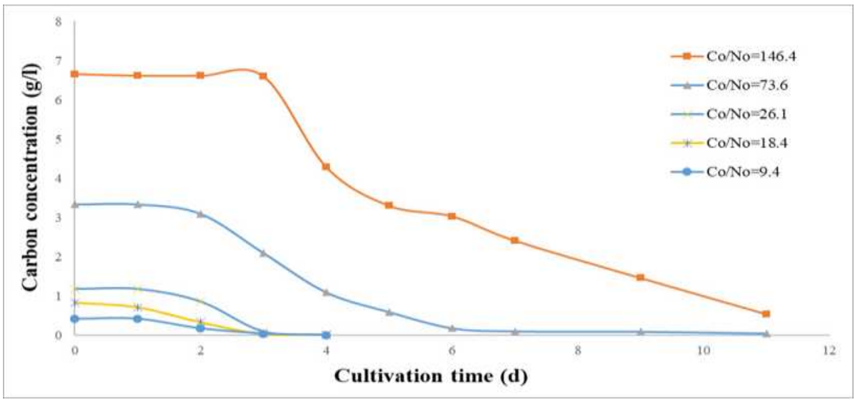

Fig. 3.1 Utilization of organic carbon as a function of cultivation time during heterotrophic growth of *C. sorokiniana* and for the initial C_o/N_o ratios as shown. Lines are drawn between data points for clarity.

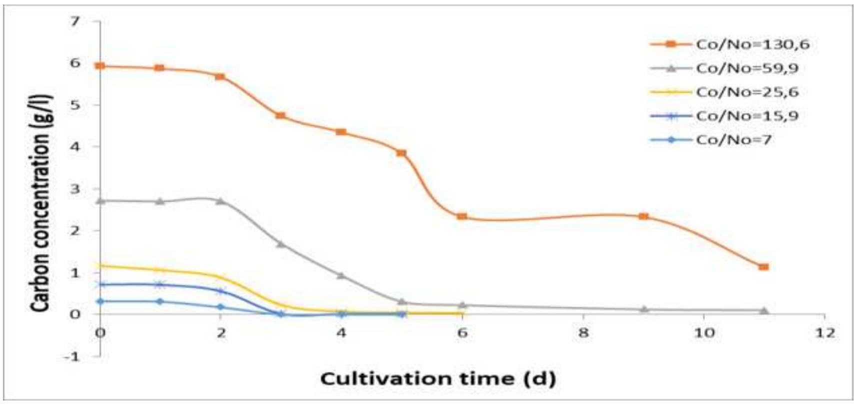

Fig. 3.2 Utilization of organic carbon as a function of cultivation time during heterotrophic growth of *C. kessleri* and for the initial Co/No ratios as shown. Lines are drawn between data points for clarity.

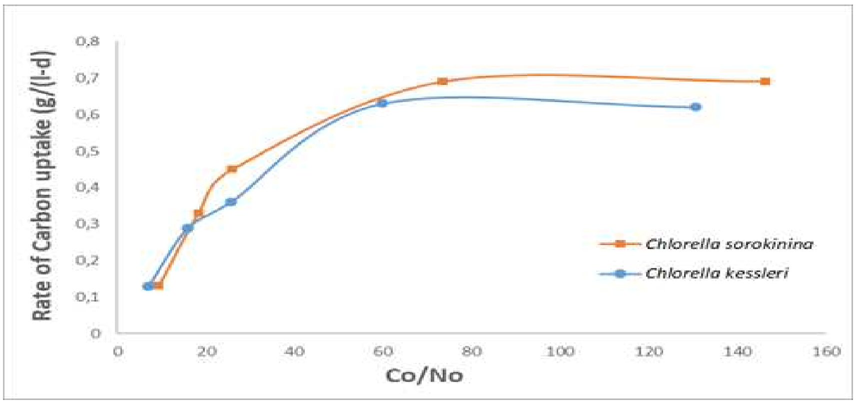

Fig. 3.3 Comparison of carbon uptake rates of two microalgae species.

It is noted from Tables 3.1 and 3.2 that as the initial carbon content increases, keeping the initial nitrogen content the same, the protein content decreases while the lipid content increases. As both nitrogen (N) and carbon (C) are needed for biomass growth, initially both C and N are used for protein, carbohydrate and lipid synthesis but at high carbon media the microalgal cells shift their metabolism to lipid synthesis. Also, as the ratio of Co/No increases, the biomass obtained per liter of growth medium at the end of each cultivation increases. However, biomass productivity at the very high initial C concentrations (Co/No>90-100) decreases slightly and this, as noted from Fig. 3.1 and 3.2, is due to the much longer cultivation times needed to assimilate the excess carbon. Biomass productivities range from a low of 0.08 g/(l-d) at Co/No=9.4 to a high of 0.27 g/(l-d) at Co/No=73.6 for *C. sorokiniana* and from a low of 0.08 at Co/No=7 to a high of 0.21 at Co/No=45.4 for *C. kessleri*. Protein and lipid productivities are obtained by multiplying the biomass productivities by the respective biomass content in protein and lipids. Therefore, the respective protein and lipid productivities range from a low of 0.013 g/(l-d) to a high of 0.097 g/(l-d) for *C. sorokiniana* and from a low of 0.09 g/(l-d) to a high of 0.059 g/(l-d) for *C. kessleri*. Biomass as well as lipid productivities are generally higher for *C. sorokiniana* compared to *C. kessleri*. It thus appears, that when the two species are compared, from a lipid productivity point of view, that *C. sorokiniana* is a more promising species for cultivation in order to produce bio-oil for biodiesel production.

Table 3.1 Biomass (P_b), protein (Pp) & lipid (P_l) productivities for *C. sorokiniana*

Co (g/L)	0.43	0.84	1.19	3.34	6.65
No (mg/L)	45.4	45.4	45.4	45.4	45.4
Co/No	9.4	18.4	26.1	73.6	146.4
C_p (%)	45.1	37.2	28.6	18.3	12.1
C_l (%)	16.8	23.7	28.2	36.1	42.9
P_b (g/(l-d))	0.08	0.12	0.25	0.27	0.22
P_p (g/(l-d))	0.036	0.045	0.072	0.049	0.027
P_l (g/(l-d))	0.013	0.028	0.071	0.097	0.094

Table 3.2 Biomass (Pb), protein (Pp) & lipid (Pl) productivities for *C. kessleri*

Co (g/L)	0.32	0.72	1.16	2.72	5.93
No (mg/L)	45.4	45.4	45.4	45.4	45.4
Co/No	7	15.9	25.6	59.9	130.6
C_p (%)	47.5	41.5	34.3	21.9	15.1
C_l (%)	10.9	18.5	24.1	28.1	32.7
P_b (g/(l-d))	0.08	0.12	0.16	0.21	0.17
P_p (g/(l-d))	0.038	0.050	0.055	0.046	0.026
P_l (g/(l-d))	0.009	0.022	0.039	0.059	0.056

Table 3.3 Table of carbon biomass yield coefficient

		Chlorella sorokiniana			
Co/No	9.4	18.4	26.1	73.6	146.4
f_C	0.50	0.51	0.51	0.53	0.54
$Y_{Cb/\Delta C}$	0.33	0.29	0.30	0.29	0.18
		Chlorella kessleri			
Co/No	7	15.9	25.6	59.9	130.6
f_C	0.48	0.49	0.50	0.50	0.51
$Y_{Cb/\Delta C}$	0.34	0.29	0.27	0.26	0.19

The total biochemical reaction occurring during the cultivation of the two species of *Chlorella* may be viewed as follows: C + MN + TE --------------→ $M_b + CO_2$ + ECC, where, C is the carbon consumed, MN and TE are the macronutrients and trace elements (micronutrients), M_b is the biomass produced and ECC are any extracellular compounds, organic or inorganic, produced by the microalgae. Therefore, as we note from Table 3.3 that only from 18% to about 35% of the organic carbon consumed ends up in the biomass of the microalgae, the organic extracellular compound produced, unless it is volatile, it should be measured in the daily measurements of total dissolved organic carbon and thus accounted for. It is also noted that excess organic carbon leads to a reduction in the carbon biomass yield coefficient. Experimental work will be conducted in order to detect volatile organic compounds but, it is believed that most of the organic carbon utilized by the algae is metabolized to produce carbon dioxide and biomass. The use of glycerol for the heterotrophic microalgal cultivation may not be attractive because glycerol prices have skyrocketed in the past 2 years because of its use in biogas producing plants and for medicinal and cosmetic applications. In biogas 52-55% of the glycerol carbon is converted to methane. In this study most of the carbon seems to be lost to carbon dioxide.

4. Conclusions

The heterotrophic growth of *C. sorokiniana* (CS) and *C. kessleri* (CK) using glycerol as the sole source of carbon proceeds very quickly as glycerol is readily absorbed and utilized by the two species. In general, as the initial ratio of Co/No increases, the carbon uptake rate initially increases sharply but, above about 60 and for both species it becomes nearly independent of the Co/No ratio. The protein content decreases, while the lipid content increases as the Co/No ratio is increased. Lipid content increases up to 42.9% and 32.7% for CS and CK respectively. In general biomass and lipid productivities were found to be higher for CS compared to CK. The carbon biomass yield coefficients for both species were found to range from 0.18 to 0.34 indicating that the majority of carbon is not utilized for biomass production. This is a major drawback for the use of glycerol for the heterotrophic microalgal cultivation since glycerol prices have skyrocketed in the past 2 years. In this study, most of the carbon seems to be lost to volatile carbon dioxide. More studies are need to determine other modes of cultivation such as semi-batch cultivation and/or mixotrophic cultivation and investigate other cultivation parameters and other microalgal species, including genetically modified species, in order to increase the carbon biomass yield coefficients. Microalgal biomass should be regarded for its wholesome value not just for its bio-oil, as the remaining biomass after bio-oil extraction, can be used for other purposes, for example as an animal feed supplement since it is a rich source of good quality protein.

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References

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis of the Ass. of Off. Analyt. Chemists Intern., 16th ed.; AOAC: Arlington, VA, USA.

Bian X., Jin W., Gu Q., Zhou X., Xi Y., Tu R., Han S., Xie G., Gao S., Wang Q. (2018). Subcritical n-hexane/isopropanol extraction of lipid from wet microalgal pastes of Scenedesmus obliquus. World Journal of Microbiology and Biotechnology 34(3), 39.

Bumbak F., Cook S., Zachleder V., Hauser S., Kovar K. (2011). Best practices in heterotrophic high-cell-density microalgal processes: achievements, potential and possible limitations. Applied Microbiology and Biotechnology 91(1), 31-46.

Huang G., Chen F., Wei D., Zhang X., Chen G. (2010). Biodiesel production by microalgal biotechnology. Applied Energy 87, 38-46.

Jones Jr, L.J.B. (1998). Plant nutrition manual CRC Press. New York.

Kong W.B., Yang H., Cao Y.T., Song H., Hua S.F., Xia C.G. (2013). Effect of Glycerol and Glucose on the Enhancement of Biomass, Lipid and Soluble Carbohydrate Production by Chlorella vulgaris in Mixotrophic Culture. Food Technology and Biotechnology 51(1), 62–69.

Liang Y., Sarkany N., Cui Y. (2009). Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnology Letters 31(7), 1043-1049.

Mata T.M., Martins A.A., Caetano N.S. (2010). Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews 14(1), 217-232.

Ogbonna J.C., Tomiyamal S., Tanaka H. (1998). Heterotrophic cultivation of Euglena gracilis Z for efficient production of α-tocopherol. Journal of Applied Phycology 10(1), 67-74.

O'Grady J., Morgan J.A. (2011). Heterotrophic growth and lipid production of Chlorella protothecoides on glycerol. Bioprocess and Biosystems Engineering 34(1), 121-125.

Qiao H., Wang G. (2009). Effect of carbon source on growth and lipid accumulation in Chlorella sorokiniana GXNN01. Chinese Journal of Oceanology and Limnology 27(4), 762-768.

Perez-Garcia O., Escalante F. M., De-Bashan L. E., Bashan Y. (2011). Heterotrophic cultures of microalgae: metabolism and potential products. Water Research 45(1), 11-36.

Perez-Garcia O., Bashan Y. (2015). Microalgal heterotrophic and mixotrophic culturing for bio-refining: from metabolic routes to techno-economics. Algal Biorefineries 61-131.

SAG (2007). Sammlung von Algenkulturen der Universität Göttingen. Culture Collection of Algae, https://www.uni-goettingen.de/Abteilung Experimentelle Phykologie und Sammlung von Algenkulturen (EPSAG), Universität Göttingen, Deutschland. Available at: http://epsag.uni-goettingen.de.

Xu H., Miao X., Wu Q. (2006). High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. Journal of Biotechnology 126(4), 499-507.

EFFECT OF MICRONUTRIENTS ON THE GROWTH RATES AND BIOMASS PRODUCTIVITIES OF *Microchloropsis gaditana*

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Abstract
The effect of the micronutrients molybdenum (Mo), cobalt (Co) and iron (Fe) on the autotrophic growth of *Microchloropsis gaditana* on the specific growth coefficient and the biomass productivity was studied. The experiments were performed in 5 L bioreactors in a greenhouse environment under the same conditions of temperature, light intensity, orientation, aeration and growth medium composition. It was found that Co, even at relatively medium concentrations, strongly inhibited the growth. Above concentrations of about 1 mg/l Co were toxic. Although Co is an essential micronutrient, it appears it favored biomass productivity at very low concentrations. On the other hand, Fe and especially Mo, even at relatively high concentrations, did not affect the growth rate of *M. gaditana*. Maximum productivities were 117, 115 and 120 mg/L/d for Mo, Co and Fe respectively at concentrations equal to 238.2, 0.02 and 10.2 mg/l respectively. Mo concentrations below about 80 mg/l did not affect the biomass productivity while, at a relatively low concentration of Fe equal to 0.41 mg/l, the productivity was reduced by 45% compared to the maximum productivity. The lag phase, about 5 days, was not affected by the concentrations of micronutrients Mo and Fe and, in all cultivations; the stationary phase was achieved in about 20 days. Maximum specific growth rate coefficients in the exponential phase were equal to 0.23, 0.23 and 0.20 d^{-1} for Mo, Co and Fe respectively.
Keywords: *Microchloropsis sp. molybdenum, cobalt, iron, biomass productivities*
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Introduction

It is well accepted that microalgae have excellent nutritional properties, as they contain high amounts of proteins, lipids, antioxidants, vitamins (such as A, B1, B2, B6, B12, C, E, biotin, folic acid), minerals (phosphorus, zinc, iron, calcium, selenium, magnesium) and are rich in pigments such as chlorophylls, carotenoids and phenols (Lubian *et al*. 2000). Some species of microalgae are rich sources of lipids with significant amounts of DHA, EPA and arachidonic acid (AA) that are essential in the diet of fish. Protein content in microalgae varies depending on the species, but also on the growing conditions. The same applies to the content in lipids. Significant amounts of polyunsaturated fatty acids (PUFAs), which are essential in the diet of fish, are also found in the biomass of microalgae (Huerlimann *et al*. 2010). Trace elements are minerals that are present in microalgal cells in extremely small amounts but, are a key component of their physiology. Iron (Fe), molybdenum (Mo), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu) and nickel (Ni) are the seven most important trace elements required by algae for their various metabolic functions (Bruland *et al*. 1991).

Iron is the most basic micronutrient as it is a component of key enzymes such as peroxidase, nitrate reductase, nitrogenase and catalase (Marchetti & Maldonado 2016). In addition, it acts as a redox catalyst in photosynthesis, contributes to nitrogen assimilation and mediates electron transfer reactions in photosynthetic organisms. Molybdenum also plays an important role in the metabolic processes of microalgae (Carvalho *et al*. 2006) but, not much work has been done on its effect on microalgal growth. Cobalt is an element which in high concentrations is toxic to microalgae. The toxicity limit varies depending on the species. In the right amounts, however, it has been shown to cause a greater accumulation of fatty acids. In this work the effect of three micronutrients, namely molybdenum (Mo), cobalt (Co) and iron (Fe) on the autotrophic cultivation of the microalgae *Michrochloropsis gaditana* was studied, specifically on the biomass growth rate and biomass productivity. The experiments were performed in a greenhouse under the same conditions of temperature, light intensity, orientation and aeration each time. Each time all the components of the medium were held the same and to study the influence of each micronutrient, only one parameter, its concentration in the growth medium, was changed while all the others were held constant.

Materials and Methods

The microalgae species *M. gaditana* (SAG strain number 2.99) was obtained from the University of Göttingen and was cultivated in Brackish Water Medium (Sammlung von Algenkulturen der Universität Göttingen (SAG), Göttingen, Germany) (= 1/2 SWES) (SAG, 2007). In each experiment the culture medium was inoculated with a standard quantity, 50 ml of microalgae inoculum. The pH was kept constant at 8.5 ± 0.3 by the addition of

HCl or NaOH solution. Air, in the form of bubbles, was introduced into the culture medium through perforated tubes placed at the bottom of each reactor. The air supply was the same in all reactors and was carried out at a rate of 300 L h^{-1} corresponding to 0.12 L CO_2 h^{-1} or 0.05 mole CO_2 h^{-1}. The experiments were performed in the greenhouse of the University of Thessaly under the same conditions of temperature, light intensity, orientation and ventilation each time. Each time all the components of the medium were the same. In order to study the influence of various micronutrients, the concentration of only one micronutrient was changed while all the other parameters such as temperature, intensity and duration of illumination, pH, macronutrients and micronutrients were constant.

Cylindrical bioreactors with a capacity of 5 L each were used for culture. The microalgae concentration was determined daily using optical density measurements at 655 nm with the use of a spectroscopy UV/Vis instrument. Three aliquots were collected daily from each culture and all measures were carried out in triplicate. At the end of each experimental period, the total production of each culture was measured (in g L^{-1}) after harvesting the biomass using centrifugation at 4000 rpm for 10 min and drying of the biomass at 40 ºC in an air circulating oven until a constant weight was attained. The specific growth rate in the exponential growth phase (μ_{exp}, which is the slope of the growth rate curve in the exponential phase) was calculated according to the relation:

$\mu_{exp} = \frac{In(\frac{\alpha_2}{\alpha_1}}{(t_2 - t_1)}$ (2.1), where, α_1 and α_2 are the absorbance readings at the beginning and the end of exponential growth phase, at time t_1 and t_2, respectively.

Results and Discussion

Figure 3.1 illustrates the absorbance of the growth medium (with respect to the blank) versus the cultivation time grown with different concentrations of Mo while, Figures 3.2 and 3.3 show the corresponding absorbances of the growth medium for *M. gaditana* grown with different concentrations of Co (Figure 3.2) and Fe (Figure 3.3). Figure 3.4 show the specific growth coefficient in the exponential phase, μ_{exp}, versus the concentrations of Mo, Co and Fe, derived from the data of Figures 3.1, 3.2 and 3.3, using equation 2.1. From Figures 3.1, 3.2 and 3.3, in the wide range of concentration values studied, 595 times for Mo, 600 times for Co and about 50 for Fe, Co seems to have the more pronounced effect on the growth of *M. gaditana*. It appears to be a strong inhibitor of its growth even at relatively very low concentrations, about 1 mg/l or lower. At 1 mg/l concentration of Co, algal growth is basically stalled. It should be mentioned that the concentration of 0.20 mg/l is the concentration of Co used in the standard growth medium (SAG 2007). This is evident from Figure 3.4 where the specific growth rate is basically zero (0.04 d^{-1}) for a Co concentration of 1 mg/l. However, at smaller concentrations it is reported to lead to an increase in lipid content. In *Chlorella vulgaris* and *Euglena oleoabundans* at higher cobalt concentrations the lipid content of the biomass increased by 25% and 63% respectively while in *Dunaliella tertiolecta* at low Co concentrations the fats increased by 25% (Battah *et al*. 2014; Chen *et al*. 2011).

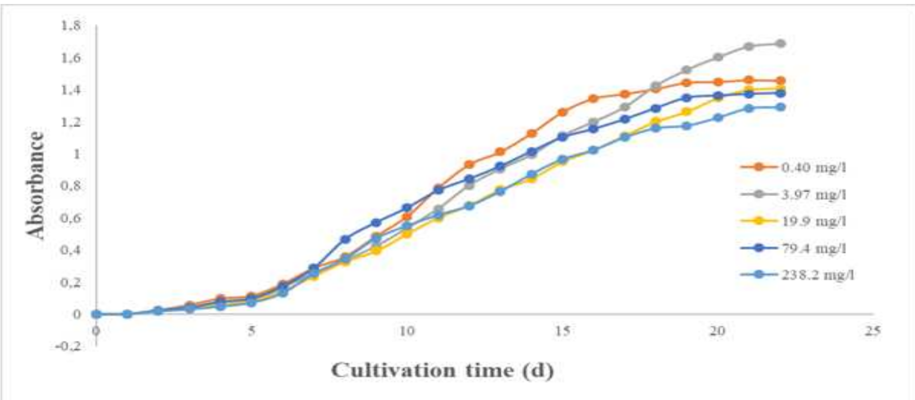

Figure 3.1 The absorbance of the growth medium (with respect to the blank) versus the cultivation time of *M. gaditana* for the concentrations of molybdenum as shown.

Mo, although its concentration was varied by nearly 595 times, does not seems to affect the growth rate. The specific growth rate in the exponential phase (μ_{exp}) was maximum at 3.97 mg/l Mo concentration, which is the concentration used in the standard Brackish Medium. However, even at a very high Mo concentration, up to 238.2 mg/l, the μ_{exp} decreases from a maximum value of 0.23 d^{-1} to 0.20 d^{-1} (Figure 3.4).

In literature, the NC-MKM strain of *Chlorella* sp. showed a significant increase in lipid content with the addition of Mo (70.3%) (Mandal *et al*. 2019). Also, in another study the addition of Mo was found to increase the lipid content of *C. vulgaris* but had no effect on *C. sorokiniana* (Ghafari *et al*. 2016). In contrast to *C. vulgaris* the species *Dunaliella tertiolecta* showed an increase in lipid content with Mo deprivation (Chen *et al*. 2011).

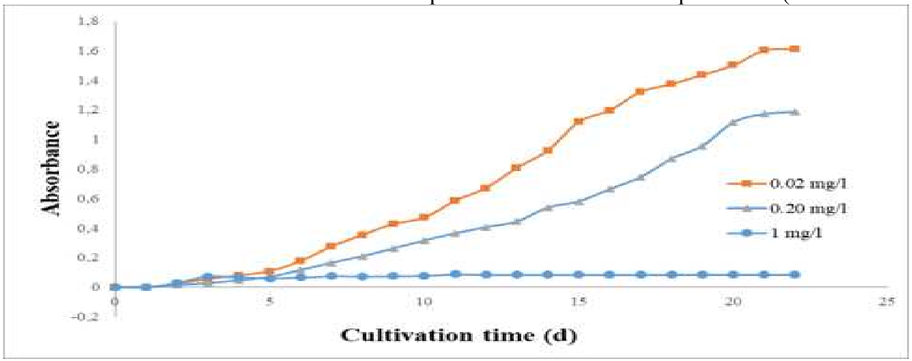

Figure 3.2 The absorbance of the growth medium (with respect to the blank) versus the cultivation time of *M. gaditana* for the concentrations of cobalt as shown.

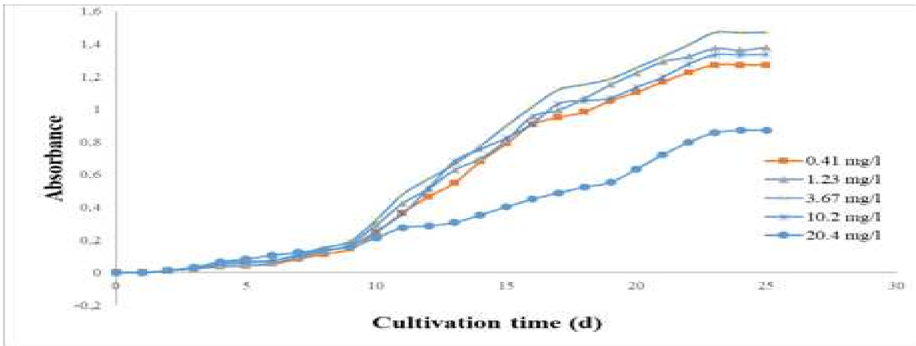

Figure 3.3 The absorbance of the growth medium (with respect to the blank) versus the cultivation time of *M. gaditana* for the concentrations of iron as shown.

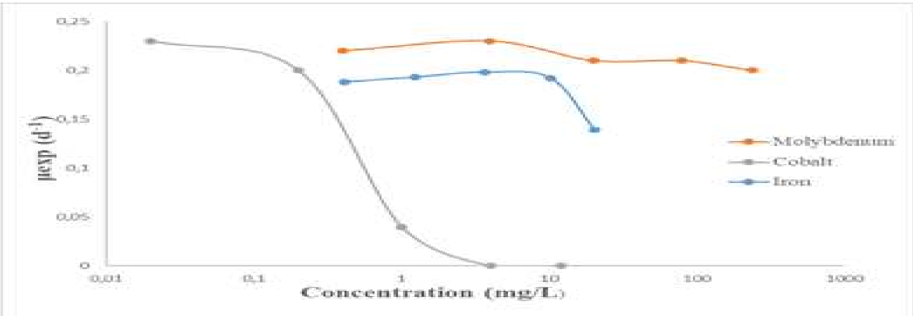

Figure 3.4 The specific growth coefficient in the exponential phase, μ_{exp}, versus the concentrations of molybdenum, cobalt and iron during the growth of *M. gaditana*.

Intermediate concentrations of Fe (Fig. 3.3), namely 20.4 mg/l i.e., about 16.6 times higher from the standard concentration of 1.23 mg/l, seem also to inhibit the growth rate. The μ_{exp} is nearly constant for Fe concentrations from 0.41 mg/l to 10.2 mg/l but, it drops to 0.14 d^{-1} for a Fe concentration of 20.4 mg/l. This reduction indicates that Fe concentration above about 10 mg/l begin to inhibit growth. Most studies in literature report that in the genus *Chlorella* the addition of Fe leads to an increase in biomass without affecting the lipid content. Specifically, in *C. vulgaris* after the addition of $FeCl_3$ in the exponential phase, its biomass increased while the lipid content remained stable, while Fe restriction increased lipid accumulation (Liu *et al*. 2008). However, no increase in lipid content was observed in *Chlorella* sp. under Fe restriction (Praveenkumar *et al*. 2012). An increase in biomass but not in the lipid content, after the addition of excess Fe to *C. vulgaris* has been observed according to Concas *et al*. (2021). The addition of Fe to the genus *Nannochloropsis* seems to have a different effect as it inhibits the growth of biomass. Savvidou *et al.* (2020) studied the effect of Fe on *N. oceanica*.

They found that biomass and lipid content were reduced when excess $FeCl_3$ was added to the growth medium but increased the % content of PUFAs and more specifically the EPA. Similar results were reported by Sabzi *et al.* (2018) where the highest value of EPA was observed for a Fe concentration equal to 0.63 mg/l, substantially smaller used in this study.

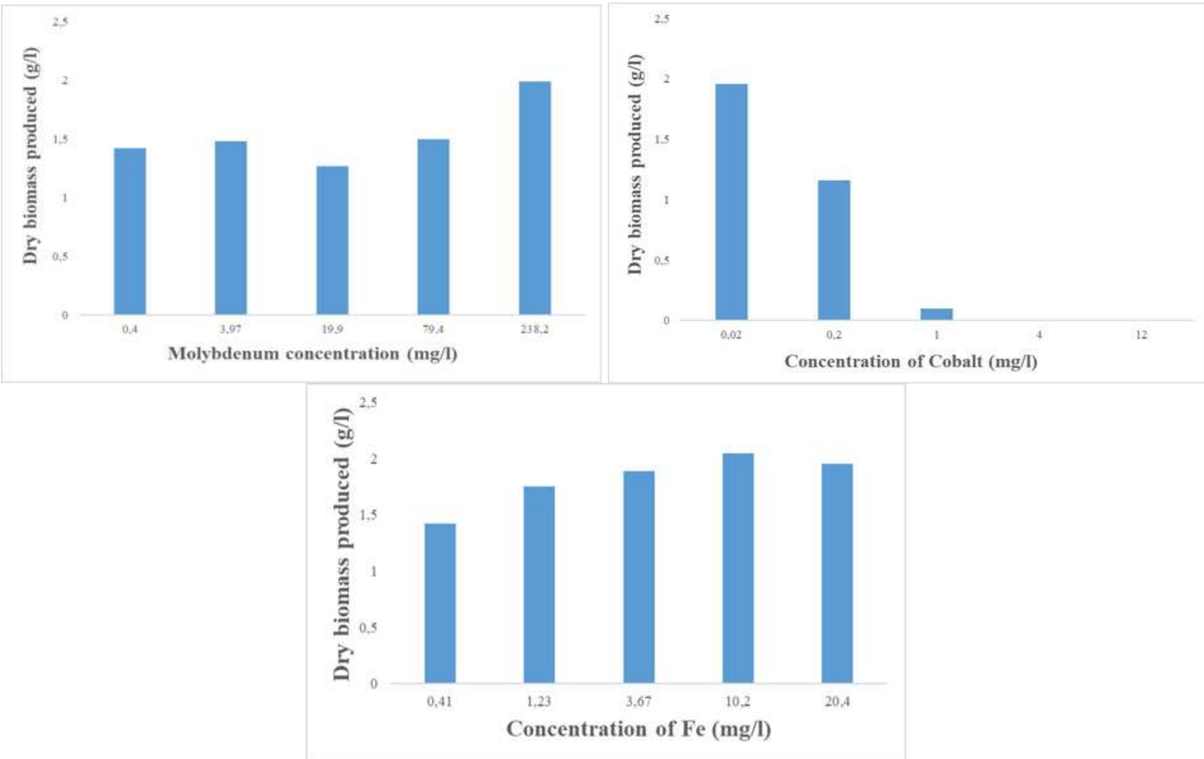

Figure 3.5a, 3.5b, 3.5c. The biomass of *M. gaditana* produced for the various concentrations of molybdenum, cobalt, and iron respectively.

Figures 3.5a, b and c show the biomass of *M. gaditana* produced (on a dry basis) in g/L for the various concentrations of Mo, Co and Fe respectively. Although the μ_{exp} is measured at a Mo concentration equal to 3.97 mg/l, biomass production is highest at the high Mo concentration of 238.2 mg/l. From Fig. 3.2, if the lag phase of about 4-5 days is excluded from the cultivation time, biomass productivities of *M. gaditana* range from a low of about 75 mg/(l-d) to a high of 117 mg/(l-d). On the other hand, the addition of excess Co leads to a stall in microalgal growth. It is interesting to note (Fig. 3.5b) that although the concentration of Co in the standard growth Brackish Medium is 0.20 mg/l, its productivity at concentrations ten times lower, i.e. at 0.02 mg/l, increases from 68 mg/(l-d) to 115 mg/(l-d), an increase of almost 70%. With respect to Fe, biomass productivities (Figure 3.6b), for a growth period also of 17 days at higher Fe concentration from the 1.23 mg/l value used in the standard growth Brackish Medium, do not seem to be affected as the Fe concentration is increased. At Fe=1.23 mg/l the productivity is 103 mg/(l-d) and it increases to 111, 120 and 115 mg/(l-d) at higher Fe concentrations equal to 3.67, 10.2 and 20.4 mg/l respectively. A lower concentration of 0.41 mg/l decreases the biomass productivity to 83 mg/(l-d). The findings in this study, with respect to biomass productivities for the effect of Fe on the growth of *M. gaditana* are different from those reported from the study of Fe on *N. oceanica* reported by Savvidou *et al.* (2020).

Conclusions

The autotrophic cultivation of the microalgal species *M. gaditana* was affected by the concentrations of the micronutrients Mo, Co and Fe. Growth was affected more severely by adding moderate amounts, about1 mg/l, of Co. Co, at small concentrations, 0.02 mg/l led to faster kinetics of growth and higher biomass productivities compared to 0.2 mg/l concentration. On the other hand, Mo even in very high concentrations did not affect the growth kinetics and the biomass productivity. Low concentrations of Fe, equal to 0.41 mg/l, led to a reduced biomass productivity compared to the maximum productivity. The lag phase, about 5 days, was not affected by the concentrations of Mo and Fe and, in all cultivations, the stationary phase was achieved in about 20 days. More study is needed in order to determine the effect of micronutrient concentration on the lipid content of the biomass as well as on the fatty acid distribution of the lipids so that lipid productivities and especially omega-3 fatty acid productivities can be determined as these fatty acids are essential as fish oil supplements.

Acknowledgements

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References

Battah M., El-Ayoty Y., Abomohra A., El-Ghany, S., Esmael, A. (2014). Effect of Mn^{2+}, Co^{2+} and H_2O_2 on biomass and lipids of the green microalga *Chlorella vulgaris* as a potential candidate for biodiesel production. Annals of Microbiology 65(1), 155-162.

Bruland K.W., Donat, J. R., Hutchins, D.A. (1991). Interactive influences of bioactive trace metals on biological production in oceanic waters. Limnology Oceanography 36, 1555–1577.

Carvalho A.P, Pontes I., Gaspar H., Malcata F.X. (2006). Metabolic relationships between macro- and micronutrients, and the eicosapentaenoic acid and docosahexaenoic acid contents of Pavlova lutheri. Enzymes Microbiology Technology 38, 358–366.

Chen M., Tang H., Ma H., Holland T., Ng K., Salley, S. (2011). Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta. Bioresource Technology 102(2), 1649-1655.

Concas A., Steriti A., Pisu M., Cao, G. (2021). Experimental and theoretical investigation of the effects of iron on growth and lipid synthesis of microalgae in view of their use to produce biofuels. Journal of Environmental Chemical Engineering 9(4), 105349.

Ghafari M., Rashidi B., Haznedaroglu, B. (2016). Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. Biofuels 9(2), 147-156.

Huerlimann R., de Nys R., Heimann K. (2010). Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnology Bioengergy 107(2), 245–257.

Liu Z-Y, Wang G-C, Zhou B-C. (2008) Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresources Technology 99, 4717–4722.

Lubián L. M., Montero O., Moreno-Garrido I., Huertas I. E., Sobrino C., González-Del Valle M., Parés G., (2000). Nannochloropsis (Eustigmatophyceae) as source of commercially valuable pigments. Journal of Applied Phycology 12(3–5), 249–255.

Mandal M., Saikia P., Chanu N., Chaurasia, N. (2019). Modulation of lipid content and lipid profile by supplementation of iron, zinc, and molybdenum in indigenous microalgae. Environmental Science and Pollution Research 26(20), 20815-20828.

Marchetti A, Maldonado M.T. (2016). Iron. In: Borowitzka MA, Beardall J, Raven JA (eds) The physiology of microalgae. Springer, Dordrecht, 233–279.

Praveenkumar R., Shameera K., Mahalakshmi G., Akbarsha M., Thajuddin, N. (2012). Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga Chlorella sp., BUM11008: Evaluation for biodiesel production. Biomass and Bioenergy 37, 60-66.

Sabzi S., Shamsaie Mehrgan M., Rajabi Islami H., Hosseini Shekarabi, S. (2018). Changes in biochemical composition and fatty acid accumulation of Nannochloropsis oculata in response to different iron concentrations. Biofuels 12(1), 1-7.

SAG (2007). Sammlung von Algenkulturen der Universität Göttingen. Culture Collection of Algae, https://www.uni-goettingen.de/Abteilung Experimentelle Phykologie und Sammlung von Algenkulturen (EPSAG), Universität Göttingen, Deutschland. Available at: http://epsag.uni-goettingen.de.

Savvidou M., Boli E., Logothetis D., Lymperopoulou T., Ferraro A., Louli V., Mamma D., Kekos D., Magoulas K., Kolisis F. (2020). A Study on the Effect of Macro- and Micro- Nutrients on Nannochloropsis oceanica Growth, Fatty Acid Composition and Magnetic Harvesting Efficiency. Plants 9(5), 660.

ELECTROSTUNNING HAS THE POTENTIAL TO DELAY FILLET DEGRADATION POST-HARVEST IN RED SEABREAM (*Pagrus major*)

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Abstract
The objective of the study was to evaluate the effect of applying electrostunning at the harvest of red seabream (*Pagrus major)* on the fillet quality by monitoring the activities of the proteolytic enzymes that are activated post-harvest and are responsible of fillet degradation, thus determining the length of shelf life. Two different electrostunning settings were compared with the conventional harvest method at two different sea temperatures. Fish were stored in conventional flake ice and sampled at various times from harvest up to 13 days post-harvest for monitoring the activity of collagenase, calpains, cathepsin B and L. The application of electrostunning settings at 1.8V/cm and 1.6m/sec prior to slaughter elicited lower activities of all proteolytic enzymes at either sea temperature and it is expected to lead to delayed proteolytic degradation along with, standing out as a more humane slaughter method.
Keywords: *Proteases, Red seabream, novel harvest methods, electrostunning*
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Introduction

Progressive loss of fish freshness post-mortem is attributed to complex combinations of biochemical and structural changes along with muscle spoilage caused by microbial activity (Chéret *et al.* 2006). The integrity of the structural proteins in myofibrils and extracellular matrix is of great importance for the quality of the fish fillet and their proteolytic cleavage results in muscle softening and creates gaping in the myocommata (Pornrat *et al.* 2007). Proteases responsible for the deterioration of the myotomia can derive from both muscle tissue and the digestive system, provided they have not been removed prior to storage (Sriket 2014). Several proteases are responsible for cleaving muscle proteins after harvest. A key role in this process is played by proteases that hydrolyze the collagen of the connective tissue (collagenases), as well as those that proteolyze muscle fibril proteins, with cathepsins and calpains being the most studied (Ntzimani *et al.* 2021). A need for a more humane method of harvest and at the same time maintaining the quality of the fillet led to exploring new harvest methods such as electrostunning, have been highlighted both from EFSA and OIE. The current harvest method is accused to create excessive stress to harvested fish before killing them by hypothermia. Electrostunning as an alternative technique has to meet requirements related with fish welfare, food quality and operates safety.

1. Materials and Methods

Whole red seabream (*Pagrus major)* was harvested from net cages in Avramar S.A facilities and stored immediately on ice. Two different electrostunning settings were compared with the conventional harvest method (**CS**); **HC,** electric field 1.8V/cm and velocity 1.6m/sec; **LC,** electric field 1.5V/cm and velocity 1.6m/sec. Sampling was performed in the same fish farm in Astakos (Aitoloakarnania, Greece) in August 2020.

Measurements of major proteases, i.e., Calpain, Collagenase, Cathepsin B and L were performed on extracted samples taken from the fillet at slaughter (day 0) and on days 1, 2, 5, 7 and 13 *post-slaughter.* Enzymes were extracted (Lakshmanan *et al.* 2005; Chéret *et al.* 2007; Teixeira *et al.* 2013) and the activity was assayed according to the Barrett & Kirschke (1981) method with minor refinements. Protein content in enzyme extracts was quantified with the Bradford (1976) method using bovine serum albumin as standard. Activity was expressed as fluorescence units (FU) change per minute per mg protein.

2. Results and Discussion

At sampling, the average water temperature was 25ºC in August 2020. Overall, cytoplasmic calpains exhibited the highest on average activity amongst the proteolytic enzymes and an early post-mortem activation (Bao *et al.* 2020; Ntzimani *et al.* 2021). Harvest by applying HC appeared to provoke the lowest on average calpain activity whereas LC provoked the highest that peaked on day 2. Collagenase activity shared a similar activation pattern with calpain, with HC provoking the lowest collagenase activity. Xu *et al.* (2017) reported a similar pattern in collagenase activity in grass carp fillets stored in ice with an activation peak early *post-mortem*. On the contrary, in Atlantic cod fillets, collagenase activity showed a gradual increase until day nine (Hernández-Herrero *et al.* 2003).

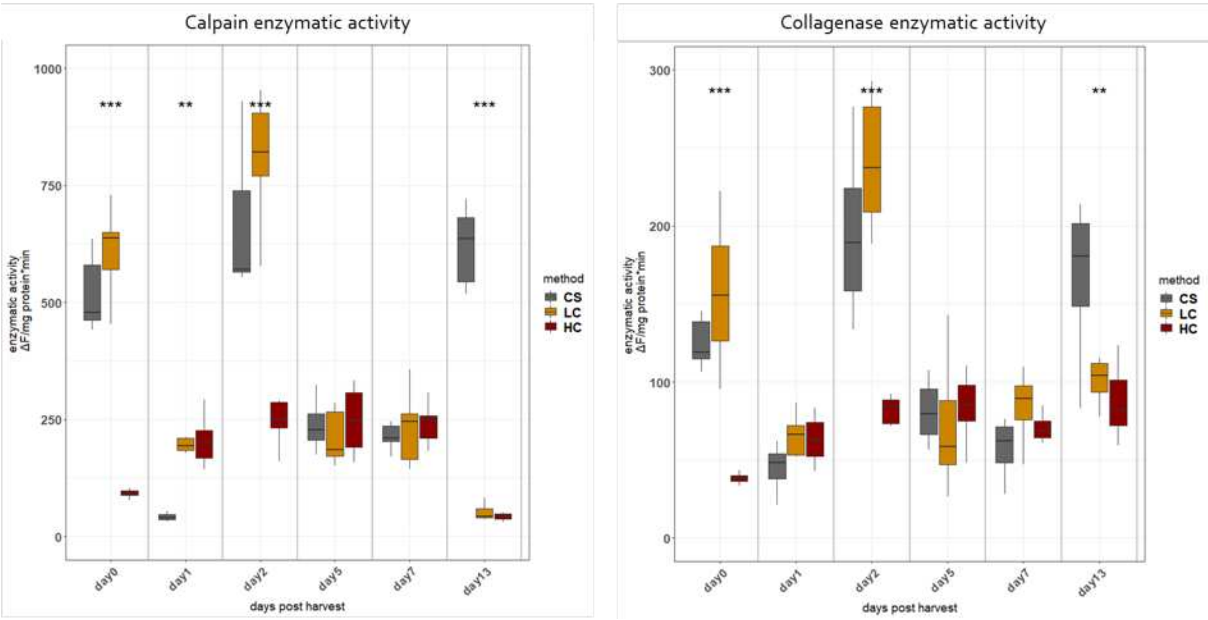

Figure 1. Enzymatic activity of Calpain and Collagenase in all harvest methods. CS (gray): Control group, LC (orange): Low current group, HC (red): High current group. Superscripts indicate statistically significant differences between treatments on each sampling day (*: p<0.05, **:p<0.01, ***:p<0.001).

Calpain and collagenase activity was significantly and positively correlated in all treatments ($0.86 < r < 0.87$). In both enzymes the highest statistically significant differences were observed on day 2 (Figure 1). A positively correlated activation of cathepsin B and L was observed regardless harvest method ($0.72 < r < 0.94$), with the lowest activities for both enzymes observed in HC group. The highest activities were observed again on Day 2 (Figure 2). Statistically significant differences were observed on both enzymes on day 2. Moreover, a correlation of these enzymes with stress and pH levels was previously described (Ohmori *et al.* 1992; Sentandreu *et al.* 2002).

Specifically, a study with Atlantic salmon showed a higher cathepsin B activity with pre-slaughter crowding stress (Bahuaud *et al.* 2010). Papaharisis *et al.* (2019) using a similar electrostunning method in red seabream found statistically significant lower mean cortisol levels in electrostunning group. Thus, we can assume that electrostunning has an effect on pre-slaughter stress and pH levels having an indirect impact on the activation of proteolytic enzymes.

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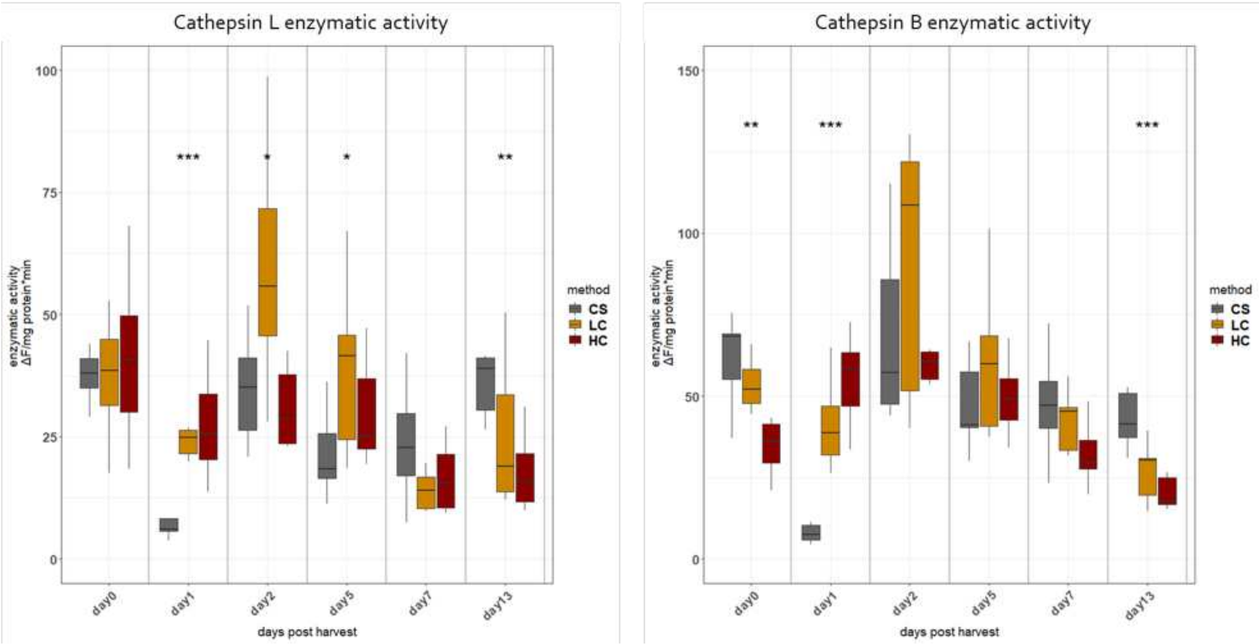

Figure 2. Enzymatic activity of Cathepsin L and Cathepsin B in all harvest methods. CS (gray): Control group, LC (orange): Low current group, HC (red): High current group. Superscripts indicate statistically significant differences between treatments on each sampling day (*: p<0.05, **:p<0.01, ***:p<0.001).

References

Bahuaud D., Mørkøre T., Østbye T.-K., Veiseth-Kent E., Thomassen M.S., Ofstad R. (2010). Muscle Structure Responses and Lysosomal Cathepsins B and L in Farmed Atlantic Salmon (Salmo Salar L.) Pre- and Post-Rigor Fillets Exposed to Short and Long-Term Crowding Stress. Food Chemistry 118(3), 602–15.

Yulong B., Wang K., Yang H., Regenstein J.M., Ertbjerg P., Zhou P. (2020). Protein Degradation of Black Carp (Mylopharyngodon Piceus) Muscle during Cold Storage. Food Chemistry 308.

Romuald C., Delbarre-Ladrat C., de Lamballerie-Anton M., Verrez-Bagnis V. (2007). Calpain and Cathepsin Activities in Post Mortem Fish and Meat Muscles."Food Chemistry 101(4), 1474–79.

Romuald C., Hernández-Andrés A., Delbarre-Ladrat C., de Lamballerie M., Verrez-Bagnis V. (2006). Proteins and Proteolytic Activity Changes during Refrigerated Storage in Sea Bass (Dicentrarchus Labrax L.) Muscle after High-Pressure Treatment. European Food Research and Technology 222(5–6), 527–35.

Hernández-Herrero M., Duflos G., Malle P., Bouquelet S. (2003). Collagenase Activity and Protein Hydrolysis as Related to Spoilage of Iced Cod (Gadus Morhua). Food Research International 36(2), 141–47.

Lakshmanan R, Patterson M., Piggot J. (2005). Effects of High-Pressure Processing on Proteolytic Enzymes and Proteins in Cold-Smoked Salmon during Refrigerated Storage. Food Chemistry 90(4), 541–48.

Ntzimani A., Angelakopoulos R., Semenoglou I., Dermesonlouoglou E., Tsironi T., Moutou K., Taoukis P. (2021). Slurry Ice as an Alternative Cooling Medium for Fish Harvesting and Transportation: Study of the Effect on Seabass Flesh Quality and Shelf Life. Aquaculture and Fisheries, March.

Pornrat S., Sumate T., Rommanee S., Sumolaya K., Kerr W.L. (2007). Changes in the Ultrastructure and Texture of Prawn Muscle (Macrobrachuim Rosenbergii) during Cold Storage. LWT - Food Science and Technology 40 (10), 1747–1754.

Teixeira B., Fidalgo L., Mendes R., Costa G., Cordeiro C., Marques A., Saraiva A., Nunes M.L. (2013). Changes of Enzymes Activity and Protein Profiles Caused by High-Pressure Processing in Sea Bass (Dicentrarchus labrax) Fillets. Journal of Agricultural and Food Chemistry 61(11), 2851–2860.

THE EFFECT OF TEMPERATURE AND INFECTION ROUTE ON in vitro HEMOCYTE PHAGOCYTOSIS ACTIVATION AFTER EXPERIMENTAL INFECTION OF COMMON OCTOPUS, *Octopus vulgaris* (CUVIER, 1797) WITH EITHER *Photobacterium damselae* subsp. *damselae* OR *Vibrio anguillarum* O1

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Abstract. The effect of temperature and infection route on hemocyte phagocytosis activation *in vitro* after experimental infection of common octopus with *Photobacterium damselae* subsp. *damselae* or *Vibrio anguillarum* O1 were investigated. Great variation was observed in the number of circulating hemocytes (1.04×10^5- 24.63×10^5 hemocytes ml^{-1} hemolymph). Temperature, time post infection, route of infection and pathogen influenced phagocytosis ability. Hemocytes of the *Photobacterium damselae* subsp. *damselae* infected specimens showed lower activation with rise of temperature and time post-infection influenced positively activation. In the *Vibrio anguillarum* O1 case, the opposite results were observed; hemocyte activation index of infected specimens found to be more elevated at higher temperature, while time post-infection, at both studied temperatures, exerted again a positive influence on activity, except of the *Vibrio anguillarum* O1 intramuscularly infected specimens in which lower activation was observed from day 3 to day 7 at 21± 0.5°C. Apparently, temperature, time post infection and pathogen are important factors in hemocyte activation.
Key words: *Octopus vulgaris, Phagocytosis, Hemocyte activation, Photobacterium damselae subsp. damselae, Vibrio anguillarum O1*
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1. Introduction

Octopus is targeted as an alternate aquatic animal for aquaculture species diversification (Vidal *et al.* 2014). There is a limited knowledge about their immune defense (Pascual & Guerra 2001) and pathologies that may occur after infection. Wild or cultured populations of octopods and fish can transmit pathogens causing infectious diseases to the cultivated organisms (Gestal *et al.* 2019), so, there could be a serious risk for production. Environmental changes, affect the action of cephalopod hemocytes and therefore their phagocytic activity (Malham *et al.* 1998; Ellis *et al.* 2011). Increase of sea surface temperature may influence the outcome of infections (Cattadori *et al.* 2005) as many infectious diseases are temperature dependent (Harvell *et al.* 2002). Photobacteriosis and Vibriosis, are fatal hemorrhagic septicemia diseases, attributed to species of *Photobacterium* and *Vibrio* (Osorio *et al.* 2018) and they represent major diseases that affect most aquatic organisms causing economic losses to the aquaculture industry (Higuera *et al.* 2013).

In this work the *in vitro* activation of the cellular immune response of experimentally infected octopus, *Octopus vulgaris* (Cuvier, 1797) with either *Photobacterium damselae* subsp. *damselae* or *Vibrio anguillarum O1* (Phdd and VAO1, hereafter, respectively) was studied, utilizing different infection routes and temperature regimes. This approach allowed us to explore the sensitivity of common octopus to these pathogens and to test whether significant changes in cellular immune responses were attributed to temperature or infection route.

2. Material and Methods

Wild octopi caught from April to May 2019 (infections with Phdd), and October to November 2019 (infections with VAO1). They were immediately placed in tanks in the ICHTHYAI wet laboratory facilities (EL83 BioExp01) and were acclimatized for 10 days at either 21± 0.5°C or 24±0.5°C. Octopus were fed defrosted or fresh teleost and crabs daily. At the end of acclimatization period, they were weighed and either infected intramuscularly (second right arm) or intravenously (right branchial heart ventricle) with either Phdd or VAO1 (1×10^9cells/ml) or with sterile 2% NaCl (same routes, negative controls). For clarity reasons the infected specimens are presented below with letter "I" and controls with letter "C". Intramuscularly infected/injected specimens with letter "M"; intravenously infected/injected specimens with letter "V". Days post-infection are marked as D0, D3and D7 (i.e., D0 CO: means Day 0, control; D3 CIM: means Day 3, control intramuscularly injected with 2% sterile NaCl; D3 IM: means Day 3, intramuscularly infected). For each infection/injection route and sampling point, a total of 3 different octopi were used (i.e., 2 sampling points x 2 infection/injection route x 2 temperature x 3 octopi = 24 individuals + 3 octopi at Day 0 = 27 octopi in total for each different pathogen). Individuals in each group were observed daily for mortality, behavioural changes, feed intake and skin lesions. The protocol for the experimental infection was approved by Decision No 5379/4-4-2017, of the competent Regional Veterinary Authority. Both bacteria were isolated from *Dicentrarchus labrax*; Phdd in 2011 in the Black Sea and it was a kind donation of Dr. Ogut

(Uzun & Ogut 2015) and VAO1 in 2012 in the Ionian Sea and it was a kind donation of Dr. Kantham Papanna (Nireus Aquaculture S.A.). On D0, 3 and 7 hemolymph from each octopus was withdrawn using syringes with Alsever's solution as anticoagulant. The hemolymph was immediately transferred into a sterile glass vial and used for hemocyte counts. Hemocyte viability and cell counting were carried out using a Neubauer chamber and the Trypan blue exclusion method (Weeks-Perkins *et al.* 1995). After counting the number of viable circulating hemocytes ml^{-1} of hemolymph, hemocytes were adjusted with Squid Ringer Solution (SRS), pH 7.5 (Castellanos-Martinez *et al.* 2014) to $1x10^5$ cells ml^{-1} in order to measure phagocytic activity. Activity was measured with a_modified Troncone *et al.* (2014) method with a 0.8% Congo red stained yeast *Saccharomyces cerevisiae* as test particle ($7x10^5$ cells ml^{-1}, at 1:7 radio of hemocytes:yeast, respectively). The phagocytosis activity of at least 40 hemocytes was recorded, in triplicate for each hemolymph sample. Positive phagocytic activity was considered when hemocytes had phagocytosed >3 particles (Hegaret *et al.* 2003). Data obtained from phagocytosis ability experiments were analyzed using Wilcoxon test, run on R Project (R Core Team., 2015). Pearson's correlation between circulating hemocytes and body weight, t-test comparisons between groups mean differences of circulating hemocytes ml^{-1} hemolymph and all graphs ($P < 0.05$ as significant level) were preformed using the IBM's SPSS Statistics software (version 20). All substantial combinations were tested. Results are expressed as the mean ± standard deviation (in text) and standard errors (in graphs) of the mean. Before any handling, infections/injections and hemolymph sampling, animals were anesthetized by immersion in 5% magnesium chloride in sea water, according to Gestal *et al.* (2019), with minor modifications.

3. Results-Discussion

Octopus vulgaris sensitivity to Phdd or VAO1: Phdd and VAO1 did not caused mortalities or disease development irrespective of route despite their implication in cephalopod infections (Gestal *et al.* 2019). Few specimens infected with VAO1 showed laziness, hyperventilation, and discoloration of the infected arm. Similar symptoms were observed in octopi infected with *V. alginolyticus* in a previous study (White *et al.* 2020). No mortalities were reported from Malham (1996 thesis) 48h after infection of octopus *Eledone cirrhosa* with $1x10^6$ cells ml^{-1} *Vibrio anguillarum*. These are encouraging results since Phdd and VAO1 are emerging pathogens of marine fish with interest in the aquaculture industry, including various species of economic importance (Terceti *et al.* 2016).

Correlation of body weight and circulating hemocytes of control specimens (Phdd or VAO1 experiments): There was no correlation between body weight and circulating hemocytes (r: 0.762, N=6, p: 0.078) of naive octopi during the experiment with Phdd (D0 C0), in contrast to the injection route controls where a strong positive relationship was calculated (r: 0.779, N=30, p: 0.001). During the VAO1 experiment, naive octopi (D0 C0) also showed no correlation between weight and circulating hemocytes (r: -0.496, N=6, p: 0.318), but there was low negative correlation between weight and circulating hemocytes when injection route controls were tested (r: -0.354, N=30, p: 0.055). It is very difficult to establish a relationship between body weight and circulating hemocytes since many factors may influence numbers of circulating hemocytes such as size, age, maturity stage, natural fluctuations and temperature, as it has been previously suggested (Stumpf & Gilbertson 1978; Malham *et al.* 1997; 1998; Carballal *et al.* 1998; Ellis *et al.* 2011).

Circulating hemocytes: Circulating hemocytes of specimens after IM and IV injection with either Phdd or VAO1 or with NaCl, ranged between $1.04x10^5$ to $24.63x10^5$ hemocytes ml^{-1} hemolymph at both experiments and temperatures (Figure 1 a, b, c, and d). This enormous variation is supported by previous studies (Rodríguez-Domínguez *et al.* 2006; Castellanos-Martinez *et al.* 2014).

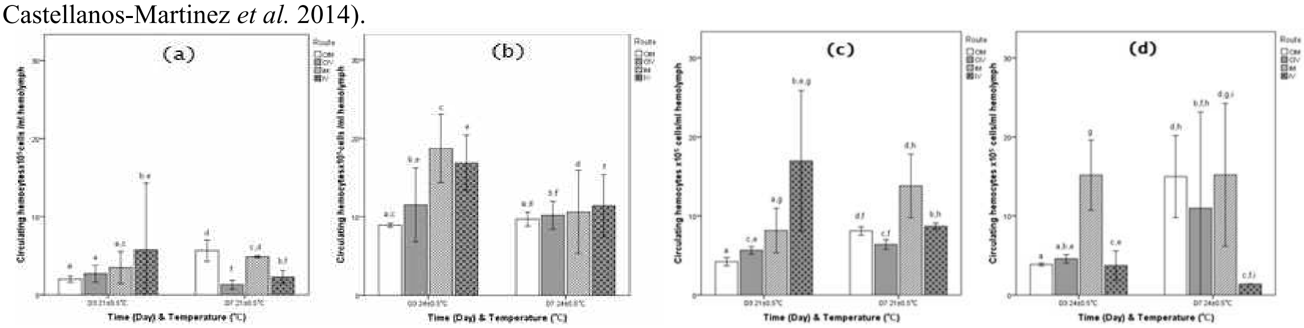

Figure 1. Circulating hemocytes after IM or IV injection with either Phdd (a and b) / VAO1 (c and d) or NaCl. a and c: at 21±0.5°C and b and d: at 24±0.5°C. D: day; CIM: controls injected IM; CIV: controls injected IV; IM: infected IM; IV: infected IV. Mean values (±SE); same letter indicates no significance (p>0.05).

Studies reported that age, weight, seasonality, reproductive cycle, heart rate, pathogen challenge, pollutant exposure, sampling stress or even the bleeding technique are biotic or abiotic parameters that can cause variations in the concentration of the hemocytes (Mosca *et al.* 2013; Hong *et al.* 2020). Malham *et al.* (1997) suggested that, during stress hemocytes it is possible to leave the circulation and may migrate to tissues that are more prone to an infection or injury. A temperature effect appeared in this study, as an increase of circulating hemocytes with rising temperature between all-time points for both routes of the specimens infected with Phdd and IM VAO1 was noted. This was not the case for IV infected groups with VAO1 indicating a possible pathogen-regulated and route-regulated response related to the temperature. This observation is supported by Van de Braak *et al.* (2002) who found reduced hemocyte concentration in the hemolymph of diseased shrimp (*Penaeus monodon*) following injection with *Vibrio anguillarum* O2.

Phagocytic ability after infection with Phdd or VAO1: Results illustrating the influence of temperature, time, and route on the phagocytosis activity after each pathogen (Phdd or VAO1) infection are presented in Figure 2a, b, c, and d.

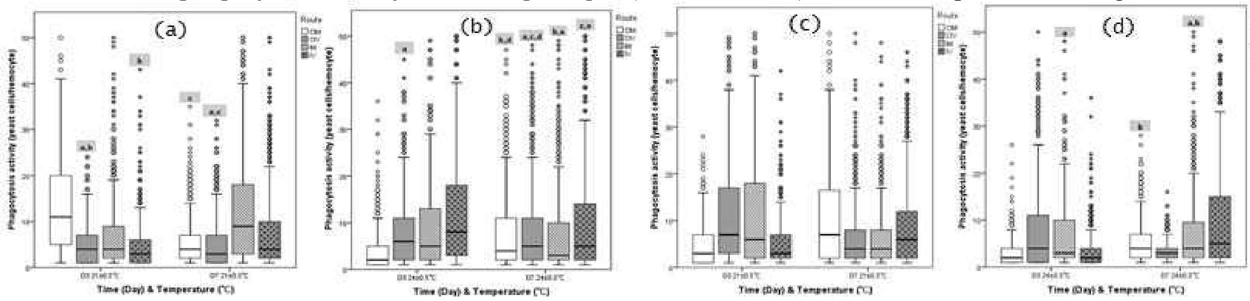

Figure 2. Phagocytosis activity (yeast cells/hemocyte) after IM or IV injection/injection with either Phdd (a and b) /VAO1 (c and d) or NaCl. a and c: at 21±0.5°C and b and d: at 24±0.5°C. D: day; CIM: controls injected IM; CIV: controls injected IV; IM: infected IM; IV: infected IV. Mean values; same letter indicates no significance (p>0.05); circles mark data outliers.

Specimens IM infected with Phdd showed higher phagocytosis activity than the IV infected group at 21±0.5°C, with significant differences. In contrast, at 24±0.5°C IM infected specimens showed lower phagocytosis activity than IV infected group with a significant difference on D3. Moreover, specimens IM infected with VAO1 showed a quick response to the pathogen at both temperatures and specimens IV infected with VAO1 presented a significantly lower response at both studied temperatures, on D3. Exactly the opposite was observed on D7. Several studies have shown that phagocytosis is a temperature dependent process in mollusks as it is inhibited at temperatures below 10°C (Carballal *et al.* 1998), while Malham *et al.* (1997) showed that pre-opsonization of bacteria, higher temperature, and longer incubation time can increase phagocytosis. The results in this study indicated that rise of temperature plays an important role and influences phagocytosis ability. However, this influence seems to be influenced also by the route of infection, time point, and pathogen. Specimens infected with Phdd and VAO1 in most cases showed elevated phagocytosis activity with rise of temperature. Since differences were observed between routes, the route of infection may have played a major role to the response. This has been reported in teleost fish where route of infection can affect host response as well as the action of the bacterium (Crumlish *et al.* 2010; Ohtani *et al.* 2019).

Activation status of hemocytes: In some instances, high levels of circulating hemocytes were not correlated with increased phagocytosis activity and vice versa. Pearson's correlations between circulating hemocytes and phagocytosis activity showed no correlation to be statistically important, apart from a specific case of Phdd at 24±0.5°C where higher circulating hemocytes levels correlated with higher phagocytosis activity with a medium positive correlation. The results of the correlations led us to calculate the "Activation Index" (AI), in order to identify the real activation status of hemocytes under the different conditions for both experiments. AI= (mean yeast cells hemocyte^{-1} value (indicating activation levels) / mean value of circulating hemocytes) x10^5. The higher the value calculated the more activated the hemocytes. Table 1 presents the activation index results.

Table 1: Activation Index for each experiment of the study

	Phdd 21±0.5°C	Phdd 24±0.5°C	VAO1 21±0.5°C	VAO1 24±0.5°C
D3CIM	7.47	0.49	1.15	0.83
D3CIV	1.74	0.73	2.22	1.97
D3IM	2.60	0.51	1.64	0.50
D3IV	0.89	0.86	0.32	1.02
D7CIM	1.02	0.90	1.35	0.36
D7CIV	4.12	0.86	1.15	0.31
D7IM	2.78	1.07	0.51	0.59
D7IV	4.30	1.00	1.12	7.76

Determination of AI (Table 1) showed that temperature involvement on hemocyte activation is important since for the Phdd infected specimens, hemocytes were more activated at 21±0.5°C instead of 24±0.5°C. In contrast, in the VAO1 case the opposite results were observed; infected specimens were more activated at higher temperature. Between time points, higher hemocyte activation was found in infected specimens in all cases; except for VAO1 IM infected group at 21±0.5°C.

Temperature can influence the bacterial growth and virulence (Lages *et al.* 2019) and it seems that it is also a key influencing factor for the physiological functions of poikilotherms since high temperature negatively affects the survival rate and growth of *O. vulgaris* (Aguado *et al.* 2001). More specific, seasonality by itself is important factor for hemocyte phagocytosis, even when comparing specimens with the same amount of infection (Castellanos-Martinez & Gestal 2018). All these lead to that hemocyte activation is influenced by many extrinsic factors. The season, temperature, pathogen, route of infection and time post infection; and intrinsic factors, such as individual physiological and immune status, as it has been previously discussed and published (White *et al.* 2020).

In conclusion, this study gives important information for a better knowledge and characterization of common octopus hemocyte mediated immunity as to date little is known about the effect of temperature on the physiology and immune response of *O. vulgaris* and generally on cephalopod hemocytes and how they respond to infections or to environmental changes.

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References

Aguado F., Rueda F.M., Egea M.A., Hernandez M.D., Faraco F., Garcia Garcia, B. (2001). Efecto de la temperatura sobre la supervivencia en el transporte, estabulacion y engorde de *Octopus vulgaris* Cuvier (1797) en el Mediterraneo occidental. In: Fernandez-Palacios, H., Izquierdo, M. (Eds.), Convergencia entre Investigacion y Empresa: Un reto para el Siglo XXI, Monografıas del ICCM no. 4. ICCM, Las Palmas de Gran Canaria. p. 174 – 179.

Carballal M.J., Villalba A., Lopez C. (1998). Seasonal Variation and Effects of Age, Food Availability, Size, Gonadal Development, and Parasitism on the Hemogram of Mytilus galloprovincialis. Journal of Invertebrate Pathology 72, 304–312.

Castellanos-Martinez S., Prado-Alvarez M., Lobo-da-Cunha A., Azevedo C., Gestal C. (2014). Morphologic, cytometric and functional characterization of the common octopus (Octopus vulgaris) hemocytes. Developmental and Comparative Immunology 44(1), 50–58.

Castellanos-Martínez S., Gestal C. (2018). Immune parameters in the common octopus (Octopus vulgaris Cuvier, 1797) naturally infected by the gastrointestinal protozoan parasite Aggregata octopiana. Aquaculture 497, 487–493.

Cattadori I.M., Haydon D.T., Hudson P.J. (2005). Parasites and climate synchronize red grouse populations. Nature 433 (7027),737–741.

Crumlish M., Thanh P.C., Koesling J., Tung V.T., Gravningen K. (2010). Experimental challenge studies in Vietnamese catfish, Pangasianodon hypophthalmus (Sauvage), exposed to Edwardsiella ictaluri and Aeromonas hydrophila. Journal of Fish Diseases 33(9), 717–722.

Ellis R., Parry H., Spicer J.I., Hutchinson T., Pipe R., Widdicombe S. (2011). Immunological function in marine invertebrates: responses to environmental perturbation. Fish &Shellfish Immunology 30(6), 1209–1222.

Gestal C., Pascual S., Guerra A., Fiorito G., Vieites J.M. (2019). Handbook of Pathogens and Diseases in Cephalopods. SprigerOpen, pp. 1-234.

Harvell C.D., Mitchell C.E., Ward J.R., Altizer S., Dobson A.P., Ostfeld R.S., Samuel M.D. (2002). Ecology - Climate warming and disease risks for terrestrial and marine biota. Science 296(5576), 2158–2162.

Hegaret H., Wikforsa H.G., Soudant P. (2003). Flow cytometric analysis of haemocytes from eastern oysters, Crassostrea virginica, subjected to a sudden temperature elevation II. Haemocyte functions: aggregation, viability, phagocytosis, and respiratory burst. Journal of Experimental Marine Biology and Ecology 29(2), 249– 265.

Higuera G., Bastías R., Tsertsvadze G., Romero J., Espejo R.T. (2013). Recently discovered Vibrio anguillarum phages can protect against experimentally induced vibriosis in Atlantic salmon, Salmo salar. Aquaculture 392–395, 128-133.

Hong H.K., Jeung H.D., Kang H.S., Choi K.S. (2020). Seasonal variations in the hemocyte parameters, gonad development, energy storage and utilization of the giant honeycomb oyster Hyotissa hyotis (Linnaeus 1758) in Jeju Island off the south coast of Korea. Aquaculture Reports17, 100299.

Lages M. A., Balado M., Lemos M.L. (2019). The Expression of Virulence Factors in *Vibrio anguillarum* Is Dually Regulated by Iron Levels and Temperature. Frontiers in Microbiology 10, 2335.

Malham S.K. (1996). Immunobiology of Eledone cirrhosa (Lamarck). Bangor University. North Wales. pp. 315.

Malham S.K., Runham N.W., Secombes C.J. (1997). Phagocytosis by haemocytes from the Lesser Octopus Eledone cirrhosa. Sociedad Espanola de Malacologia. Iberus 15(2), 1-11.

Malham S.K., Coulson C.L., Runham N.W. (1998). Effects of repeated sampling on the haemocytes and haemolymph of Eledone cirrhosa (Lam.). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 121(4), 431–440.

Mosca F., Narcisi V., Calzetta A., Gioia L., Finoi M.G., Latini M., Tiscar P.G. (2013). Effects of high temperature and exposure to air on mussel (Mytilus galloprovincialis, Lmk 1819) hemocyte phagocytosis: Modulation of spreading and oxidative response. Tissue and Cell 5(3), 198-203.

Ohtani M., Villumsen K.R., Strøm H.K., Lauritsen A.H., Aalbæk B., Dalsgaar, I., Nowak B., Raida M.K., Bojesen A.M. (2019). Effects of fish size and route of infection on virulence of a Danish Yersinia ruckeri O1 biotype 2 strain in rainbow trout (Oncorhynchus mykiss). Aquaculture 503, 519–526.

Osorio C.R., Vences A., Matanza X.M., Terceti M.S., DiRita V.J. (2018). Photobacterium damselae subsp. damselae, a Generalist Pathogen with Unique Virulence Factors and High Genetic Diversity. Journal of Bacteriology 200 (15), 2-18.

Pascual S., Guerra A. (2001). Vexing question on fisheries research: the study of cephalopods and their parasites. Iberus 19 (2), 87–95.

R Core Team. (2015). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rodríguez-Domínguez H., Soto-Búa M., Iglesias-Blanco R., Crespo-González C., Arias-Fernández C., García-Estévez J. (2006). Preliminary study on the phagocytic ability of Octopus vulgaris Cuvier, 1797 (Mollusca: Cephalopoda) haemocytes in vitro. Aquaculture 254(1-4), 563–570.

Stumpf J.L., Gilbertson D.E. (1978). Hemocytes of Biomphalaria glabrata: Factors Affecting Variability. Journal of Invertebrate Pathology 32(2), 177-181.

Terceti M. S., Ogut H., Osorio C.R. (2016). Photobacterium damselae subsp. damselae, an Emerging Fish Pathogen in the Black Sea: Evidence of a Multiclonal Origin. American Society for Microbiology. Applied and Environmental Microbiology 82(13), 3736-3745.

Troncone L., De Lisa E., Bertapelle C., Porcellini A., Laccetti P., Polese G., Di Cosmo A. (2014). Morphofunctional characterization and antibacterial activity of haemocytes from Octopus vulgaris. Journal of Natural History 49 (21-24), 1457-1475.

Van de Braak C.B.T., Botterblom M.H.A., Taverne N., van Muiswinkel W.B., Rombout J.H.W.M., van der Knaap W.P.W. (2002). The roles of haemocytes and the lymphoid organ in the clearance of injected Vibrio bacteria in Penaeus monodon shrimp. Fish & Shellfish Immunology 13, 293–309.

Vidal E.A., Villanueva R., Andrade J.P., Gleadall I.G., Iglesias J., Koueta N., Rosas C., Segawa S., Grasse B., Franco-Santos R.M., Albertin C.B., Caamal-Monsreal C., Chimal M.E., Edsinger-Gonzales E., Gallardo P., Le Pabic C., Pascual C., Roumbedakis K., Wood J. (2014). Cephalopod culture: current status of main biological models and research priorities. Advances in Marine Biology 67, 1-98.

Wang Y.J., Hu M.H., Cheung S.G., Shin P.K.S., Lu W.Q., Li J.L. (2012). Immune parameter changes of hemocytes in green-lipped mussel Perna viridis exposure to hypoxia and hyposalinity. Aquaculture 356, 22–29.

Weeks-Perkins B.A., Chansue N., Wong-Verelle D. (1995). Assays of immune function in shrimp phagocytes: techniques used as indicators of pesticide exposure. In: Stolen, J.S., et al., Techniques in Fish Immunology 4. Immunology and Pathology of Aquatic Invertebrates. SOS Publications, Fair Haven, N.J., pp. 223–231.

White D.M., Valsamidis M.A., Bakopoulos V. (2020). Hemocyte phagocytosis bindng intensity of experimentally infected *Octopus vulgaris* (Cuvier, 1797) with *Photobacterium damselae* subsp. *piscicida*. Proceedings of the Webinar on Aquaculture and Fisheries, Theme: Trends in Aquaculture and Fisheries, pp. 12.

TRANSCRIPTOME COMPARISON OF RESISTANT AND SUSCEPTIBLE SEA BASS (*Dicentrarchus labrax*) CHALLENGED WITH NERVOUS NECROSIS VIRUS REVEALS A MARKED CONTRAST IN GENE EXPRESSION LEVELS IN A TIME-COURSE STUDY

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Abstract
Nervous necrosis virus (VNN) is a pathogen affecting more than 120 different species from marine and freshwater environments, causing high mortality and morbidity of cultured species. The host genetic background to disease resistance has been reported for European sea bass (*Dicentrarchus labrax*), and is correlated with high mortality rates in some families and low mortality in others. However, the molecular and immunological basis for this resistance is not yet fully known. Aim of the present study was the global comparison of the gene expression profiles of resistant and susceptible European sea bass following experimental infection with VNN. Sea-bass from one NNV-resistant and one NNV-susceptible families were challenged with VNN and sampled at 3 hours, 48 hours and 14 days post-challenge, followed by re-infection and sample collection after 7 days. The viral titer was determined in both resistant and susceptible fish at all time-points. Gene expression profiles were recorded and analyzed revealing that while a clear response was observed in both resistant and susceptible fish, there were striking differences between the two phenotypes. The resistant family showed substantially higher numbers of both up-regulated and down-regulated transcripts than the susceptible family in all time-points prior re-infection. That observation was reversed after re-infection with NNV where the susceptible fish had more intense gene up- and down- regulation compared with the resistant fish. Notably, the up-regulated transcripts abundance in the resistant family at the initial time-point (3h) is extremely high. In both families, the lower de-regulated transcript numbers are observed 14 days post infection.
Keywords: *Dicentrarchus labrax, nervous necrosis virus, transcriptome, RNA-Seq, disease resistance*
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1. Introduction

The European sea bass (*Dicentrarchus labrax*) is a teleost fish found in the Mediterranean, the Atlantic Ocean and Black seas. It is an economically important species, ranked fourth in European aquaculture production and, although undergoing domestication for the last three decades, selective breeding programs are still in their infancy (Tine *et al*. 2014). Nervous necrosis virus (NNV) is a pathogen causing high mortality and morbidity of cultured species. NNV is part of the genus Betanodavirus and is a member of the Nodaviridae family, with a genome consisting by two positive-sense ssRNA molecules (RNA1 and RNA2), in a non-enveloped capsid. NNV is the causing agent of viral nervous necrosis (VNN), or vacuolating encephalopathy and retinopathy (VER) or encephalomyelitis species. The clinical symptoms of VNN infection include abnormal swimming behavior (spiral swimming, whirling, horizontal looping or darting), loss of appetite, swim bladder hyperinflation and coloration abnormalities (pale or dark), resulting eventually in the death of infected hosts (Bandín *et al.* 2020; Doan *et al.* 2017; Costa *et al.* 2016). Susceptibility of European sea bass to VNN is well-known. Interest towards selective breeding as a tool to enhance genetic resistance in this species has increased sharply due to the major threat represented by VNN for farmed sea bass. Traditional pedigree-based breeding programs require individual phenotyping for mortality, which can be difficult for routine implementation. Such difficulties can be overcome by developing genomic and transcriptomic tools for the prediction of selection candidates for the traits of interest.

2. Materials and Methods

Two families of healthy, non-vaccinated fish, with different rates of mortality (resistant:~16% & susceptible:~72%) were chosen. NNV was originally isolated from naturally infected *D. labrax* fishes (genotype: RGNNV (Toubanaki *et al*. 2015). Sea bass were challenged by intramuscular injection with 10^{11} $TCID_{50}$/mL of Nodavirus-containing supernatant (100 μL) or were mock-challenged with 100 μL PBS (negative control group). Sampling was performed at 3 and 48 hours post infection (hpi) and 14 days post infection (dpi), followed by 7 days post re-infection (dpri). At each time point fish were anesthetized and weighed. Blood, head kidney (HK) and brain (B) tissues were removed aseptically and tissues were stored in RNA later. Mortality was recorded daily and RT-qPCR (Baud et al. 2015) was used for viral load quantification. The specific anti-NNV IgM levels were determined on each group sera utilizing a sandwich-ELISA (Bakopoulos *et al.* 1997). Total RNA was extracted from HK by TriZol protocol, the quantity of the extracted RNA was evaluated by Nanodrop spectrophotometer and its quality was assessed by Bioanalyzer (Agilent). cDNA libraries were prepared with Ion Total RNA-Seq Kit v2 kit (Thermo Fisher Scientific) and RNA-sequencing was performed with Ion Torrent technology (Ion S5XL, Thermo Fisher Scientific). For each biological group, 3-5 individuals were used, resulting to a total of 60 samples/ libraries. Raw reads were assessed for quality control (fastq_screen & multiQC), and were trimmed. Reads were aligned to sea bass reference genome seabass_V1.0 (GCA_000689215.1). The differential expression of genes was identified with metaseqR2 (Bioconductor package).

3. Results – Discussion

To evaluate the difference in gene expression profiles between resistant and susceptible European sea bass, a subset of the most susceptible and the most resistant families from a previous study were examined (Bakopoulos V., unpublished data). The family-specific mortality of the 'genetically susceptible' family was 72.3% while the 'genetically resistant' family had 16% mean mortality. Typical signs of the disease were observed in NNV-infected fish from day 2, including erratic swimming, disorientation and skin discoloration. The first mortalities for the susceptible and the resistant family were recorded on day 2 and 4, respectively. Mortalities reached a peak on days 5-7 while they were minimized after days 15 and 18 for the resistant and susceptible family, respectively. No mortalities were registered in negative control group. Viral RNA was detected in NNV-infected fish 3 dpi while the highest levels of viral load were observed on 7 dpi. Viral load was higher on average in susceptible than resistant fish. The viral load at the final time point (28 dpi) was not eliminated suggesting that the surviving fish were still carrying virus particles. The results demonstrate that animals with disparate genetic resistance can become infected and that viral infection and replication occurs in resistant genotypes, implying that genetic resistance cannot be entirely due to an inability of the virus to enter the cells of the host.

A broad-level gene expression comparison of genetically resistant and susceptible families at 3 hpi, 48 hpi, 14 dpi and 7 dpri was performed using RNA-seq. RNA extracted from head kidney homogenates were analyzed in 3-5 biological replicates per infection status (NNV-infected or control) per time-point per family figure 1). The samples quality and clustering indicates confirmed the analysis reliability.

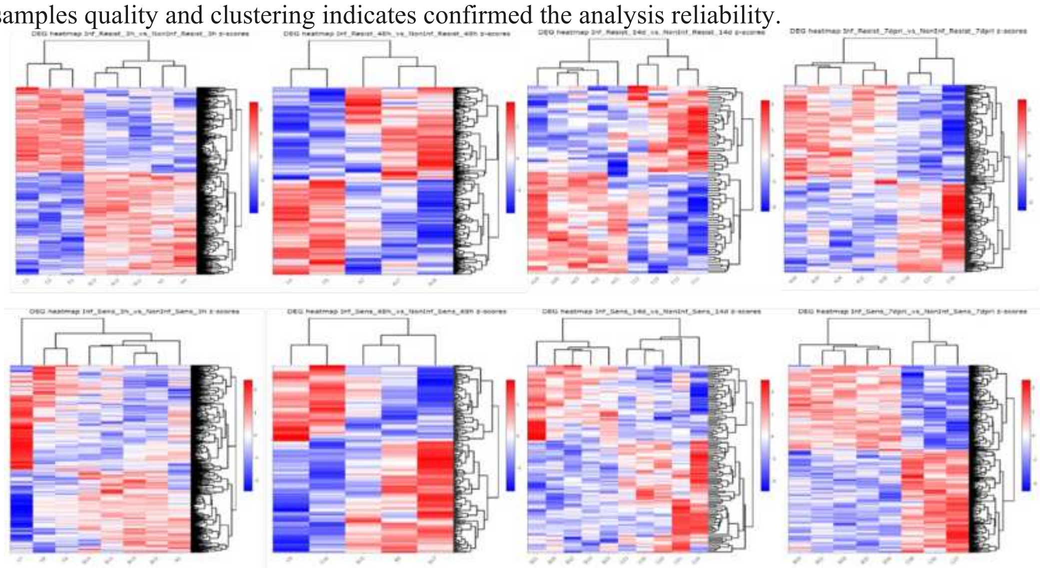

Figure 1. Heatmap plots of global gene expression response in resistant and susceptible (sensitive) families following NNV infection. One heatmap plot of every transcript for each of the eight comparisons are shown.

With an initial analysis, the gene expression values of NNV-infected samples at each time-point and genotype were compared to the respective controls (such that ‘up-regulation’ refers to a significantly higher gene expression signal in NNV-infected fish) (figure 2). A striking pattern of global gene expression differences between the families was evident, in all time points. The resistant family showed substantially higher numbers of both up-regulated and down-regulated transcripts than the susceptible family in all time-points prior re-infection. That observation was reversed after re-infection with NNV where the susceptible fish had more intense gene up- and down- regulation compared with the resistant fish. Notably, the up-regulated transcripts abundance in the resistant family at the initial time-point (3h) is extremely high. In both families, the lower de-regulated transcript numbers are observed 14 days post infection.

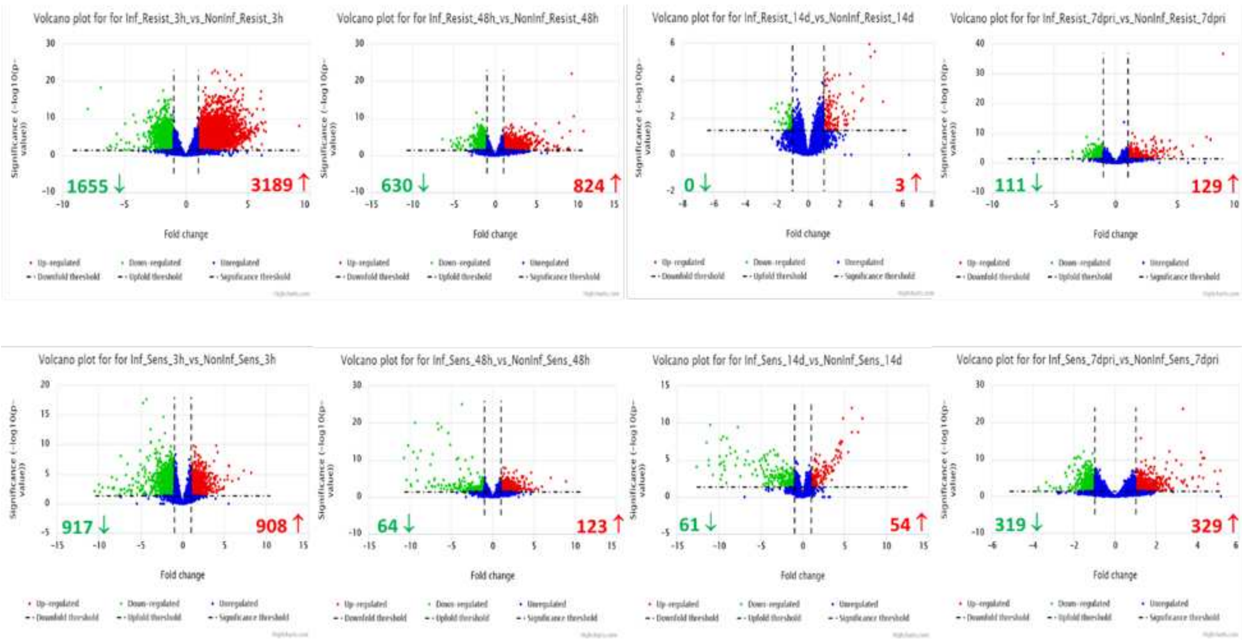

Figure 2. Volcano plots of global gene expression response in resistant and susceptible families following NNV infection. Volcano plots of the log2 fold change versus log10 p-value of every transcript for each of the eight comparisons are shown. Transcripts with p values < 0.05 (significant) are shown in red (up-regulated) or green (down-regulated), while those with p values > 0.05 are shown in blue. The number of significant down-regulated (green) and up-regulated (red) transcripts for each comparison are shown in the corners of each volcano plot, based on the FDR (false discovery rate or adjusted p-value threshold < 0.05).

In order to characterize genes that are associated with NNV resistance in the studied time points, the gene sets which were up-regulated in each family were compared with Venn diagrams. The genes that are uniquely expressed in the resistant family at each time point are shown in figure 3. The number of transcripts that are uniquely up-regulated in the resistant family is significantly higher at the earlier time point (3h) (3709 genes) that all following time points. The up-regulated transcript are less abundant 48 hours post infection (2023 genes) and are at the lower observed point 14 days post infection (319 genes). The number of up-regulated genes expression seems increased again 7 days post re-infection (728 genes). The number of uniquely up-regulated transcripts in the sensitive family is also higher at the earlier time point (3h) (1286 genes) and subsequently lowers with 827 γονίδια 48 hpi and 319 genes 14dpi. The number of up-regulated genes expression is strikingly increased 7 days post re-infection (1168 genes).

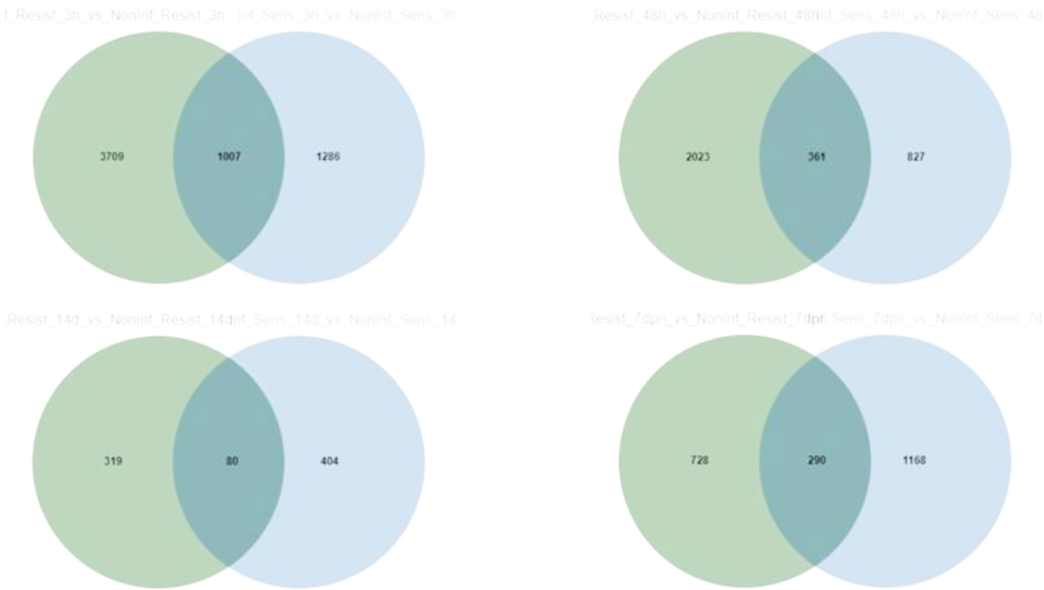

Figure 3. Venn diagrams of global gene expression response in resistant and susceptible families following NNV infection. Venn diagrams at each studied time point contain transcripts with p values < 0.05 (significant) shown in green for the resistant family and shown in blue for the sensitive family. The number of uniquely up-regulated transcripts for each family are shown inside the respective cycle.

The direct comparison of uniquely up-regulated genes on the resistant family for the three time-points prior re-infection, i.e. 3 hpi, 48 hpi and 14 dpi revealed 9 commonly overexpressed genes (figure 4).

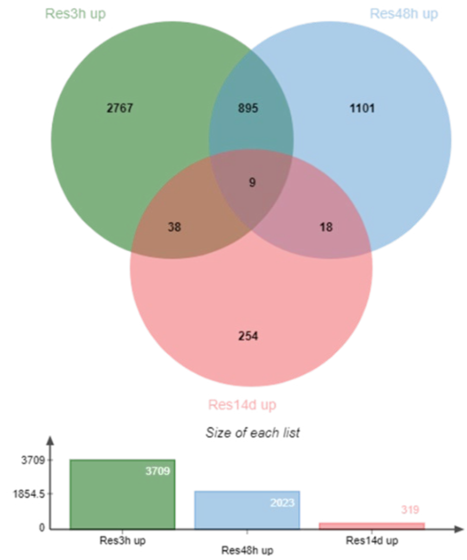

Figure 4. Venn diagrams of up-regulated gene expression in resistant family following NNV infection. Venn diagrams at each studied time point contain transcripts with p values < 0.05 (significant) shown in green for 3 hpi, blue for 48 hpi and red for 14 dpi. The number of uniquely up-regulated transcripts for each family are shown inside the respective cycle.

These nine proteins include the neutral alpha-glucosidase c (GANC), upf0692 protein c19orf54 homolog, spindle and kinetochore-associated protein 3 (SKA3), nadh-ubiquinone oxidoreductase 75 kda mitochondrial-like (NDUFS1), lysosome-associated membrane glycoprotein 3 (LAMP3), protein zwilch homolog (ZWILCH), immunoglobulin light chain, serine threonine-protein kinase endoribonuclease ire1(ERN1) and dna-directed rna polymerase iii subunit rpc7-like (POLR3GL). Most of these genes and their function have not been studied in fish. Studies in human reports that neutral α-glucosidase has a hydrolytic and transferase activity. It catalyzes hydrolysis, releasing α D-glucose and acting on the non-reducing end of the molecule. The UPF0692 family includes human protein C19orf54 but their function is not known. Spindle and kinetochore-associated protein 3 functions as part of a network that coordinates checkpoint signaling from the microtubule-binding sites within a kinetochore by laterally linking the individual binding sites. They suggested that this network plays a major role in silencing the spindle checkpoint when chromosomes are aligned at metaphase to allow timely anaphase onset and mitotic exit. The core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) catalyzes electron transfer from NADH through the respiratory chain, using ubiquinone as an electron acceptor. It is essential for catalyzing the entry and efficient transfer of electrons within complex I, plays a key role in the assembly and stability of complex I and participates in the association of complex I with ubiquinol-cytochrome reductase complex (Complex III) to form supercomplexes. Lysosome-associated membrane glycoprotein 3 may play a role in dendritic cell function and in adaptive immunity. It has been suggested that it plays an important role in viral post-entry steps. Protein zwilch homolog is essential component of the mitotic checkpoint, which prevents cells from prematurely exiting mitosis. Its function related to the spindle assembly machinery is proposed to depend on its association in the mitotic RZZ complex. Serine/threonine-protein kinase and endoribonuclease acts as a key sensor for the endoplasmic reticulum unfolded protein response (UPR). Accumulation of misfolded proteins in the endoplasmic reticulum causes release of HSPA5/BiP, allowing the luminal domain to homodimerize, promoting autophosphorylation of the kinase domain and subsequent activation of the endoribonuclease activity. Acts as an upstream signal for ER stress-induced GORASP2-mediated unconventional (ER/Golgi-independent) trafficking of CFTR to cell membrane by modulating the expression and localization of SEC16A. Finally, the DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Specific peripheric component of RNA polymerase III which synthesizes small RNAs, such as 5S rRNA and tRNAs.

Future work will confirm the genes functional significance by the enrichment of the present gene lists and pathway analysis.

Acknowledgements

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References

Bakopoulos V., Volpatti D., E., R., Galleotti M., Adams A. (1997). Development of an ELISA to detect *Pasteurella piscicida* in culture and in 'spiked' fish tissue. Aquaculture 153, 359-366.

Bandin I., Souto S. (2020). Betanodavirus and VER Disease: A 30-year Research Review. Pathogens, 9, 106. **Not appearing in the text**

Baud M., Cabon J., Salomoni A., Toffan A., Panzarin V., Bigarré L. (2015). First generic one step real-time Taqman RT-PCR targeting the RNA1 of betanodaviruses. Journal of Virological Methods 211, 1-7.

Costa Z.C., Thompson K.D. (2016). Understanding the interaction between Betanodavirus and its host for the development of prophylactic measures for viral encephalopathy and retinopathy. Fish & Shellfish Immunology 53, 35-49.

Doan Q.K., Vandeputte M., Chatain B., Morin T., Allal F. (2017). Viral encephalopathy and retinopathy in aquaculture: a review. Journal of Fish Diseases 40, 717-742.

Tine M., Kuhl H., Gagnaire P.A., Louro B., Desmarais E., Martins R.S., Hecht J., Knaust F., Belkhir K., Klages S., Dieterich R., Stueber K., Piferrer F., Guinand B., Bierne N., Volckaert F.A., Bargelloni L., Power D.M., Bonhomme F., Canario A.V., Reinhardt R. (2014). European sea bass genome and its variation provide insights into adaptation to euryhalinity and speciation. Nature Communications 5, 5770.

Toubanaki D.K., Margaroni M., Karagouni E. (2015). Development of a Novel Allele-Specific PCR Method for Rapid Assessment of Nervous Necrosis Virus Genotypes. Current Microbiology 71, 529-39.

HISTORY OF THE INTRODUCTION OF THE NON-NATIVE SPECIES *Pagrus major* (TEMMINCK AND SCHLEGEL, 1843) IN GREEK AQUACULTURE

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Abstract
The Non Native Species *Pagrus major* was introduced in Greece for aquaculture purposes in 1986. The annual production of red porgies from aquaculture in Greece concerning both species *P. major* and *P. pagrus* have increased from 37 kg in 1993 to a mean value of 1740 t during 2014-2017 registering an impressive average increase between 2012 and 2016 of 75.5% per year. Given that *P. major* specimens were already caught in the wild it is of great interest to further investigate the impact of its presence in the marine environment of Greece.
Keywords: *Pagrus major, Mariculture, Greece*
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1. Introduction

Red sea bream *Pagrus major* (or *Chrysophrys major* Temminck & Schlegel 1843) is farmed in the eastern Mediterranean Sea since 1985. Nowadays, this species is sold fresh in the Greek market as a fish farming product. The historical review of the introduction and farming of *P. major* in Greece was conducted from peer-reviewed scientific literature, relevant gray literature and direct contacts with professionals in the aquaculture sector. The annual production of red porgies from aquaculture in Greece was derived from FAO (FAO-FishStatPlus 2008, in Pavlidis and Mylonas 2011), FEAP (Souto & Villanueva 2003) and the Statistical Service of Greece (HELSTAT 1992–2018) data concerning both *P. major* and *P. pagrus* species, which are apparently not distinguished by the corresponding services. To understand the spatial distribution of red porgies aquaculture in the Greek seas, data of fry supply in farming units during the last three years were also provided by the main porgies' hatcheries in Greece.

2. Breeding efforts for red porgies in Greece

Red porgies production from the aquaculture in Greece both for *P. major* and *P. pagrus*, have been increased from 37 kg in 1993 to a mean value of 1740 t during 2014-2017 (HELSTAT 1992–2018). This data series exhibited a long-term gap of data during 1995-2011, as there are no available records for the periods from 1995 to 1998 and from 2000 to 2011. This seems paradoxical for a business activity of a high price product in the Eastern Mediterranean (Boudet *et al*. 2004 in Pavlidis & Mylonas 2011), but it could be explained by interruptions observed in the production of red porgies due to lack of know-how in animal husbandry, nutrition, reproduction and the problems faced due to coloring that made questionable the sustainability of the breeding of porgies during 1990's. This lack of knowledge mainly referred to the native species *P. pagrus* and, despite the intensive research efforts to cover it (Kentouri *et al*. 1995; Kokokiris 1998), as can be seen from the result, led to its total replacement in the fish industry by *P. major*, already present in Mediterranean farms and farms (Barbato & Corbari 1995; Kraljevic & Dulcic 1999). In particularly, farming of *P. major* in Greece began at 1986 with the supply of eggs from an aquaculture unit of southern Italy into a hatchery in Ithaka island, Ionian Sea (P. Kolios, personal communication, August 7, 2018).

The produced juveniles were supplied from fish farms of the Evian Gulf, the Gulf of Corinth and the Sagiada region (North Ionian). In addition, during 1991-92, *P. major* juveniles from the Maricoltura Italia Fish Breeding Station (Monfalcone, GO) was introduced for breeding in a fish farm of the Gulf of Corinth. Subsequently, fish from these units, selected on the basis of their satisfying growth rate and appearance, formed the stocks of red porgies breeders that exist today in most Greek marine hatcheries. A detailed list related to the main steps on the farming of this species in East Mediterranean Sea is given in Table 1. Nowadays, this species is sold fresh in the Greek market as a fish farming product. Recently, a formal request from the Hellenic Republic for amendment of Annex IV of Council Regulation (EC) No 708/2007 concerning the use of alien and locally absent species in aquaculture in order to add *P.major* in the above list has been submitted.

Table 1. List of the main references related to aquaculture of *Pagrus major* in Greece.

FAO fishing areas	Country	Year	Species	Activity	Referenceς
GSAs20	Greece	1986-1990	*P. major*	Farming	Sweetman, 1992
GSAs20	Greece	1994	*P. major*	Farming	Le Breton & Marques, 1995
GSAs17, GSAs20	North Adriatic Sea and Greece	1991	*P.major* ♂ *x D.dentex* ♀ hybrids	Farming	Argenton *et al*., 1998

3. The rapid increase in production in recent years

Annual production recorded systematically from 2011 onwards, reached in 2016 the largest amount of 3041 t, registering an impressive average increase between 2012 and 2016 of 75.5% per year. During the last two years there has been a great increase in the production of red porgy fry from Greek hatcheries, which will lead to high productions in the following years derived from large crops. From interviews conducted to hatchery executives, the expected production of juveniles of *P. major* for 2018 will reach 12 million pieces. From a spatial point of view, our research revealed that the large number (77.7%) of *P. major* juveniles produced in Greece during the last three years was channeled to fish farms located in the Ionian Sea (GSA 20), while the large production of fry is carried out in hatcheries located in the gulf of Evian in the Aegean Sea (GSA 22). However, it is difficult to predict the future quantities of porgies' crops, because the strategies of the companies are different and also under redesign in terms of marketable fish sizes.

4. First records for presence in the wild

So far, *P. major* specimens caught in the Mediterranean marine environment have been reported by Dulcic & Kraljevic (2007) in the Adriatic Sea, by Cladas *et al*. (2019) in the Ionian Sea and by Kampouris *et al*. (2020) in the Thermaikos Gulf in the North Aegean Sea. However *P. major* is already mentioned in the updated check list of the fish species in Greek seas (Papaconstantinou 2014) based on "unverified information that exotic fish species (for instance *P. major*) …. eventually other species too…., are used largely in marine fish farms in Greece" (Corsini-Foka & Economidis 2007). Given that *P. major* is a non-native species, it is of great interest to investigate the impact of its presence in the marine environment of Greece, with the aim of answering the key question if this species is capable of establishing viable populations in the region. The lack of information from the fisheries sector makes it difficult to evaluate the imprint of the presence of this non-native species in the Eastern Mediterranean.

This may be due to the difficulty of visual distinction of *P. major* from the native congener *P. pagrus* which has the same basic reddish coloration of the body and the same shape. In fact, the color differences, mainly those of the caudal fin that address the respective identification keys (Whitehead *et al*. 1986; Nakabo 2002), are not easily distinguishable, particularly in farmed fish, in which the quality and intensity of coloration differ significantly from the applied techniques of breeding, harvesting and pre-slaughter (Foscarini 1988; Lin *et al.* 1998a; 1998b; Papaharisis *et al*. 2019), as well as the duration of ice storaging (Pavlidis *et al.* 2006; Erikson & Misimi 2008; Vardanis *et al.* 2011; Erikson *et al.* 2019).

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References

Argenton F., Barbaro A., Belvedere P., Bortolussi M., Colombo L., Dalla Valle L., Francescon A., Libertini A., Vianello S. (1998). Towards an integrati on between chromosome set manipulation, intergeneric hybridization and gene transfer in marine fish culture. In: "Genetics and breeding of Mediterranean aquaculture species" Bartley D.M., Basurco B. (eds), Zaragoza, CIHEAM Cahiers Options Méditerranéennes 34, 77 -122.

Barbato F., Corbari L. (1995). New species in Italy. Aspects économiques de la production aquacole. Zaragoza, CIHEAM Cahiers Options Méditerranéennes 14, 123-128.

Cladas Y., Spala K., Doudoumis V., Ketsilis-Rinis V., Batargias C., Koutsikopoulos C. (2019). Presence Confirmation of Non-Native Species Pagrus major (Temminck and Schlegel, 1843) in the Eastern Mediterranean. Oceanography & Fisheries Open access Journal 9(2), 555756.

Corsini-Foka M., Economidis P.S. (2007). Allochthonous and vagrant ichthyofauna in Hellenic marine and estuarine waters. Mediterranean Marine Science, 8 (1), 67 - 89.

Dulcic J., Kraljevic M. (2007). On the record of red seabream Pagrus major (Temminck and Schlegel, 1843) (Osteichthyes:Sparidae) in the Adriatic Sea. Scientia Marina 71(1), 15-17.

Erikson U., Truong H.T.M., Le D.V., Pham P.D., Svennevig N., Phan V.T. (2019). Harvesting procedures, welfare and shelf life of ungutted and gutted shortfin pompano (Trachinotus falcatus) stored in ice. Aquaculture 498, 236–245. https://doi.org/10.1016/j.aquaculture.2018.06.085

Foscarini R. (1988). A Review: Intensive Farming Procedure for Red Sea Bream (Pagrus major) in Japan. Aquaculture 72, 191-246.

HELSTAT (1992–2018). Results of the sea fishery survey by motor boats for the years 1990-2017. Athens, Greece: Hellenic Statistical Authority of Greece. http://www.statistics.gr/el/statistics

Kampouris T.E., Economidis P.S., Batjakas I.E. (2020). First record of Pagrus major (Temminck & Schlegel, 1843) (Perciformes: Sparidae) from east Mediterranean Sea and the northernmost Mediterranean record of Por's goatfish Upeneus pori Ben-Tuvia & Golani, 1989 (Perciformes: Mullidae) from Thermaikos Gulf, North-West Aegean Sea, Greece. Cahiers de Biologie Marine 61, 253-258. https://doi.org/10.21411/CBM.A.F4BCFF17

Kentouri M., Pavlidis M., Papandroulakis N., Divanach P. (1995). Culture of the red porgy, Pagrus pagrus in Crete. Present knowledge, problems and perspectives. Proceedings of the seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), Nicosia, Cyprus, 14-17 June 1995. Paris: CIHEAM/MEDRAP II, 1995. Cahiers Options Mediterranean. 16, 65-75.

Kokokiris, L. (1998). The reproductive cycle and hermaphrodite pattern in Pagrus pagrus. PhD thesis, University of Crete, pp. 219.

Kraljevic M, Dulcic J. (1999) Intergeneric hybridization in Sparidae (Pisces: Teleostei) Dentex dentex ♀x Pagrus major ♂ and P. major ♀x D. dentex ♂. Journal of Applied Ichthyology 15, 171-175.

Le Breton A., Marques A. (1995) Occurrence of a histozoic Myxidium infection in two marine cultured species: Puntazzo puntazzo C. and Pagrus major. Bulletin of the European Association of Fish Pathologists 15, 210–212.

Lin M.Q., Ushio H., Ohshima T., Yamanaka H., Koizumi C. (1998a). Effect of low temperature treatments on K+-induced melanosome aggregation in melanophores of cultured red sea bream. Nippon Suisan Gakkaishi (Japanese Edition) 64(2), 280-285.

Lin M.Q., Ushio H., Ohshima T., Yamanaka H., Koizumi C. (1998b). LWT - Skin Color Control of the Red Sea Bream (Pagrus major). Food Science and Technology 31(1), 27-32.

Nakabo, T. (2002). Fishes of Japan with pictorial keys to the species. English edition I. Japan: Tokai University Press.

Papaharisis L, Tsironi T, Dimitroglou A, Taoukis P, Pavlidis M. (2019). Stress assessment, quality indicators and shelf life of three aquaculture important marine fish, in relation to harvest practices, water temperature and slaughter method. Aquaculture Research 1–13. https://doi.org/10.1111/are.14217

Papaconstantinou C. (2014). Fauna Graeciae: An updated checklist of the fishes in the hellenic seas. Hellenic Centre for Marine Research (HCMR), ISBN: 978-960-9798-04-4, 338 p.

Pavlidis M.A., Mylonas C.C. (2011). Sparidae: Biology and aquaculture of gilthead sea bream and other species. Sparidae, Biology and Aquaculture of Gilthead Sea Bream and other Species. Online ISBN:9781444392210. https://doi.org/10.1002/9781444392210

Pavlidis M., Papandroulakis N., Divanach P. (2006). A method for the comparison of chromaticity parameters in fish skin: preliminary results for coloration pattern of red skin Sparidae. Aquaculture 258, 211–219.

Souto F.B., Villanueva R.X.L. (2003). European Fish Farming Guide. European Union, European Social Fund, ISBN: 84-453-3470-0.

Sweetman J.W. (1992) Larviculture of Mediterranean Marine Fish Species: Current Status and Future Trends. Journal of the World Aquaculture Society 23 (4), 330-337

Vardanis G., Sfichi-Duke L., Tort L., Divanach P., Kotzabasis K., Pavlidis M. (2011). The use of biochemical, sensorial and chromaticity attributes as indicators of post-mortem changes in commercial-size, cultured red porgy Pagrus pagrus, stored on ice. Aquaculture Research, 42, 341–350. https://doi.org/10.1111/j.1365-2109.2010.02628.x

Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nielsen J., Tortonese E. (1986). Fishes of the North-eastern Atlantic and the Mediterranean. UNESCO, Paris. Vols. I-III: 1473 p.

HUMORAL IMMUNE RESPONSE OF SEA BASS (*Dicentrarchus labrax*) WITH NATURAL RESISTANCE TO THE RGNNV GENOTYPE OF B-NODAVIRUS

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Abstract
Viral encephalopathy and retinopathy (VER) caused by b-nodavirus, RGNNV genotype, seriously affects sea bass ((*Dicentrarchus labrax*) intensive aquaculture in the Mediterranean. Studies in our laboratory and elsewhere have shown that sea bass has natural resistance to the virus. The aim of this study was to analyze the humoral immune response of a resistant to VER family of sea bass and correlate it with the expression of the IgHM gene. Groups of fish were either infected with b-nodavirus or injected with sterile PBS (- control) or with lipopolysaccharide (LPS) (+ control for immune response against bacteria) or with polyriboinosinic:polyribocytidylic acid (pI:c) (+ control mimicking a viral infection). Samples from cephalic kidney and blood were taken on Day 0 (hours 0, 3, 6 and 12 post-infection/injection – pi, hereafter) and on Days 1-4, 7, 14, 21 and 28pi. Analysis of specific anti-b-nodavirus antibody (Ab) levels in sera showed a significant increase at D14-D28 in the infected group that correlated well to increased expression of the IgHM gene. Similar were the findings for total Ab levels which were also correlated to the IgHM expression. These data could assist in elucidating further the differences between naturally resistant and sensitive to VER fish.
Keywords: *Dicentrarchus labrax*, *b-nodavirus, Viral encephalopathy and retinopathy, humoral immune response*
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1. Introduction

Betanodaviruses has been reported to cause disease to many commercially important fish species worldwide (Frerichs *et al*. 1996). Regarding prevention of fish viral nervous necrosis (VNN hereafter), various approaches have been tested experimentally such as DNA vaccines (Sommerset *et al*. 2005), heat-inactivated viral particles (Breuil & Romestand, 1999), recombinant capsid protein vaccines (Yuasa *et al*. 2002) and virus-like particles (Thiery *et al*. 2006) with or without success. Natural resistance of sea bass has been reported before (Doan *et al*. 2017) in wild populations with good prospective for the selective breeding of this trait and in other fish species Bangera *et al*. (2011). There are many studies on the humoral immune response of sea bass either after infection or immunization (Breuil & Romestand 1999; Yamashita *et al*. 2005; Liu *et al*. 2006; Pakingking *et al*. 2009; 2010; Lai *et al*. 2014), but all of them have been performed with fish populations of variable genetic background. The aim of this study was to analyze the humoral immune response of a resistant to VER family of sea bass and correlate it with the expression of the IgHM gene.

Materials & Methods

Fish, Facilities, Virus: Sea bass (*Dicentrarchus labrax*), healthy, unvaccinated, N=500, average weight 45.74g, from a b-nodavirus (RGNNV genotype)-resistant family were transported by Nireus Aquaculture S.A. to the wet laboratory (ICHTHYAI) of the Department of Marine Sciences, University of The Aegean [permit EL83 BioExp 01, according to the Presidential Decree 56/2013 conforming to Dir 2010/63/EE (Decision No 4053/14-3-2017 of the competent Regional Veterinary Authority]. Fish were maintained in a closed recirculated sea water system with solids filtration, disinfection (UV), biological filter and aeration. Water temperature maintained at 22-23°C during acclimatization of the stock and at 27.0-27.2°C during challenge. Photoperiod at 12h light:12h dark, salinity 3.8-3.9%, dissolved O_2 >4.8mg/l, pH 8, total ammonia nitrogen and nitrite <0.05ppm and <0.5ppm, respectively, and nitrate levels <40mg/l. A strain of b-nodavirus, RGNNV genotype isolated from a cultured European sea bass outbreak in 2012 in Western Greece, was utilized.

Injections & sampling: Required number of fish was transported to the allocated tank system where temperature was gradually raised to 27.0-27.2°C and then fish were acclimatized for a further 7 days. Fish handling, injections, blood collection, etc. were performed under anesthesia using 0.2% phenoxyethanol. Fish were injected intramuscularly (i.m.) in the dorsal muscle with the following solutions creating 4 groups: a) Group NNV: Fish (N=230) i.m. infected (Vimal *et al*. 2014) with 100μl of virus solution containing $7x10^6$ $TCID_{50}$/ml; b) Group (-) control: Fish (N=70) were i.m. injected with 100μl of sterile PBS; c) Lipopolysaccharide group (LPS) (+) control for immune response against bacteria: Fish (N=70) were i.m. injected with 100μl of a solution containing 2.5mg/ml bacterial (*E.coli*) LPS in 0.15M PBS; d) Group pI:c (+) control mimicking viral infection: Fish (N=70) were i.m. injected with 100μl of a solution containing 2.5mg/ml Polyinosinic: polycytidylic acid in 0.15M PBS. Fish from each group were randomly distributed in 3 tanks. Fish were monitored twice daily and mortalities were recorded. Protocol of experimental infection approved by Decision No 5379/4-4-2017, of the competent Regional Veterinary Authority. Samples from cephalic kidney and blood were taken on Day 0 (hours 0, 3, 6 and 12 post-infection/injection – pi, hereafter) and on Days 1-4, 7, 14, 21 and 28pi. Blood was allowed to clot for 10min at room temperature and then overnight at 4°C and then it was centrifuged at 750 x g for 10min to separate the serum.

Cause of death & preparation of rabbit and sea bass anti-betanodavirus polyclonal serum: Confirmation b-nodavirus presence in brain and eye samples from fish that died post-infection in the NNV group was performed with RT-PCR according to Dalla Valle *et al*. (2000). To exclude a bacteriological cause of death, microbiological sampling from cephalic kidney and identification of bacteria was performed following routine procedures. Rabbit and sea bass anti-betanodavirus polyclonal serum were prepared according to Bakopoulos *et al*. (1997a) with slight modifications. Rabbit and sea bass serum was titrated against b-nodavirus antigen using a simple indirect ELISA.

Measurement of specific anti-b-nodavirus and total sea bass serum antibodies & of B lymphocyte (IgHM) related gene expression: Sera from each group were utilized in a modified sandwich-ELISA analysis according to (Bakopoulos *et al*. 1997b) for the determination of specific anti-betanodavirus IgM levels. Total antibody levels of all serum samples and groups were measured using a simple indirect ELISA. Total RNA was extracted from HK and 5μg RNA were used for cDNA synthesis. SYBR Green-based real-time PCR reactions were performed in triplicate to assess IgHM expression.

Statistical analysis: All statistical differences between individual serum results (either specific a-betanodavirus or total antibodies) within and between time-points and groups were assessed with one-way ANOVA followed by Tukey multiple comparison test.

Results & Discussion

Fish mortality: Mortalities were recorded only from the infected NNV group. Affected fish showed the typical symptoms of viral nervous necrosis (VNN) (Breuil *et al*. 1991; Munday *et al*. 2002). Mortalities initiated on day 4 pi, after a sharp increase reached highest levels between days 6 and 8 pi and then after a sharp decrease, low daily mortality the following days was observed until the conclusion of the experiment and this pattern is supported by previous studies ((Thiery *et al*. 2006; Bakopoulos *et al*. 2018a; b). Out of a total of 230 individuals in the NNV group, 48 animals succumbed to the infection representing 20.83% of the population. Similar infectious doses has caused in general sea bass populations either 57.3% (Bakopoulos *et al*. 2018a) or 55-81.7% (Bakopoulos *et al*. 2018b) or 80.5% cumulative mortality (Thiery *et al*. 2006), but the lower mortality in this study is attributed to the natural resistance of the fish utilized. Natural resistance of sea bass has been reported before (Doan *et al*. 2017) in wild populations with good prospective for the selective breeding of this trait and in other fish species Bangera *et al*. (2011; 2013). Betanodavirus was detected in all the samples taken from dead fish, while microbiological sampling did not reveal the presence of any bacterial pathogen.

Humoral immune response: Specific anti-b-nodavirus Abs in the NNV group fluctuated insignificantly at low levels during the first 4 days post-infection and they gradually increased from D7 onwards (Fig. 1a). Specific anti-b-nodavirus Ab levels of all time-points from D0 to D7 were significantly lower compared to D14, 21 and 28. Non-specific and very low anti-b-nodavirus Ab activity remained at similar levels with fluctuations across all time-points (Fig.1b) in the (-) control with no significant differences between time-points. Reactions of Abs against b-nodavirus Ags in the pI:c group (Fig. 1c) were not-significant between time points and are considered as non-specific cross-reactions and they were very low at most time-points. In the latter group, after a short increase of activity during the first 12h post-injection, Ab levels dropped and fluctuated at similar levels until D4 when an increase in activity was observed at D7.

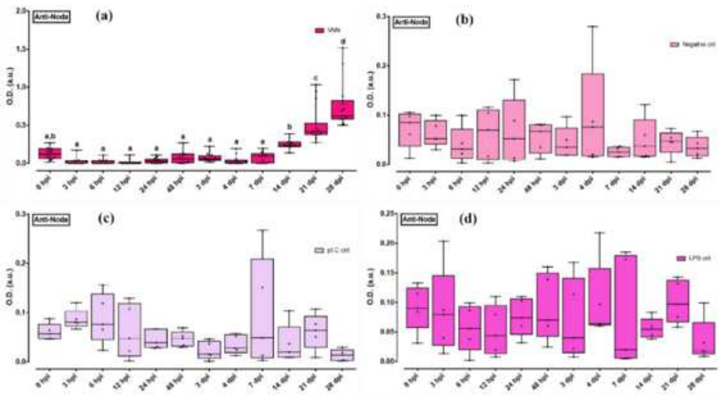

Figure 1. Specific anti-betanodavirus O.D. levels of all groups (a): NNV; (b): (-) control; (c): pI:c control; (d): LPS control are given as bounds of box and whisker plots with min-to-max values. All statistical differences were assessed with one-way ANOVA followed with Tukey multiple comparison test. Different lower-case letters denote statistically significant differences.

Most if not all of this activity may be attributed also to natural Abs that bind to b-nodavirus Ags. Insignificant fluctuations of very low anti-b-nodavirus Ab binding activity was observed between time-points in the LPS group (Fig.1d), with levels raising during D2 to D7 post-injection, after a slight drop the first 24h. There were no significant differences of specific anti-b-nodavirus Ab levels in the LPS group between time-points. Specific anti-b-nodavirus Abs in group VNN after day 14 pi were significantly higher in comparison to the negative control group, as well as in comparison to the LPS and pI:c groups and correspond well with an elevation of the IgHM gene expression on D7 and D14 pi. In the (-) control group no elevation of the latter gene was measured, hence the insignificant specific anti-b-nodavirus levels measured. IgHM gene expression in the pI:c group elevated 6h and on D3 pi and may account for the (insignificant) fluctuations of anti-b-nodavirus Ab activity in this group on 6h and D7 pi. Finally, the expression of IgHM gene in the LPS group increased between D2 to D4 p.i. and this may be responsible for the slight increase of specific anti-b-nodavirus activity evidenced in the LPS group the same period. The motif of humoral immune response reported here in the VNN group is supported by the findings of (Pakingking *et al*. 2009; 2010). Interestingly, great variation was noted in the levels of specific anti-b-nodavirus activity in the VNN group, especially during those time-points where this activity was elevated. A reason for this fluctuation could be individual variation in the humoral immune response against the virus which is something evidenced in many other studies (Breuil & Romestand, 1999; Yamashita *et al*. 2005; Liu *et al*. 2006; Pakingking *et al*. 2009; 2010; Lai *et al*. 2014). This may very well be due to differences in various genetic loci among related individuals. This hypothesis is also supported by the observation of non-responding fish in the study of Thiery *et al*. (2006), by the mortalities recorded, as well as by research that has been undertaken in our laboratory on natural disease resistance of European sea bass families (Bakopoulos *et al*. 2018a) and certainly demands additional research and clarification. Some very low anti-b-nodavirus activity has been observed in the (+) and (-) control groups (pI:c, LPS and negative control, respectively). Such activity has been reported before from adjuvants or irrelevant to the virus antigens (Wedrychowicz *et al*. 1992; Acosta *et al*. 2004; Haugland *et al*. 2005; Lee *et al*. 2018). These cause a non-specific stimulation of the immune system, innate or adaptive (such as pI:c or LPS in our study), supporting our observation.

Total Ab levels in the NNV group (Fig. 2a) showed a gradual decrease from 0h to D1, an increase during D2 to D3, dropped on D4 and D7 and then increased gradually during the following days D14 to D21. Ab levels during D14 to D28 were significantly higher when compared to the other time-points, except for D3 post-infection. Ab levels at D28 were significantly higher when compared to D14. The observed pattern is related to the expression of the IgHM gene in this group. Similarly, total Ab levels in the (-) control group (Fig. 2b), rather the lack of any change, correlates exactly to the lack of expression of the IgHM in this group. An increased expression of the IgM gene observed in the pI:c group 6h p.i. may be responsible in the observed increase in total Ab levels during time-points D3-D7 (Fig. 2c); pI:c is a viral mimic since it has similar stimulating effects of viral dsRNA and apparently the activation it exerts influences also IgHM expression in the short term.

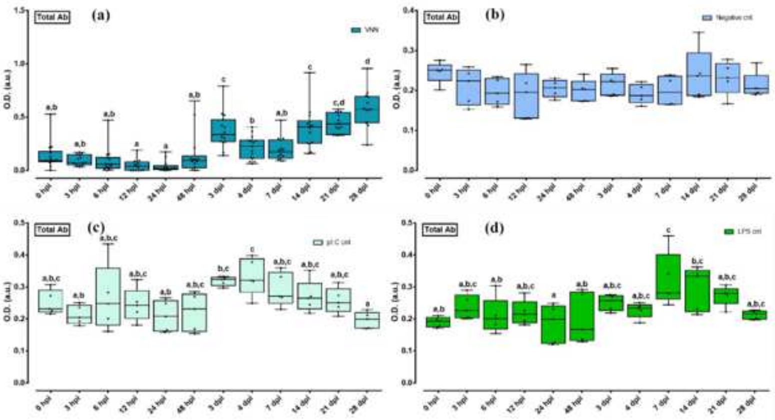

Figure 2. Total Ab O.D. levels of all groups (a): NNV; (b): (-) control; (c): pI:c control; (d): LPS control are given as bounds of box and whisker plots with min-to-max values. All statistical differences were assessed with one-way ANOVA followed with Tukey multiple comparison test. Different lower-case letters denote statistically significant differences.

Total Ab levels of the LPS group fluctuated at similar levels from the first time-point to D4 post-injection (Fig. 2d). On D7 increased and the following time-points dropped gradually. The D7 increase in total Ab levels was significant compared to time-points 0h to D2 post-injection and this increase correlates well to an increase in IgHM gene expression during D3 to D4 p.i.

Acknowledgements

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References

Acosta, F., Lockhart, K., Gahlawat, S.K., Real, F., Ellis, A.E. (2004). Mx expression in Atlantic salmon (*Salmo salar* L.) parr in response to *Listonella anguillarum* bacterin, lipopolysaccharide and chromosomal DNA. Fish Shellfish Immunol. 17(3), 255-263.

Bakopoulos, V., Palaiologos, A., Kantham, P., Kottaras, L., Kyvelou, V., Dimitroglou, A., et al. (2018a). Selective breeding and disease resistance of sea bass, *Dicentrarchus labrax*, against viral nervous necrosis. 3rd International Congress on Applied Ichthyology & Aquatic Environment, Hydromedit 2018, Volos, Greece, 8-11 November 2018.

Bakopoulos, V., Palaiologos, A., Poulos, K., Malatestas, K., Dimitrakopoulou, E., Spinos, E., Kokkoris, G.D. (2018b). Efficacy testing of anti-VNN vaccine formulations against the RGNNV b-nodavirus genotype in European sea bass (*Dicentrarchus labrax*). 3rd International Congress on Applied Ichthyology & Aquatic Environment, Hydromedit 2018, Volos, Greece, 8-11 November 2018.

Bakopoulos, V., Volpatti, D., Adams, A., Galleotti, M., Richards, R.H. (1997a). Qualitative differences in the immune response of rabbit, mouse and sea bass, *Dicentrarchus labrax*, L, to *Photobacterium damsela* subsp. *piscicida*, the causative agent of fish Pasteurellosis. Fish Shellfish Immunol. 7, 161-174.

Bakopoulos, V., Volpatti, D., Papapanagiotou, E., Richards, R.H., Galeotti, M., Adams, A. (1997b). Development of an ELISA to detect *Pasteurella piscicida* in culture and in "spiked" fish tissue. Aquaculture 156(3/4), 359-365.

Bangera R., Ødegård J., Præbel A.K., Mortensen A., Nielsen H.M. (2011). Genetic correlations between growth rate and resistance to vibriosis and viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). Aquaculture 317, 67–73.

Bangera R., Ødegård J., Nielsen H.M., Gjøen H.M., Mortensen A. (2013). Genetic analysis of vibriosis and viral nervous necrosis resistance in Atlantic cod (*Gadus morhua* L.) using a cure model. Journal of Animal Science 91, 3574–3582.

Breuil G., Bonami J.R., Pepin J.-F., Pichot Y. (1991). Viral infection (picorna-like virus) associated with mass mortalities in hatchery-reared sea-bass (*Dicentrarchus labrax*) larvae and juveniles. Aquaculture 97, 109-116.

Breuil, G., Romestand, B. (1999). A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of the sea bass, *Dicentrarchus labrax* (L.): application to the screening of spawners in a sea bass hatchery. J. Fish Dis. 22, 45-52.

Dalla Valle, L., Zanella, L., Patarnello, P., Paolucci, L., Belvedere, P., Colombo, L. (2000). Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. J. Fish Dis. 23, 321-327.

Doan Q.-K., Vandeputte M., Chatain B., Haffray P., Vergnet A., Breuil G., Allal F. (2017). Genetic variation of resistance to Viral Nervous Necrosis and genetic correlations with production traits in wild populations of the European sea bass (*Dicentrarchus labrax*). Aquaculture 478, 1–8.

Frerichs, G.N., Rodger, H.D., Peric, Z. (1996). Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, Dicentrarchus labrax. J. Gen. Virol. 77, 2067-2071.

Haugland, Ø., Torgersen, J., Syed, M., Evensen, Ø. (2005). Expression profiles of inflammatory and immune-related genes in Atlantic salmon (*Salmo salar* L.) at early time p.v. Vaccine 23, 5488-5499.

Lai, Y-X., Jin, B-L., Xu, Y., Huang, L-J., Huang ,R-Q., Zhang, Y., Kwang, J., et al. (2014). Immune responses of orange-spotted grouper, *Epinephelus coioides*, against virus-like particles of betanodavirus produced in *Escherichia coli*. Vet. Immunol. Immunopath. 157, 87-96.

Lee, Y.J., Lee, J.Y., Jang, Y.H., Seo, S-U., Chang, J., Seong, B.L. (2018). Non-specific effect of vaccines: Immediate protection against respiratory syncytial virus infection by a live attenuated influenza vaccine. Frontiers Microbiol. 9(83), 1-11.

Liu, W., Hsu, C-H., Chang, C-Y., Chen, H-H., Li, C-S. (2006). Immune response against grouper nervous necrosis virus by vaccination of virus-like particles. Vaccine 24, 6282-6287.

Munday, B., Kwang, J., Moody, N.J. (2002). Betanodavirus infections of teleost fish: a review. J. Fish Dis. 25, 127-142.

Pakingking Jr, R., Bautista, N.B., de Jesus-Ayson, E.G., Reyes, O. (2010). Protective immunity against viral nervous necrosis (VNN) in brown-marbled grouper (*Epinephelus fuscogutattus*) following vaccination with inactivated betanodavirus. Fish Shellfish Immunol. 28, 525-533.

Pakingking Jr, R., Seron, R., dela Pena, L., Mori, K., Yamashita, H., Naka, T. (2009). Immune responses of Asian sea bass, *Lates calcarifer* Bloch, against an inactivated betanodavirus vaccine. J. Fish Dis. 32, 457-463.

Sommerset, I., Skern, R., Biering, E., Bleie, H., Fiksdal, I.U., Grove, S., Nerland, A.H. (2005). Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination. Fish Shellfish Immunol. 18, 13-29.

Thiery, R., Cozien, J., Cabon, J., Lamour, F., Baud, M., Schneemann, A. (2006). Induction of a protective immune response against Viral Nervous Necrosis in the European sea bass *Dicentrarchus labrax* by using Betanodavirus Virus-Like Particles. J. Virol. 80(20), 10201-10207.

Vimal, S., Madan, N., Farook, M.A., Nambi, K.S.N., Majeed, S.A., Rajkumar, T., et al. (2014). Production of recombinant vaccine using capsid gene of nodavirus to protect Asian sea bass, *Lates calcarifer* (Bloch, 1790), Aquaculture 418-419, 148-154.

Wedrychowicz, H., Tait, A., Bairden, K., Holmes, P.H. (1992). Immune responses of sheep to surface antigens of infective larvae of *Ostertagia circumcincta*. Parasite Immunol. 19, 249-266.

Yamashita, H., Fujita, Y., Kawakami, H., Nakai, T. (2005). The Efficacy of inactivated virus vaccine against Viral Nervous Necrosis (VNN). Fish Path. 40(1), 15-21.

Yuasa, K., Koesharyani, I., Roza, D., Mori, K., Katata, M., Nakai, T. (2002). Immune response of humpback grouper, Cromileptes altivelis (Valenciennes) injected with the recombinant coat protein of betanodavirus. J.Fish Dis. 25, 53-56.

GROWTH PERFORMANCE OF SEABASS (*Dicentrarchus labrax*) AND ROCKET PLANT (*Eruca sativa*) IN A BRACKISH AQUAPONICS SYSTEM USING THE NUTRIENT FILM TECHNIQUE (NFT)

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Abstract
The aim of the study was the effect of salinity on the growth performance and survival of sea bass (*Dicentrarcus labrax*) and rocket plant in a nutrient film technique (NFT) brackish aquaponic system under three different salinities (1.5 ppt, 5 ppt and 7 ppt). Three nutrient substrate aquaponic systems (NFT) of 375.2 L, were used, which were consisting of three fish tanks of 109.2 L, a hydroponic pipe nutrient film (NFT) of 16,6 L and were supported by a sump filter of 100 L. In total 90 juvenile seabass were used (average initial weight of 3.92 ± 0.83 g and initial length of 7.62 ± 0.47 cm), and 12 rocket plants (average initial height of 10.7 ± 1.2 cm) in NFT hydroponic pipes for 45 days. The findings of this study showed that salinity affects significantly the growth performance of rocket plants indicated that 1.5 ppt was better salinity to grew rocket plants in an NFT aquaponics system. Significantly higher final height, percentage height increase was observed in salinity 1.5 ppt compared to the salinities 5 ppt and 7 ppt, respectively ($p<0.05$). Despite that, no significant differences were reported in the growth performance of sea bass in three salinities ($p >0.05$). There was a high survival rate for both seabass and rockets plants. The present study highlights the importance of growing rocket plants and sea bass in a brackish aquaponic system using 1.5 ppt salinity.
Keywords: *Brackish aquaponic system, NFT, growth performance, Dicentrarchus labrax, Eruca sativa.*
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1. Introduction

Aquaponic systems are characterized in many studies as an ecofriendly, sustainable, and innovative method for producing food in a close recirculating aquaculture system (RAS) (Endut *et al*. 2010; Fronte *et. al*. 2016; Vlahos *et al*.2019; Stathopoulou *et al*. 2021). It combines the aquaculture of aquatic organisms (finfishes, inverterbrates), the hydroponic culture of plants (vegetables, herbs) and bacteria which use to increase the filter capacity and efficiency. Accumulated nutrients (ammonia, nitrite, nitrate, phosphorus) are recycled through the water and absorbed by the plants for their growth. Nutrient Film Technique (NFT) is used for plants and herbs aquaponics in PVC pipes creating a grow bed channel which enhance solid waste removal (Nelson 2008; Lennard 2021). According to Lennard (2021), NFT is only suitable for small vegetable species because the grow bed cannot support high quantity of roots and provides high oxygen flow rate to the plant roots that leads to increase the plant production. The roots of the plants in the NFT aquaponics system are in constant contact with nutrient solution, creating a thin film of depth ranging from 0.076 cm to 0.254 cm. Effective solid waste removal plays an important role for the NFT aquaponic system to avoid clogging the effluent of the NFT nutrient film pipe and making it difficult for water to pass through. The flow rate of water (nutrient solution) is estimated to be between 400 mL/min and 1500 mL/ min. For better operation of the system, the nutrient pipe should be placed slightly inclined.

Brackish aquaponics system may combine rearing Mediterranean euryhaline species of fish such as sea bream, sea bass or Mugilidae species and plant species that are adapted to salinity (halophytes, herbs or vegetables, algae) which exhibit high commercial nutritional value (Fronte *et al*. 2016; Vlahos *et al.* 2019). The aim of the present study was the effect of salinity on the growth performance and survival of sea bass (*Dicentrarcus labrax*) and rocket plant in a NFT brackish aquaponic system under three different salinities (1.5 ppt, 5 ppt and 7 ppt).

2. Material and Methods

The experiment was conducted at the experimental facility of the Laboratory of Aquaculture -Aquarium unit, Department of Animal Production, Fisheries and Aquaculture (University of Patras, Greece). Sea bass juveniles and rocket plants arrived from a local nursery facility and the laboratory of plant physiology of the Department of Plants production in Mesollognhi, respectively. The adaptation period for both fish and plants in the experimental salinities was 30 days and took place gradually. In fish tanks salinity was reduced by 5 ppt once a week while at the plants saltwater was added every three days.

After the successful adaptation of the seabass to the three salinities (1.5ppt, 5ppt and 7 ppt), 90 juvenile (10 individual /aquarium) sea bass (*D. labrax*) individuals, with an average body weight of 3.92 ± 0.83 g and an average body length of 7.62 ± 0.47 cm, were placed in the three aquaponic fish tanks. 12 rocket plants (4 plants /NFT hydroponic bed) evenly were placed in each hydroponic NFT pipe. Fish and plants were divided into three treatments under three different salinities 1.5 ppt, 5 ppt and 7 ppt, respectively. The three salinities were chosen as a criterion for rocket plant growth performance and survival. The salinity of 1.5 ppt was chosen as the ideal salinity that the rocket plant grew well according to dos Santos & Rolim (2011). The salinities of 7 ppt and 5 ppt were chosen as the higher and medium salinity that affects the adaptation and growth performance of the rocket plant, respectively.

Each salinity treatment consisted of three rectangular fish tanks (40 cm x 35 cm x 26 cm) with 36.4 L volume, three hydroponic cultivation pipes (Φ 100 mm) creating a volume of 16.6 L as described by Nelson (2008) and Lennard (2021) as a nutrient film technique methods cultivation. Nine autonomous closed recirculation aquaponic systems were constructed with a total volume of 375.2 L per system. Each aquaponic system was supported by a biological sump filter of a total volume of 100 L, which consisted of a mechanical filter, and a biofilter section and having a significant contribution in the nitrification process by increasing filter capacity and efficiency. In the last part of the filter a pump (Aquametic, 22 W, 1200 L / h) was placed to supply the aquaponic systems with the water through the filter, creating a flow rate of 38±9.6 m^3/d with a water filtrarion speed of 0,73 cm/min through the aquaponic system (fish tank and NFT pipe). Also, in each aquaponic system a 300 watts lamp (AQUA MEDIC aqua sun light NG 2 X 150W + 2 X T5 54W) was placed at a distance of 60 cm from the hydroponic NFT pipe to ensure the appropriate exposure of plants light. Photoperiod was adjusting to be 9 h light : 15 h dark (winter photoperiod). The active photoperiod radiation of the lamps was set up to be at 400-500 μmol m^2 sec^{-1}.

The fish were fed three times per day with 5% of their body weight by hand with a commercial pellet diet (formula ONE Marine Pellet, ocean nutrition medium pellet 1.5 mm) (crude protein 41,1%, crude fat 9%). The feeding rate was adjusted to fish weight every two weeks. Fish tanks were cleaned, and uneaten food and faeces were removed every day by siphoning. At the end of the experiment, fish were anaesthetized with an 0.20 mg/L MSS 222, and final fish body weights and lengths were measured. Oxygen levels were adjusting up to 75-85% saturation for the whole experimental period. At the end of the experiment growth performance indicators were measured for fish according to the following: SGR (%/day)= $[(\ln W_{fin}-\ln W_{in}) / \Delta t]x100$, WG (gr)= $W_{fin}-W_{in}$, FCR= Food offered (gr) / weight gain (gr), DFI (%/d)= 100x[(food offered/weight gain) / feeding days], Condition factor (K) = (WxL^{-3}) x100 where W is the bodyweight of the fish (gr) and L is the total length of the fish (cm) and S= [(final number of fish - initial number of fish) / initial number of fish] x100 (Mente *et al*. 2016). The growth rate of the plants was calculated according to the equation (Endut *et al.* 2010): plant growth (cm/d) = height of plant/day, plant growth (G%) =(Final plant height -Initial plant height) *100/initial plant height. The Volumetric ammonia rate/ m^3filter media/d (VTR) was calculated using the equation: VTR (gr TAN/m^3/d) = $(TAN_{in}-TAN_{out})*Q/V$, where TAN_{in} and TAN_{out} (mg/L) was the TAN influence and effluent of the filter bet, Q was the flow rate (L/h) and V was the volume of the filter media (m^3). Water parameters such as total ammonia nitrogen (Tan), nitrite, nitrate ion, iron, and phosphorous were analyzed once a week using a spectrophotometry analyzer (HACH3800). pH also was measured twice a week using a multimeter (HACK 40QH). Data were checked for comparisons of the means (one-way ANOVA), normality (Kolmogorov-Smirnov), homogeneity (Levene test). They were considered statistically significant at $p<0.05$ (Zar 1999). Statistical analyses were carried out using the software package IBM SPSS Statistics V25.

3. Results

The results of the physicochemical parameters for the whole experimental period are presented in Table 1. There were no significant statistical differences (ANOVA, $p>0.05$) between the means of $NO_3^-N_{in}$, $NO_3^-N_{out}$, $PO_4^{=}{}_{out}$, pH, Fe, T°C in all treatments. TAN_{in} was statistically significantly higher (ANOVA, $p<0.05$) in the aquaponic system with 1.5 ppt and 5 ppt than those in 7 ppt salinity, respectively (Table 1). $PO_4^{=}{}_{in}$ was statistically significant higher ($p<0.05$) at 1.5 ppt salinity level than those in 5 ppt and 7 ppt salinities, respectively. VTR (gr TAN/m^3/d) was statistically significantly higher at 1.5 ppt and 7 ppt treatment in comparison to the 5ppt salinity treatment (Table 1). Fish and plant growth performance are presented in Table 2. At the start of the experiment, there were no statistical significant differences in the means of the seabass initial body weights and lengths and rockets plants initial height ($p>0.05$) for all treatments. At the end of the experiment, there were no significant statistical differences in the means of the fish final body weight, final length, weight gain, coefficient factor (K_{in}, K_{out}), body weight increase (%) in all treatments, respectively ($p>0.05$). Specific growth rate (SGR) was statistically significantly better at 7 ppt than the 1.5 ppt and 5 ppt salinity treatments. Survival rate for seabass was 90%, 80% and 93.3% in 1.5 ppt, 5 ppt and 7 ppt salinity treatments, respectively. FCR and DFI at the end of the experiment were no statistical significant ($p>0.05$) different between the salinity treatments (Table 2). Rocket growth performance (final Height, dH and % G) was statistically significantly higher at 1.5 ppt than 5 ppt and 7 ppt salinities (Table 2).

Table 1. Rearing conditions TAN_{in} , $NO_3^-N_{in}$, $NO_3^-N_{out}$, $PO_4^{=}{}_{in}$, $PO_4^{=}{}_{out}$, pH, Fe, T°C in the fish tank and the hydroponic NFT pipe of the brackish aquaponic system over the experimental period of 45 days.

	1.5 ppt	**5 ppt**	**7 ppt**
TAN_{in} (mg/L)	0.08 ± 0.01^a	0.08 ± 0.01^a	0.04 ± 0.01^b
VTR (gr TAN/m^3/d)	5.96 ± 2.08^a	5.21 ± 1.35^b	6.85 ± 0.69^a
$NO_3^-N_{in}$ (mg/L)	28.64 ± 7.43^a	15.37 ± 3.84^a	6.80 ± 2.84^a
$NO_3^-N_{out}$ (mg/L)	25.09 ± 5.66^a	12.34 ± 2.89^a	5.46 ± 1.70^b
$PO_4^{=}{}_{in}$ (mg/L)	3.68 ± 0.42^a	1.55 ± 0.72^b	0.32 ± 0.06^b
$PO_4^{=}{}_{out}$ (mg/L)	$0.31 \pm 0,02^a$	0.37 ± 0.10^a	0.17 ± 0.4^a
Fe_{in} (mg/L)	0.10 ± 0.01^a	0.09 ± 0.02^a	0.09 ± 0.01^a
T °C	22.7 ± 0.02^a	22.7 ± 0.27^a	22.7 ± 0.27^a
pH	7.6 ± 0.07^a	7.7 ± 0.27^a	7.7 ± 0.07^a

Data were expressed as mean ± S.E.M (n=10). Means in a row followed by the same superscript are not statistically significantly different ($p>0.05$).

Table 2. Seabass and rocket plant growth performance of the brackish aquaponic system over the experimental period of 45 days.

	1.5 ppt	**5 ppt**	**7 ppt**
Initial average weight (W_{in}, gr)	3.82 ± 0.17^a	4.14 ± 0.16^a	3.89 ± 0.10^a
Final average weight (W_{d45}, gr)	7.84 ± 0.37^a	8.53 ± 0.35^a	7.72 ± 0.24^a
Survival (S%)	90 ± 10.0^a	80 ± 10.0^a	93.3 ± 3.3^a
Weight gain (WG_{d45}, gr)	4.09 ± 0.29^a	4.62 ± 0.28^a	3.91 ± 0.17^a
Initial Coeficient factor (K_{in} gr/m^3)	0.93 ± 0.04^a	0.93 ± 0.05^a	0.88 ± 0.03^a
Final Coeficient factor (K_{d45} gr/m^3)	1.19 ± 0.08^a	1.17 ± 0.07^a	1.14 ± 0.04^a
Specific growth rate (SGR_{d45} %/d)	2.11 ± 0.11^a	2.23 ± 0.09^a	1.90 ± 0.04^b
Body weight increase (% BWI_{d45})	114.35 ± 11.95^a	121.22 ± 7.74^a	102.32 ± 3.80^a
Food conversion ratio (FCR_{d45})	1.23 ± 0.12^a	1.05 ± 0.06^a	1.23 ± 0.08^a
Daily food intake (DFI_{d45} %/d))	2.95 ± 0.3^a	2.62 ± 0.15^a	3.08 ± 0.19^a
Initial average length (L_{in}, cm)	7.5 ± 0.07^a	7.7 ± 0.09^a	7.7 ± 0.07^a
Final average length (L_{d45}, cm)	8.8 ± 0.13^a	9.1 ± 0.12^a	8.8 ± 0.09^a
Initial average height (Hin)	11.1 ± 0.58^a	10.2 ± 0.85^a	10.9 ± 0.42^a
Final average height (Hfin)	14.0 ± 0.27^a	10.9 ± 0.76^b	11.4 ± 0.37^b
Increase plant height (dH)	2.9 ± 0.63^a	0.7 ± 0.27^b	0.5 ± 0.20^b
Growth plant increase (% G)	27.0 ± 7.23^a	7.6 ± 2.93^b	4.6 ± 1.71^b

Data were expressed as mean ± S.E.M (n=30). Means in a row followed by the same superscript are not statistically significantly different ($p>0.05$).

4.Discussion

The present study investigated, to the knowledge of the authors for the first time, the production of sea bass and rocket plant for 45 days in a brackish aquaponics system. To date, only a few studies have been conducted on the use of brackish aquaponic systems (Kotzen *et al*. 2010; Pantanella & Colla 2013; Vlahos *et al*. 2019), in comparison to many studies that have research results on fresh water aquaponic systems (Fronte *et al*. 2016; Stathopoulou *et al.* 2021). Sea bass juveniles and rocket plants are among the edible species of Mediterranean aquaculture and agriculture due to their commercial value and interest (dos Santos & Rolim 2001; Barbosa *et al*. 2020). The results of the present study demonstrated that sea bass growth performance (SGR) was similar in 1.5 ppt and 5 ppt and lower in the 7 ppt salinity treatments, respectively. Tasiou (2019) reported that SGR in both aquaponic system with 8ppt and 14 ppt salinities was 1.90 ± 0.05 %/d (SGR 8ppt) and 1.83 ± 0.04 %/d (SGR 14ppt), respectively. Eroldoğan & Kumlu (2002) reported that seabass on growing in fresh water and in brackish water (10 ppt and 20 ppt) grew better compared with sea bass growing in 30 ppt and 40 ppt. Nozzi *et al.* (2016) reported that seabass grew in a freshwater aquaponics system observed better weight gain (WG) to those (seabass) growing in a saltwater aquaponics system. Moreover, the results showed that they were no statistical significant differences in the means of the seabass body weight increase (% BWI) between all treatment and was similar to those reported by Islam *et al*. (2020a;b) and Tasiou (2019). Pantanella & Colla (2013), and Waller *et al.* (2015) suggested that sea bass grew on average from 32 g to 54 g, at a SGR of 1.5 %/day and FCR of 0.93 in the 16 psu salinity aquaponics system for 35 days. Rocket plant growth performance (height and number of lateral branches) was better in 1.5 ppt (2.7 mS/cm) than 5 and 7 ppt salinities. According to the results of the present study growth performance and rocket, yields were affected by salinity. Increasing salinity up to 5 ppt (8.4 mS/cm) and 7 ppt (11.5 mS/cm) restricts rockets crop production compared to salinity of 1.5 ppt (2.7 mS/cm).

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References

Barbosa P.T.L., Povh.,J.A., do Nascimento Silva., A. L., Ventura., A.S., Stringhetta., G. R., Laice., L.M., de Oliveira ., A.F., de Carvalho., T.A., Filho., R.A.C.C. (2020). Performance of Nile Tilapia and vegetables Grown in Different Aquaponic Volumes. Journal of Agricultural Studies. 8 (4): 497-506. ISSN 2166-0379.

Chartzoulakis, K., Klapaki G. (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Scientia Horticulturae 86: 247-260.

dos Santos, A. N., and Rolim, M. M. (2011). Produção de rúcula em Sistema hidropônico NFT utilizando água salina do Semiárido-PE e rejeito de dessalinizador. Revista Brasileira de Ciências Agrárias (Agrária) Brazilian Journal of Agricultural Sciences, 6(1), 147-155.

Endut A., Jusoh A., Ali N., Wan-Nik W., Hassan A., (2010). A Study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresour. Technol. 101: 1511-1517.

Eroldoğan, O.T.; Kumlu, M., (2002). Growth performance, body traits and fillet composition of the European sea bass (*Dicentrarchus labrax*) reared in various salinities and fresh water. Turkish J. Vet. Anim. Sci., 26, 993–1001.

FAO (2013). On-farm feeding and feed management in aquaculture. Fisheries and Aquaculture Technical Paper. Food and Agriculture Organization Of the United Nations Rome, No. 583. 67 pp. ISBN 978-92-5-107978-2.

Fronte, B., Galliano, G., Bibbiani, C, (2016). *From freshwater to marine aquapoinic: new opportunities for marine fish species production*. 4th Conference with International Participation Conference VIVUS-on Agriculture, Ennvironmentalism, Horticulture and Floristics, Food Production and Processihg and Nutritioh «With Knowledge and Experience to New Entrepreneurial Opportunities» 20th and 21st April 2016, Biotechnical Centre Naklo, Strahinj 99, Naklo. Slovenia.

Islam, M.J., Kunzmann, A., Bogner, ¨ M., Meyer, A., Thiele, R., James Slater, M., Slater, M. J., (2020a). Metabolic and molecular stress responses of European seabass, *Dicentrarchus labrax* at low and high temperature extremes. Ecol. Ind. 112, 106118 https://doi.org/10.1016/j.ecolind.2020.106118.

Islam, M.J., Kunzmann, A., Thiele, R., Slater, M.J., (2020b). Effects of extreme ambient temperature in European seabass, *Dicentrarchus labrax* acclimated at different salinities: growth performance, metabolic and molecular stress responses. Sci. Total Environ. 735, 139371. https://doi.org/10.1016/j.scitotenv.2020.139371.

Kotzen b. Appelbaum S., (2010). *An Investigation of Aquaponicw Using Brackish Water Resources in the Negev Desert.* Journal of Applied Aquaculture, 22:297-320.

Lennard, W., (2021). WITHDRAWN: A comparison of the effect of starting nitrate concentration on fish growth, plant production and water quality within a research-scale, recirculating aquaponics system. Aquaculture and Fisheries.

Mente, E., Solovyev, M.M.,Vlahos,N., Rotllant, G., Gisbert,E (2016). Digestive enzymes activity during initial ontogeny and after feeding diets with different protein sources in zebra cichlid *Archocentrus nigrofasciatus*. Journal of World Aquaculture Society, 48, 5, 831-848.

Nelson, R.L.(2008). Aquaponic Food Production: Growing Fish and Vegetables for Food and Profit. Nelson and Pade, Wisconsin.

PantanellaE., Colla G. (2013). Saline aquaponics opportunities for integraed marine aquaculture. International aquaponic conference: Aquaponics and global food security, 19-21. *University of Wisconsin-Stevens Point,* pp 52.

Stathopoulou, P., Berillis, P., Vlahos, N., Nikouli, E., Kormas, K., Levizou, E., Katsoulas, N. and Mente, E., (2021). Freshwater-adapted sea bass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. Peer J, 9, p.e11522.

Tasiou K. (2019). The production process of sea bass in an aquaponic system. MSc thesis. Department of Ichthyology and Aquatic Environment, University of Thessaly, pp. 85.

Vlahos N., Levizou E., Stathopoulou P., Berillis P., Antonopoulou E., Bekiari V., Krigas N., Kormas K ., and Mente E. (2019). An Experimental Brackish Aquaponic System Using Juvenile Gilthead Sea Bream (*Sparus aurata*) and Rock Samphire (*Crithmum maritimum*). Sustainability 11(18). DOI: 10.3390/su11184820.

Waller, U, Buhmamn, A.K., Emst, A., Hanke, W., Kulakowski. A., Wecker, B., Orellana, i., (2015). Papenbrock, J. Integrated multi-tropic aquaculture in a zero – exchange recirculation aquaculture system for marine fishand hydroponic halophyte production. Aquac. Int. 2015, 23, 1473-1489.

Waller, U., Buhmann, A.K., Ernst, A., Hanke, V., Kulakowski, A., Wecker, B., Orellana, J., Papenbrock, J. (2015). Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac. Int. 5, 23, 1473–1489.

Zar J. H. Biostatistical Analysis; Prentice-Hall: London,UK.,, (1999); P. 718.

SPECIFIC IMMUNE RESPONSE OF SEA BASS (*Dicentrarchus labrax*) IMMUNIZED WITH VARIOUS ANTIGENS OF *Photobacterium damselae* subsp. *piscicida, Vibrio anguillarum* O1 and B-NODAVIRUS

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Abstract
Mediterranean aquaculture suffers significant losses every year due to diseases of various aetiology (infectious and non-infectious). Infectious diseases are attributed mainly to viral, bacterial and parasitic diseases. From these diseases, viral encephalopathy and retinopathy, caused by b-nodavirus, and photobacteriosis and vibriosis, caused by the bacteria *Photobacterium damselae* subsp. *piscicida* and *Listonella anguillarum*, respectively, are the most important. Although, there are various vaccines available in the market, monovalent or bivalent, there are no trivalent products able to offer sufficient protection against all three diseases. The aim of this study was to evaluate the specific immune response of sea bass after immunization against various antigen preparations from these pathogens with a view to identify preparations that could be combined in a trivalent vaccine. Sera collected during various time-points post-immunization, were tested with ELISA and results indicated that best specific antibody levels were produced against the extracellular products of both bacterial pathogens, followed by whole cells, capsular polysaccharide and lipopolysaccharide. The latter antigen of *L. anguillarum*, did not raised a good immune response and may not be appropriate for inclusion in vaccines.
Keywords: *Dicentrarchus labrax*, *photobacteriosis, vibriosis, viral encephalopathy and retinopathy, specific immune response*
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1. Introduction

Viral encephalopathy and retinopathy (VER) is causing serious losses to fish stocks of sea bass in the Mediterranean. To the mortalities caused by VER, additional losses are experienced by Gram (−) bacteria, mainly *Vibrio* (*Listonella*) *anguillarum* (hereafter VaO1) (Sorensen & Larsen 1986; Toranzo & Barja 1990) and *Photobacterium damselae* subsp. *piscicida* (Phdp, hereafter) (Bakopoulos *et al.* 1995; 2015), respectively. Both pathogens are endemic to the marine environment and both European sea bass and sea bream (*Sparus aurata*) are protected to some extent by vaccination, usually using bivalent vaccines (Spinos *et al*. 2017). Ideally, sea bass and bream aquaculture needs a polyvalent vaccine formulation which will protect simultaneously against all three diseases. The aim of this study is to measure the specific humoral immune response of sea bass after immunization with various single antigen (Ags) group formulations, such as extracellular products (ECPs), capsular polysaccharide (CPS) and lipopolysaccharide (LPS) and b-Nodavirus (Noda), with a view to identify potential Ags (and doses) that could be utilized in a trivalent vaccine against all three diseases.

Materials & Methods

<u>Fish, Facilities, Pathogens, Antigen preparation</u>: Sea bass, healthy, unvaccinated, N=360, average weight 78g, were transported by Nireus Aquaculture S.A. to the wet laboratory (ICHTHYAI) of the Department of Marine Sciences, University of The Aegean [permit EL83 BioExp 01, according to the Presidential Decree 56/2013 conforming to Dir 2010/63/EE (Decision No 4053/14-3-2017 of the competent Regional Veterinary Authority]. Fish were maintained in a closed recirculated sea water system with solids filtration, disinfection (UV), biological filter and aeration. Water temperature maintained at 22.5ºC. Photoperiod at 12h light:12h dark, salinity 3.8-3.9%, dissolved O_2 >4.8mg/l, pH 8, total ammonia nitrogen and nitrite >0.05ppm and >0.5ppm, respectively, and nitrate levels <40mg/l. For the preparation of antigens (Ag), the following pathogens were used: a) an isolate Phdp isolated from sea bass in 2019 in western Greece; b) an isolate of VaO1 (which was a kind donation of Dr Papanna, Nireus Aquaculture S.A.; c) a strain of Noda, RGNNV genotype isolated from a cultured European sea bass outbreak in 2012 in Western Greece; the culture was a kind donation from Dr Katagouni, Hellenic Pasteur Institute. Phdp was grown in specific media (Bakopoulos *et al.* 2003a) and VaO1 in iron limitation media (Bakopoulos *et al.* 1997a). Whole cells (WC), ECPs and CPS from bacteria were prepared as described by Bakopoulos et al (2003b and references therein), while LPS was prepared according to Nomura & Aoki (1985). Noda solution was heat-

inactivated for 1h at 60°C, then concentrated using cellulose membrane and polyethylene glycol 8,000MW. Protein content of Ag solutions was measured with the BIORAD protein assay method, while saccharide content with the acid-sulfuric method.

Immunizations & sampling: Fish were anesthetized with 0.2% phenoxyethanol; each Ag solution was mixed 70:30 v:v with sterile liquid paraffin; each fish received 100μl of the resulting solution intraperitoneally (i.p.). For each dose of Ag, 15 fish were immunized and blood was collected on day (D) 0 (pre-immune), D4, D28 and D35 post-immunization (p.i.). The following Ag solutions were used: a) Phdp WC; 10^9, 10^8 and 10^7 cells/ml, b) VaO1 WC; 10^9, 10^8 and 10^7 cells/ml, c) VaO1 ECPs; 350, 175 and 87.5μg/ml protein, d) Phdp ECPs; 530, 265 and 132.5μg/ml protein, e) Phdp CPS; 98.6, 75 and 55μg/ml sugar, f) VaO1 LPS; 310, 156 and 79μg/ml, g) Phdp LPS; 112, 82 and 61μg/ml sugar, h) Noda: 200, 150 and 100μg/ml protein and i) sterile 2% NaCl (negative controls).

ELISA analysis: Sera from each group were utilized in a modified sandwich-ELISA analysis according to (Bakopoulos et al. 1997b) for the determination of specific anti-betanodavirus IgM levels. For all the other Ag groups a simple indirect ELISA was utilized. All statistical differences between individual serum results within and between time-points and groups were assessed with one-way ANOVA.

Results & Discussion

Phdp: Great variation between replicates of the same group was noted (Fig. 1). During D4, specific Ab levels of all sera against WC of Phdp was negligible. On D28, specific Ab reaction in the sera prepared against 10^9 WC/ml of Phdp was elevated, in contrast to the sera prepared against 10^8 and 10^7 WC/ml of Phdp. On day 35, specific Ab levels of sera raised against 10^9 WC/ml of Phdp remained unchanged, while for the 10^8 and 10^7 WC/ml

Phdp groups average values were raised with best reaction achieved by the 10^8 WC/ml Phdp group. Specific sea bass Ab levels against Phdp WC 10^8 cells/ml at D35 were significantly higher in comparison to the D28 Phdp WC 10^8 & 10^7 cells/ml groups. No effect of Ag dose was evident at D35 since there was no statistical difference between groups. Phdp WC are able to raise good levels of specific Abs and this is supported by previous studies (Bakopoulos *et al*. 1997c).

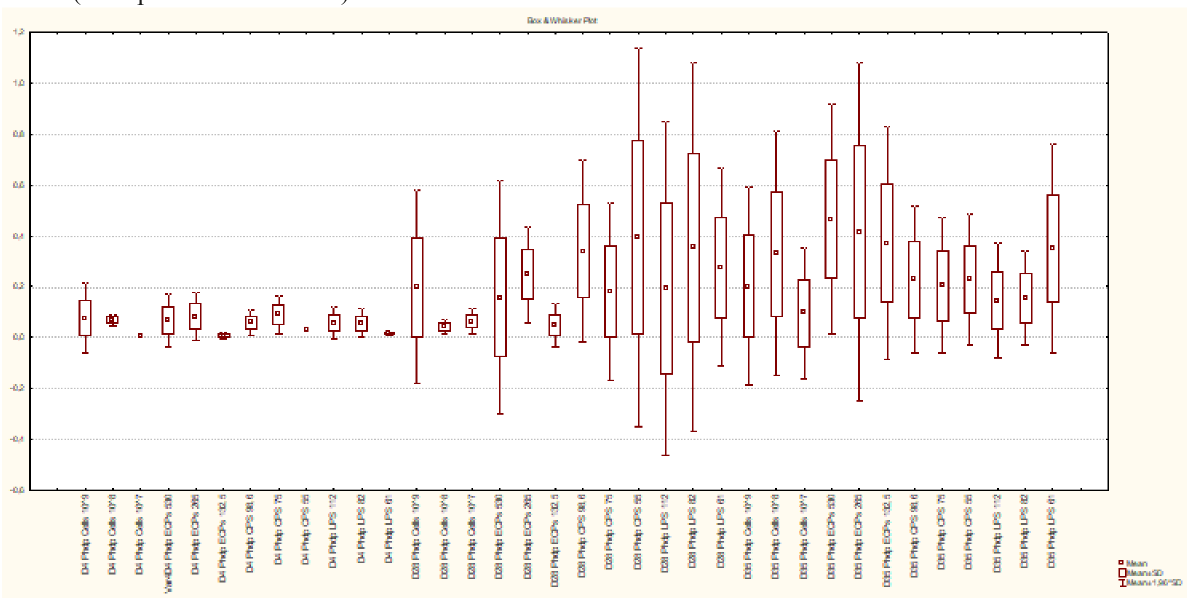

Figure 1. Specific antibody levels in sea bass sera prepared against different Ags of Phdp and tested against WC of Phdp. OD values from which the average OD value of D4 controls has been abstracted. Only data from responding individuals are incorporated.

At D28, elevation of specific Ab levels was noted in the sera prepared against 530, 265 and 132.5 μg/ml protein ECPs of Phdp, with the best mean value obtained by the 265 μg/ml protein group and the worse by the 132.5 μg/ml protein group. At D35, specific Ab levels of groups 530, 265 and 132.5 μg/ml protein ECPs of Phdp were raised in comparison to D28 and these levels were proportional to the amount of ECPs protein Ag administered to each group. Specific Ab levels in the sera of group 265 μg/ml protein ECPs D28 differed significantly from the group 132.5 μg/ml protein ECPs at the same time-point. At time-point D28, specific Ab levels in the sera of group 132.5 μg/ml protein ECPs was significantly lower from group 265 μg/ml protein ECPs. Finally, specific Ab levels in the sera of group 132.5 μg/ml protein ECPs at D28 were significantly lower when compared to all groups at D35. Although there was no significant difference between groups at D35, mean values did show an effect of Ag dose. Apparently, ECPs contain released of secreted WC Ags and that is why reactions against WC of Phdp are achieved. Phdp ECPs are capable of raising a strong, protective, specific immune response and this is also supported by the Magarinos *et al*. (1999) findings. On D28, elevation of specific Ab levels was noted in the sera prepared against 98.6, 75 and 55 μg/ml sugar CPS of Phdp with the best levels observed from the 55 μg/ml sugar CPS group followed by the 98.6 μg/ml sugar CPS group. At D35, no further elevation of specific Ab levels of the 98.6, 75 and 55 μg/ml sugar CPS of Phdp groups was noted, on the contrary, they seem to drop and stabilize at the same level. No statistical difference was calculated when specific Ab levels of all groups at D28 & D35 were compared with levels of groups at both time-points. No Ag dose effect was noted. Regarding the LPS groups on D28, elevation of specific Ab levels was noted in the sera prepared against 112, 82 and 61 μg/ml sugar LPS groups with best levels achieved by the 82 and followed by the 61 μg/ml sugar LPS group. At D35, no further elevation of specific Ab levels in the sera prepared against 112, 82 and 61 μg/ml sugar LPS groups Phdp was noted (on the contrary, they seem to drop and stabilize at the same level), except for the 61 μg/ml sugar LPS group that showed elevation of the mean value in comparison to D28. Strong reactions against LPS have been reported before (Bakopoulos *et al*.1997c) supporting our results. Highest reactions were achieved from the 55 μg/ml sugar CPS group and the 82 μg/ml sugar LPS group at D28 and from all ECPs groups and the 61 μg/ml sugar LPS group at D35. Pair-wise statistical comparisons indicate that Phdp ECPs is a strong Ag leading to the production of higher levels of specific Abs in comparison to Phdp WC, CPS and LPS. CPS and LPS groups responded stronger earlier (i.e. D28) and this needs to be considered during the selection of Ags.

<u>VaO1</u>: In general, specific Ab levels in sea bass sera raised against the Ags used in these experiments (WC, ECPs and LPS), did not reach high levels (Fig. 2). Interestingly, reactions were seen as early as D4 post-immunization from the 310 μg/ml sugar LPS group against WC of VaO1. At D28, negligible specific Ab levels were noted from 10^9 and 10^7 WC/ml VaO1 groups, showing a small increase from D4, and mean Ab levels increased at D35 only for the 10^9 and 10^8 WC/ml VaO1 groups, while for the 10^7 WC/ml VaO1K group, they dropped. Responding and non-responding fish were observed in all cases. Mean specific Ab levels in groups 350, 175 and 87.5 μg/ml protein ECPs VaO1 increased when compared to D4 levels and levels were proportional to the protein content used in immunizations. A further increase (at least for some individuals) of mean specific Ab levels for the ECPs groups was noted at D35.

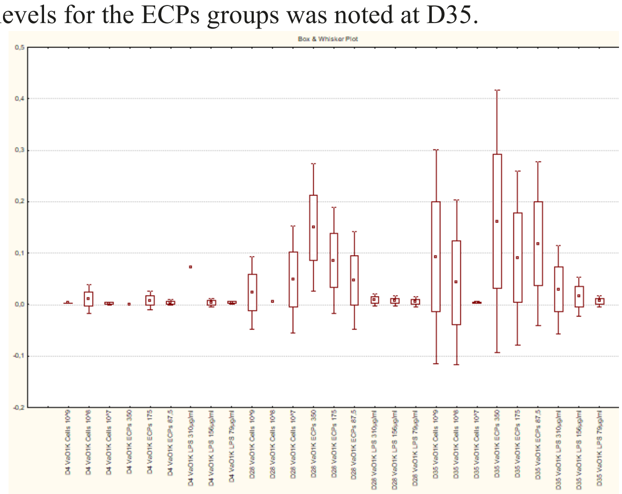

Figure 2. Sea bass sera prepared against various Ags of VaO1K and tested against WC of VaO1K. OD values from which the average OD value of D4 controls has been abstracted. Only data from responding individuals are incorporated.

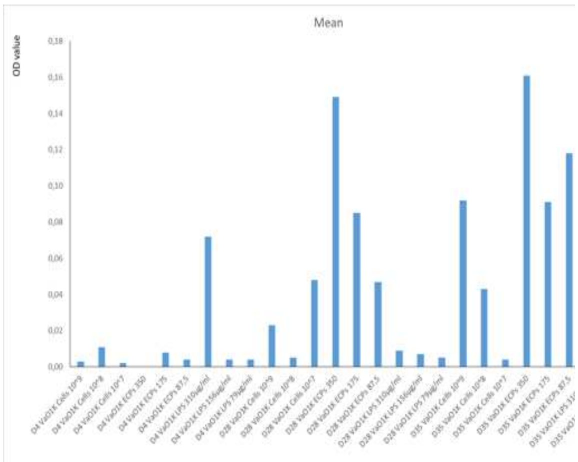

Figure 3. Mean OD values of responding individuals in each group and time-point.

As in the case of Phdp, VaO1 ECPs seem to contain Ags that are present in WC and shed into the culture medium. Regarding the groups immunized with LPS, almost no specific Ab levels were found at time-point D28, while a low, proportional to the Ag concentration used in immunizations, increase in Ab levels was noted for the 310 and 156 μg/ml sugar LPS groups at D35. Although LPS of *Vibrio* spp. is the base for serotyping species, it does not seem to be a strong Ag for sea bass and this is supported also by the work of (Spinos *et al*. 2017). Best reactions (Fig. 3) were noted from groups immunized with various concentrations of ECPs protein, followed by the groups immunized with WC. ECPs of VaO1 are apparently strong Ags in contrast to VaO1 LPS. VaO1 cells did cause elevation of specific Ab levels at D35 and surprisingly very early reactions (D4) were noted for the high LPS dose group.

Nodavirus: Even at D4 post-immunization some specific anti-Noda Ab activity was observed from the group immunized with the low 100μg/ml protein of Noda (Fig. 4). On D28 best activity was observed from the 100μg/ml protein group. Finally at D35 best reactions were achieved by the two lower Ag doses groups. In many cases, non-responsive individuals were identified and this is not an unusual phenomenon (Thiery *et al*. 2006). The highest anti-Noda activity was found at D35 group Noda 150, followed by the Noda 100 group at the same time-point, indicating that the Ag dose of 200μg/ml protein of Noda may be immunosuppressive (Fig. 5).

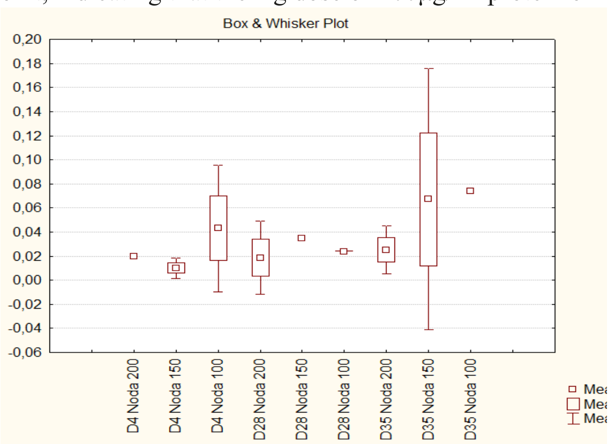

Figure 4. Sea bass sera prepared against various Ag concentrations of b-Nodavirus particles and tested against b-Nodavirus particles. OD values from which the average OD value of D4 controls has been abstracted. Only data from responding individuals are incorporated.

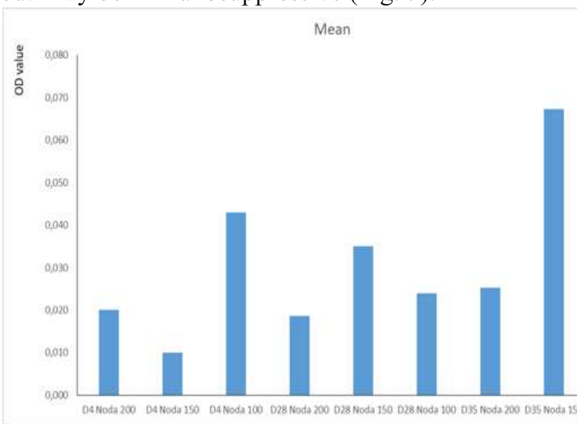

Figure 5. Mean OD values of responding individuals in each group and time-point.

Pairwise statistical comparisons between Ag groups and time-points showed no differences in all groups at D4 and D28 time-points. Specific anti-Noda Ab levels of group D4 Noda 150 were significantly lower compared to the D28 Noda 100 group. Similarly, Ab levels of group D35 Noda 200 were significantly lower compared to D35 Noda 100, indicating a reverse correlation between Ag dose and mean Ab activity, especially on D35 post-immunization, and that 100μg/ml viral protein may be more appropriate for immunizing sea bass.

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References

Bakopoulos V., Adams A., Richards R.H. (1995). Some biochemical properties and antibiotic sensitivities of *Pasteurella piscicida* isolated in Greece and comparison with strains from Japan, France and Italy. Journal of Fish Diseases 18, 1-7.

Bakopoulos V., Adams A., Richards R.H. (1997a). The effect of iron limitation growth conditions on the cell and extracellular components of the fish pathogen *Pasteurella piscicida*. Journal of Fish Diseases 20(4), 297-305.

Bakopoulos V., Nikolaou I., Kalovyrna N., Amirali E., Kokkoris G., Spinos E., 2015. Prevention of fish photobacteriosis. Comparison of the efficacy of intraperitoneally administered commercial and experimental vaccines. Mediterranean Marine Science 16(2), 385-392.

Bakopoulos V., Pearson M., Volpatti D., Gousmani L., Adams A., Galeotti M., Dimitriadis G.J. (2003a). Investigation of media formulations promoting differential antigen expression by *Photobacterium damsela* subsp. *piscicida* and recognition by sea bass, *Dicentrarchus labrax*, (L.), immune sera. Journal of Fish Diseases 26(1), 1-13.

Bakopoulos V., Volpatti D., Adams A., Galleotti M., Richards R.H. (1997c). Qualitative differences in the immune response of rabbit, mouse and sea bass, *Dicentrarchus labrax*, L, to *Photobacterium damsela* subsp. *piscicida*, the causative agent of fish Pasteurellosis. Fish and Shellfish Immunology 7, 161-174.

Bakopoulos V., Volpatti D., Gusmani L., Galeotti M., Adams A., Dimitriadis G.J. (2003). Vaccination trials of sea bass, *Dicentrarchus labrax* (L.), against *Photobacterium damsela* subsp. *piscicida*, using novel vaccine mixtures. Journal of Fish Diseases 26(2), 77-90.

Bakopoulos V., Volpatti D., Papapanagiotou E., Richards R.H., Galeotti M., Adams A. (1997b). Development of an ELISA to detect *Pasteurella piscicida* in culture and in “spiked” fish tissue. Aquaculture 156(3/4), 359-365.

Magarinos B, Romalde JL, Barja JL, Nunez S, Toranzo AE. (1999). Protection of gilthead seabream against pasteurellosis at the larval stages. Bulletin of the European Association of Fish Pathologists 19, 159-161.

Nomura J., Aoki T. (1985). Morphological analysis of LPS from Gram negative fish pathogenic bacteria. Fish Pathology 20, 193–197.

Sorensen, U.B., Larsen, J.L., 1986. Serotyping of *Vibrio anguillarum*. Applied and Environmental Microbiology 51(3), 593-597.

Spinos E., Kokkoris G.D., Bakopoulos V. (2017). Prevention of sea bass (*Dicentrarchus labrax*, L. 1758) photobacteriosis and vibriosis. Long term efficacy study of intraperitoneally administered bivalent commercial vaccines. Aquaculture 471, 172-184.

Thiery R., Cozien J., Cabon J., Lamour F., Baud, M., Schneemann A. (2006). Induction of a protective immune response against Viral Nervous Necrosis in the European sea bass *Dicentrarchus labrax* by using Betanodavirus Virus-Like Particles. Journal of Virology 80(20), 10201-10207.

Toranzo A.E., Barja J.L. (1990). A review of the taxonomy and seroepizootiology of *Vibrio anguillarum* with special reference to aquaculture in the northwest of Spain. Diseases of Aquatic Organisms 9, 73-82.

THE PROGRESS OF ECONOMIC PERFORMANCE OF SEABASS & SEABREAM AQUACULTURE PRODUCTION SYSTEMS IN GREECE

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Abstract

Marine aquaculture in Greece was favored by ideal conditions as one of its main characteristics is the length and diversity of its coastline (the 4th longest coastline of the European Union with a length of about 16,000 km), and its innumerable islands (approximately 2,500, of which 165 are inhabited). The strong growth of the aquaculture sector in Greece has led to changes in the structure, organization, and operation of enterprises in the sector, the development of technology and production methodology and a change of perception regarding the new development model set by the legal framework of the European Union and international competitiveness. In Greece, the dominant species of national aquaculture production are sea bream and sea bass. This paper will analyze the development of production, costs, and cost structure of production as well as the productivity of sea bream and sea bass aquaculture in Greece over the past two decades.

Keywords: *Sea bass, sea bream, aquaculture, cost, economic performance, fry production.*

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1. Introduction

According to data from European Commission reports, (STECF, 2018), EU aquaculture production is mainly concentrated in 5 countries: Spain (21%), France (15%), Italy (14%), the United Kingdom (14%), and Greece (10%), making up 74% of the sales volume. These 5 countries are responsible for 73% of the sales value in EU. Greece is the main producer of seabream and seabass covering 47% of the total EU value. Considering the importance of those two cultured species, this paper attempts a study of the evolution of seabass & seabream aquaculture economic performance throughout a four-decade period, covered by the respective bibliography. According to it, sea bass and sea bream aquaculture in Greece demonstrates production for the first time in 1983 starting a dynamic course in terms of production volume and income.

2. Material and Methods

This paper studies the progress of seabass and seabream aquaculture economic performance in Greece based on data from research papers regarding the early years of the industry, and data from Hellenic Statistical Authority and European Commission aquaculture sector research reports for Greece for the 2002-2018 period. It utilizes data of production volume and production cost of fry and fully grown fish, average price, as well as marine water aquaculture labor data, since seabass and seabream production combined corresponds to almost 99% of total marine fish production in Greece.

3. Results

3.1 Sea bass and sea bream production in Greece

Data on aquaculture production of sea bass and sea bream in Greece for the period before 2002 is presented in various published surveys and papers (Stephanis 1995; Karagiannis & Katranidis 2000; Papoutsoglou 2000; Karagiannis *et al*. 2008). According to them, aquaculture production of sea bass and sea bream is demonstrated for the first-time in 1983. During the first five years of the 1990-decade, with aquaculture intensification, sea bass and sea bream combined production in Greece rose from 1,6 thousand tons in 1990 to 17,5 thousand tons in 1994 (Tab.1).

Table 1: Sea bass & sea bream production during 1980-1995 (in tonnes).

Year/species	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Sea bass & sea bream	0	1	3	53	89	105	200	500	1.600	2.459	4.845	9.500	13.500	17.553

Following this early period and according to Hellenic Statistical Authority, the production of sea bass and sea bream in Greece for the 2002-2018 is as presenting in Figure 1:

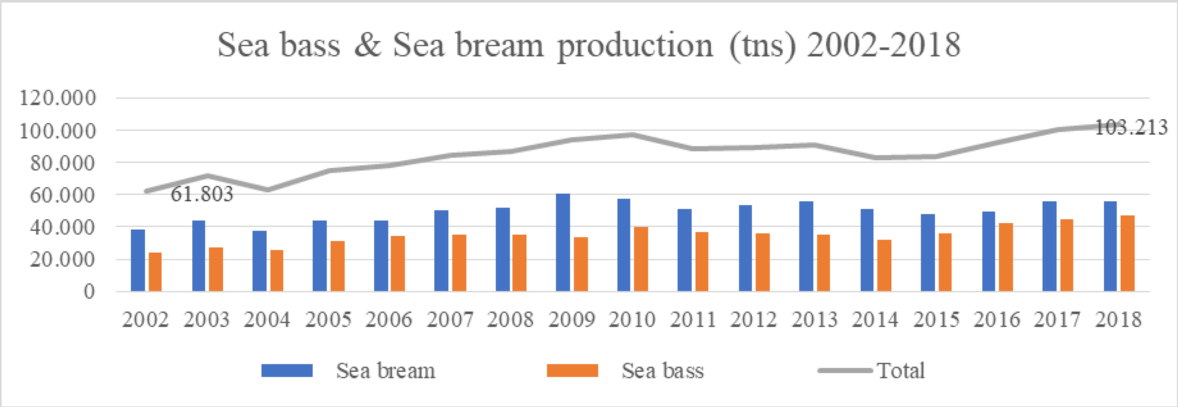

Figure 1. Sea bass & sea bream production in Greece during 2002-2018.

Regarding the production of sea bass & sea bream fry production, according to Stephanis (1995), the respective volumes for the 1990-1994 period were as presenting in Table 2.

Table 2: Sea bass & Sea bream fry production during 1990-1994

Year	**1990**	**1991**	**1992**	**1993**	**1994**
Fry (millions)	14	23	37	60	70

For the 2002-2018 period, the sea bass and sea bream fry production volumes, according to HAS are presenting in Figure 2.

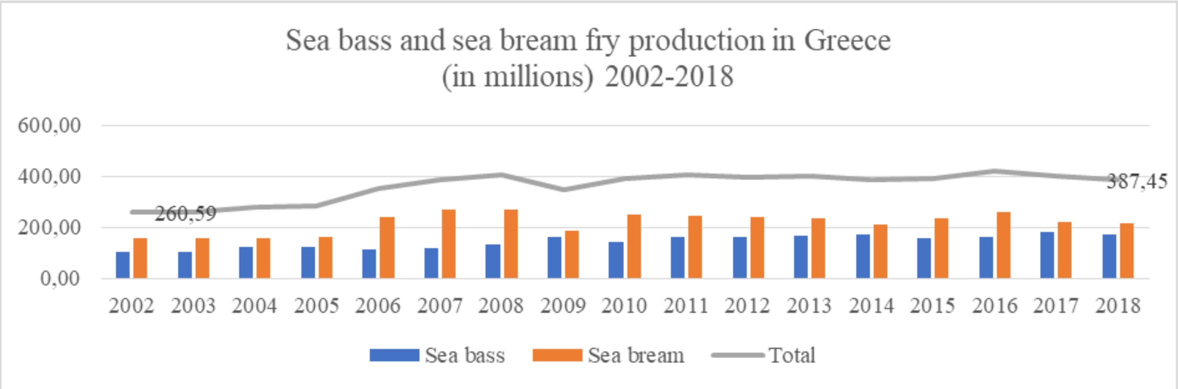

Figure 2. Sea bass & Sea bream fry production in Greece during 2002-2018.

During the 1980s-decade, sea bass/sea bream aquaculture production was dependent on fry imports that led to additional production costs (Stephanis 1995). The intensification of sea bass & sea bream aquaculture required increased amounts of fry and consequently domestic production of fry was developed to supply the ever-increasing raw material demand of the industry. Therefore, the development of the sector and the resulted increase of Greek sea bass & sea bream production was based on the implementation of technological innovations throughout the various stages of production, especially of the larval stage which is considered as crucial in fish production (Pappas *et al*. 2011). As shown in Figure 2, the sector's fry production demonstrated an almost 150% increase during 2002-2018 period.

3.2. Production costs

3.2.a. Production costs of hatcheries/nurseries units

In order to assess any possible changes in the fry production cost structure, corresponding cost structure data was used for years 1994 and 2016 and presenting in Figure 3 (Stephanis 1995; STECF 2018).

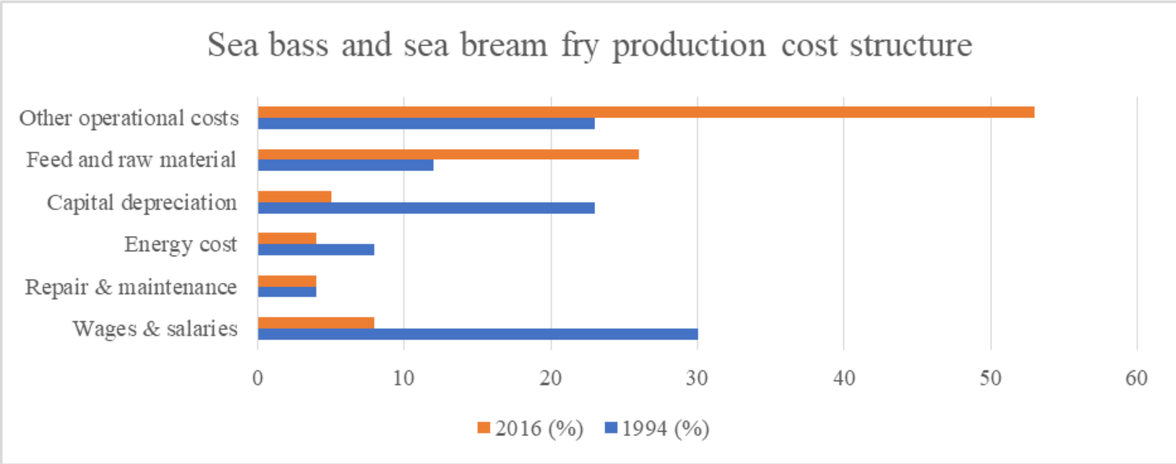

Figure 3. Sea bass and sea bream fry production cost structure.

In the recent years, due to the consolidation of sea bass and seabream aquaculture production industry, many enterprises, especially the large aquaculture industries, hold two or three parallel activities (cages, hatcheries nurseries etc.) therefore the sector's cost structure of fry production has changed due to the different company sizes and difficulties and different practices in cost allocation methods (STECF 2018). Regarding the difference of wage values, the large companies that are vertically integrated and operate their own fry production units, demonstrate significantly higher production capacity and labor productivity (Stephanis 1994).

Furthermore, a study on larval production cost in three hatcheries with different fry production capacity (Pappas *et al.* 2011), demonstrated significant differences on total cost per fry (37%) and variations of cost structure depending on the size of the units, where labor corresponds to 30-40% of the total cost, live feed 18-25%, and larval-brood stock expenses fluctuating from 7-16.8% of the total production cost.

3.2.b. Production costs of sea bass & sea bream

Regarding the production costs of sea bass and seabream aquaculture sector, the following table demonstrates the sector's cost structure for 1989, 1994 and 2016 (Stephanis 1994; STECF 2018).

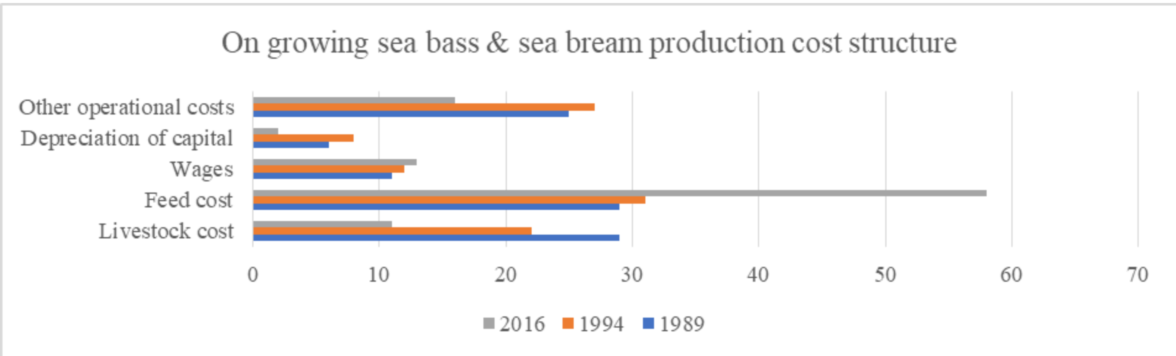

Figure 4. On growing sea bass & sea bream production cost structure.

The drop of livestock cost percentage reflects the development of enterprises' own hatcheries/nurseries units to lower the production cost and increase the production volumes.

3.2.c. Labor Productivity of marine water aquaculture

The evolution of labor productivity of marine water aquaculture for the years 2002-2018 was calculated as ton per employee using the Hellenic Statistical Authority data for the respective years and is as presenting in Figure 5.

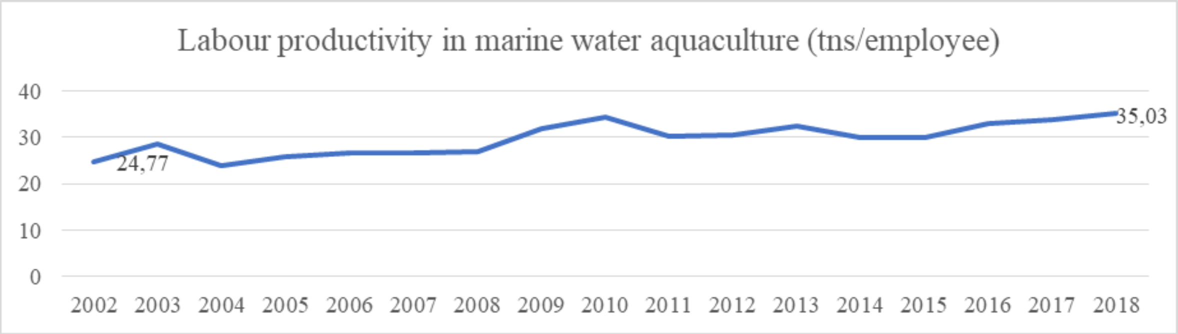

Figure 5. Evolution of marine water aquaculture labor productivity during 2002-2018

The development of medium and large size aquaculture industries with investments in research and innovation in addition to the use of improved fish feeds, lead to continuous increase of labor productivity for the seawater aquaculture sector in Greece.

3.3. Prices

According to Stephanis (1994), the prices of farmed sea bass and sea bream dropped almost 53% during the 1989-1994 period starting at around 14€/kg and ending up below 6€/kg. According to the Hellenic Statistical Authority, for the period of 2002-2018, the price evolution for sea bass and sea bream market size fish is in Figure 6.

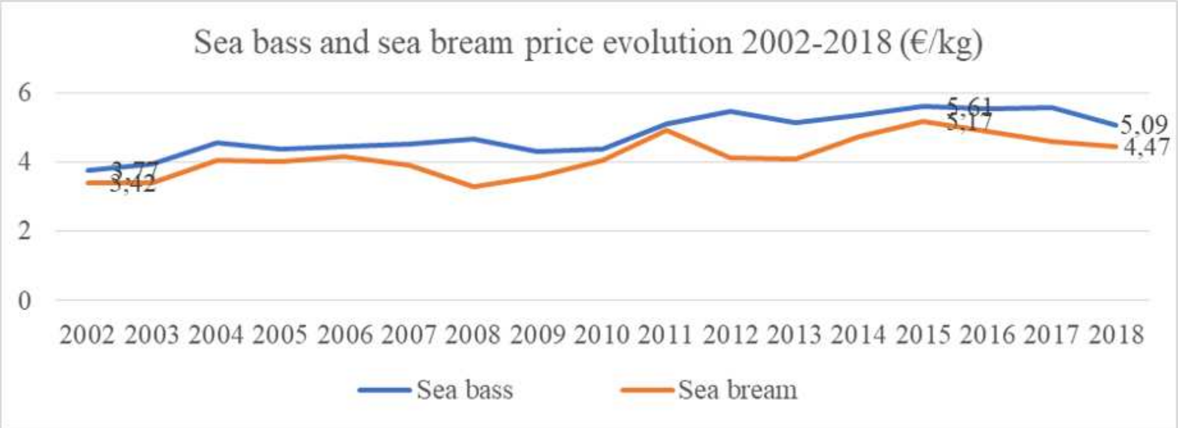

Figure 6: Sea bass and sea bream price evolution 2002-2018.

As we can be seen in Figure 6, for the period 2002-2018, there is an almost 33% total price increase for both species, despite a sharp drop in the first years of economic crisis. However, after the peak year of 2015, a decrease for the next 3 years may be indicative of increased competition and supply by countries outside EU (Federation of Greek Maricultures 2016).

4. Discussion

During the last two decades, sea bass and sea bream aquaculture has established Greece as the top producer in Europe, demonstrates production activity in almost all regions of the country, holds the largest proportion of the total quantity of sea bass and sea bream produced in the EU and shows exports to Italy, Spain, Russia, and the United Kingdom. Comparison of the sea bass & sea bream aquaculture economic and production indicators during the four-decade period shows that the sector managed to achieve continuous increase of production through modern management, technology investments, vertical integration, and improvement of labor productivity, despite the deep impact of the economic crisis in all aspects of Greece's economic life. The fact that aquaculture is considered a priority of EU's Blue Growth Strategy, highlights the importance of the Greek sea bass & sea bream sector in terms of future state income, employment, and sustainable development.

References

European Commission, 2016. *Tapping into blue growth: the way forward for European aquaculture*. Brussels, European Union.

Federation of Greek Maricultures, 2016. *Aquaculture in Greece*, Recovered from https://www.fgm.com.gr/uploads/file/FGM_ENG_2016.pdf.

Hellenic Statistical Authority, 2019. *Survey on Aquaculture*, recovered from https://www.statistics.gr/en/statistics/

Karagiannis, G. & Katranidis, S. 2000. A Production Function Analysis of Seabass and Seabream Production in Greece. Journal of the world aquaculture society Vol. 31, No. 3, pp. 297-305.

Karagiannis, G., Katranidis S. & V. Tzouvelekas 2000. Measuring technical, allocative and cost efficiencies of seabass and seabream farms in Greece. *Aquaculture Economics & Management* 4:3-4 DOI: 10.1080/13657300009380269, pp. 191-207.

Pappas, N., Panagiotaki, P., Matsiori, S., Golomazou, E., Malandrakis E., Martsikalis P., Dadali, O., Exadactylos, A. and Neofytou, N. 2000. Cost evaluation of sea bream and sea bass larval production in Greek hatcheries. Conference: Aquaculture Europe 2011, European Aquaculture Society (EAS*)*, pp 838-839 At: Rhodes, Greece

Papoutsoglou, S., 2000. Monitoring and regulation of marine aquaculture in Greece, licensing\ regulatory control and monitoring guidelines and procedures. Journal of Applied Ichthyology 16, pp. 167-171.

STECF - Scientific, Technical and Economic Committee for Fisheries, 2018. *Economic Report of the EU Aquaculture sector (STECF-18-19)*, Luxembourg: Publications Office of the European Union.

Stephanis, J., 1995. Economic viability of production systems seabass - seabream in Greece (industrial scale). Aspects économiques de la production aquacole Cahiers Options Méditerranéennes; n.14, pp. p. 65-77.

HEMOLYMPH ACTIVITIES AFTER EXPERIMENTAL INFECTION OF COMMON OCTOPUS, *Octopus vulgaris* (CUVIER, 1797) WITH *Photobacterium damselae* subsp. *piscicida*

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Abstract

Total proteins, lysozyme activity and hemolytic activity of *Octopus vulgaris* cell-free hemolymph were detected after experimental infection with the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Octopus vulgaris* hemolymph has higher concentration of proteins compared to other mollusks (210.52±18.89 mg/ml and 168.60±5.65mg/ml, at 21±0.5°C and 24±0.5°C, respectively, in baseline controls). At 21±0.5°C the infected groups showed significant higher levels of total proteins from D3 to D7, in contrast to the observed similar levels of protein during both time-points at 24±0.5°C. Lysozyme assay results showed that route of infection, day post infection and temperature may play a key role in lysozyme activity. Hemolytic activity was determined in cell-free hemolymph samples of *Octopus vulgaris*, and hemolysis rate ranged at low levels at both experimental temperatures. Results may assist in elucidating the repertoire of defense mechanisms of common octopus against pathogens.

Key words: *Octopus vulgaris, Proteins, Lysozyme, Hemolytic activity, Photobacterium damselae subsp. piscicida*

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1. Introduction

Host response against invading pathogens have a great variety of defensive strategies (Kasumyan *et al.* 2021); one of these is the humoral immune (non-cellular) defense. It is a very important immune mechanism for all organisms; especially for cephalopods as they lack a specific immune response and do not possess immunological memory (Castellanos-Martinez & Gestal 2013) like other invertebrates. According to experimental studies, humoral defense of cephalopods, is described to be supported by a variety of biochemical constituents (Castillo *et al*. 2015) of hemolymph. This unique kind of body fluid it is composed mainly of water, but also of organic compounds and inorganic salts (respiratory agents, proteins, enzymes, lipids and carbohydrates) (Machałowski & Jesionowski 2021 and references therein).

To date, little is known about the non-cellular hemolymph constituents of common octopus, *Octopus vulgaris* (Cuvier 1797). Proteins are important components of the humoral defense of all species with highest concentrations found in tissues exposed to microorganisms (Castellanos *et al*. 2014). The major protein constituent of hemolymph is the respiratory pigment hemocyanin with amounts to about 98% of the total protein present in octopod blood (Aguila *et al*. 2007). Lysozyme, an enzyme found also in hemolymph, attacks the bacteria cell wall (Gestal & Castellanos 2015). This enzyme was first described in octopod hemolymph and tissues samples of *Eledone cirrhosa* by Malham et al. (1998), after injection with *Vibrio anguillarum* and later by Grimaldi *et al*. (2013), after intramuscular injection of titanium dioxide nanoparticles in *O. vulgaris* specimens.

Lytic activity of body fluids plays an important role in host innate immune defense. Achieves lysis of pathogens by the large number of plasma proteins that work together (Nayak *et al*. 2018). Hemolysis of vertebrate erythrocytes by invertebrate cell-free hemolymph has not studied before. Key *et al*. (2002) and Houyvet *et al*. (2018) studied the hemolytic activity caused by salivary gland extracts from the octopus, *Eledone cirrhosa* and the hemolytic activity of antimicrobial peptides of cuttlefish, respectively.

The objective of the present study is to investigate the *in vitro* humoral response of experimentally infected octopus, *O. vulgaris*, with *Photobacterium damselae* subsp. *piscicida* (Phdp hereafter), using different infection routes and temperature regimes, in relation to climate change. Specifically, protein concentration, lysozyme activity and hemolytic activity in cell-free hemolymph were investigated. It is expected that the results will provide important data on how the common octopus copes with contamination by the common fish pathogen, Phdp.

2. Material and Methods

<u>Octopods sampling, infections/injections routine and hemolymph samples collection</u>: A total of 54 wild octopi were caught from October to November of 2017. Specimens were immediately placed in tanks at

ICHTHYAI wet laboratory facilities (EL83 BioExp01). Then were acclimatized for 10 days at either 21± 0.5°C or 24±0.5°C. They were fed daily with defrosted or fresh crabs and teleost. After the acclimatization period all octopods were weighed and either infected intramuscularly (i.m., hereafter) at the second right arm or intravenously (i.v., hereafter) at the right branchial heart ventricle, with Phdp $1x10^9$cells/ml or with sterile 2% NaCl (same routes, negative controls). For clarity reasons the following abbreviations were used: I: infected specimens; C: control specimens; M: i.m. injected; V: i.v. injected. The day post-injection/infection is marked as D0, D3and D7. For each injection/infection route and sampling point, a total of 3 different octopi were used and in total 27 octopi for each different temperature. Individuals were checked daily for skin lesions, behavioural changes, feed intake and mortality. On D0, 3 and 7 hemolymph was withdrawn from each specimen using syringes pre-filled with anticoagulant (Alsever's solution). After collection, the hemolymph was immediately transferred into a sterile glass vial and stored at 4°C, until all time-point samples were collected. Hemolymph was then centrifuged to separate cells (750xg for 3 minutes at 4°C) (Thermo Scientific, Heraeus, Pico 21 centrifuge). The cell-free hemolymph was aliquoted and stored at -85°C for future tests. Octopods utilized in the study were 89.5% males and 11.5% females, and weight ranged from 0.475 to1.085kg. The protocol for the experimental infection was approved by Decision No 5379/4-4-2017, of the competent Regional Veterinary Authority. The bacteria were isolated from *Dicentrarchus labrax*; Phdp in 2012 at the Island of Evoia, Greece, and it was a kind donation of Dr. Kantham Papanna (Nireus Aquaculture S.A.).

Total proteins: The BIORAD protein assay used which is based on the Bradford method (Bradford, 1976) and following the manufacturer's instructions. Bovine serum albumin (BSA) (Sigma) was used as the standard protein. All the samples collected were analyzed in triplicates.

Lysozyme assay: Lysozyme activity was measured utilizing the ability of the enzyme to lyse the bacterium *Micrococcus lysodeikticus* (Ellis 1990), with some modifications. Samples were incubated at 30°C and the optical density (OD here after) was read at 450 nm at 0 and 5min. For positive control, hemolymph was replaced by hen egg white lysozyme (serial dilutions starting at 1.6 mg ml^{-1} in PBS) and for negative control, buffer replaced hemolymph. Units of lysozyme activity were defined as follows (Sigma-Aldrich 2021):

$$\text{Units/ml enzyme} = \frac{(\Delta A_{450}/\text{min Test} - \Delta A_{450}/\text{min Blank})\ (df)}{(0.001)\ (0.1)}$$

where:
df = dilution factor
0.001 = change in absorbance (ΔA_{450}=OD_{450}) as per the Unit Definition (One unit of lysozyme causes a decrease in absorbance OD_{450} of 0.001 min^{-1} (Ellis 1990))
0.1 = volume (in milliliters) of the 0.4 mg ml^{-1} *M. lysodeikticus* suspension in phosphate buffer

Hemolysis assay: The hemolytic activity in cell-free hemolymph was measured using 5 x 10^8 cells ml^{-1} sheep red blood cells (RBC hereafter) in 90μl Mg^{2+}-EGTA-Gelatin-HEPES buffer (pH: 7.3-7.4; MEGH hereafter) as described by Tort *et al.* (1996) and Zelek *et al.* (2018) with modifications. Samples were incubated at 20-22°C for 90 min and then the reacting solution was centrifuged at 600x*g* for 5min, at 4°C. Supernatant was then collected, and hemolysis was determined by measuring the OD at 405nm. Complete (100%) and no (0%) hemolysis was determined by adding either distilled water or MEGH buffer in sheep RBC suspension, respectively. Hemolytic activity (ACH) was reported as the percentage lysis and it was calculated according as follows:
Lysis=100*(Absorbance (Abs) sample-Abs background)/ (Abs max-Abs background) % (Zelek *et al.* 2018).
The optical density of hemolymph and MEGH blank was also measured at 405nm.

Statistical analysis: All measurements were made in triplicate and checked for normality using the Kolmogorov-Smirnov test (Norusis 2004). All graphs and statistics were preformed using the IBM's SPSS Statistics (version 20) software. All substantial combinations were tested (same bacterium, day, route but different temperature, same bacterium, route, temperature but different day and same route, day, temperature but different state of animals, infected and non-infected). Results are expressed as the mean ± standard deviation (in text) and standard errors (in graphs) of the mean. A p value of ≤0.05 was considered as significant.

3. Results-Discussion

Total Proteins: The concentration of protein in hemolymph after IM or IV injection with either Phdp or NaCl at 21±0.5°C and 24±0.5°C is illustrated in Figure 1nd b, respectively. Hemolymph's total proteins of D0 controls at 21±0.5°C and 24±0.5°C were 210.52±18.89 mg/ml and 168.60±5.65mg/ml, respectively) (Data not shown in Figure). As shown in Figure 1a, at 21±0.5°C proteins in CIM-CIV control groups dropped from D3 to D7, while infected groups showed significant higher levels of total proteins. Proteins of infected groups, irrespective of route on D7 was significantly higher than control groups. Hemolymph's total proteins at 24±0.5°C (Figure 1b) showed a different pattern. Proteins in IM-IV infected groups ranged in similar levels from D3 to D7 in contrast to the significant higher and lower levels observed in CIM group and CIV group respectively.

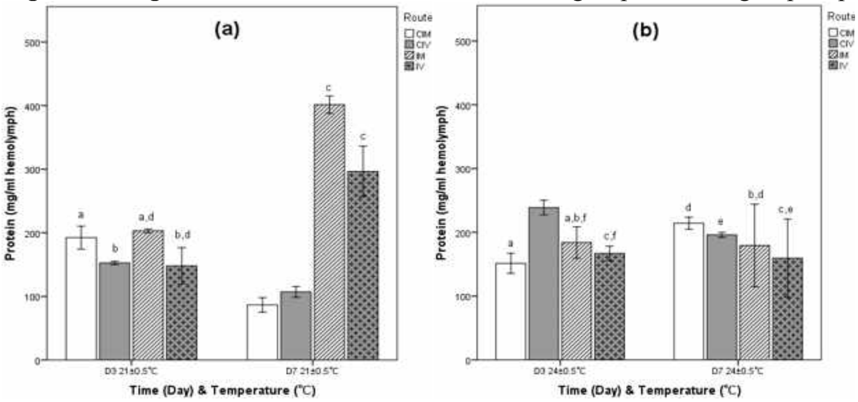

Figure 1. Concentration of protein (mg ml^{-1} hemolymph) after IM or IV injection with either Phdp or NaCl. (a): at 21±0.5°C and (b): at 24±0.5°C. D: day; CIM: controls injected IM; CIV: controls injected IV; IM: infected IM; IV: infected IV. Mean values; same letter indicates no significance (p>0.05).

Apparently, higher temperature influenced in a negative manner the response of infected specimens, in respect to protein synthesis in the hemolymph. Our results are supported by Lorenzon *et al*. (2011) who demonstrated that blood protein levels fluctuate with changes in environmental and physiological conditions in crustaceans, but differ from Pascual *et al.* (2020) who showed that protein concentration is not influenced by seasonal changes. Rogener *et al*. (1987) demonstrated that total protein in hemolymph of *O. vulgaris* non-injected or infected, was high, supporting our measurements.

Lysozyme assay: Lysozyme activity in hemolymph was detected in all samples and is illustrated in Figure 2a and b, respectively. Hemolymph's lysozyme of D0 controls was calculated at 43.99±12.42 and 79.33±16.61 Units ml^{-1}at 21±0.5°C and 24±0.5°C, respectively (data not shown in Figure).

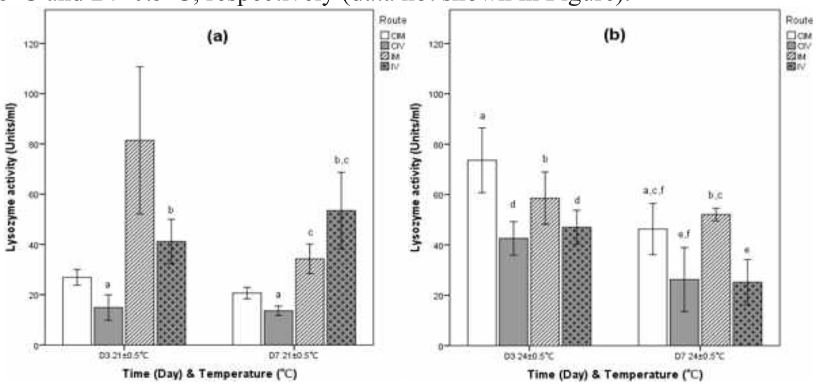

Figure 2. Lysozyme activity (Units ml^{-1}) after IM or IV injection with either Phdp or NaCl. (a): at 21±0.5°C and (b): at 24±0.5°C. D: day; CIM: controls injected IM; CIV: controls injected IV; IM: infected IM; IV: infected IV. Mean values; same letter indicates no significance (p>0.05).

Lysozyme activity, at 21±0.5°C of CIM group and IM group, dropped significantly from D3 to D7, while CIV group and IV group ranged in similar levels. Activity of infected groups, irrespective of route on D3 and D7 was significantly higher than control groups. At 24±0.5°C (Figure 2b), activity in CIM and IM groups ranged in similar levels from D3 to D7 with a tendency to drop, in contrast to the significant lower levels observed in CIV and IV groups. From the results it seems that higher temperature had a deleterious effect on lysozyme activity of infected groups, irrespective of route, while at lower temperature, a significant drop was observed in the IM and a tendency of (not-significant) elevation in the IV group, over time. Malham *et al.* (1998), in contrast to our findings, observed no changes over time in either control or infected specimens with bacteria (*Vibrio anguillarum*).

Furthermore, it was reported that lysozyme activity appears to act non-specifically against a wide range of pathogens in *E. cirrhosa* and hypothesized that live bacteria injected into *E. cirrhosa* were either cleared from the circulation or rendered non-viable within 4 hours. Grimaldi *et al*. (2013) observed a significant increase in lysozyme activity of *Octopus vulgaris* 4 h after intramuscular injection of titanium dioxide nanoparticles with levels returning to baseline levels in less than 24 h, suggesting that the observed effects are of short term. Our results indicated that lysozyme activity in hemolymph can be found for longer periods than previously reported and that temperature, route of infection and probably the pathogen can affect it.

Hemolytic activity: Results on hemolytic activity in cell-free hemolymph are illustrated in Figure 3a and b. Activity of D0 controls was 2.15±0.63 % and 4.48±1.65 %, at 21±0.5°C and 24±0.5°C, respectively (data not shown in Figure).

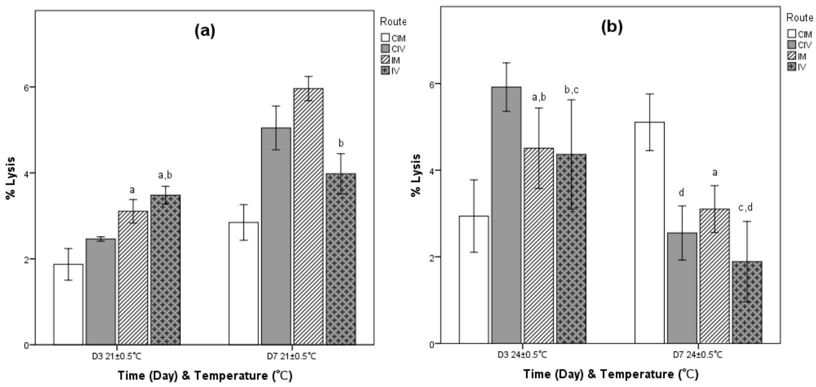

Figure 3. Hemolytic activity (%) after IM or IV injection with either Phdp or NaCl. (a): at 21±0.5°C and (b): at 24±0.5°C. D: day; CIM: controls injected IM; CIV: controls injected IV; IM: infected IM; IV: infected IV. Mean values; same letter indicates no significance (p>0.05).

The hemolytic activity at 21±0.5°C increased significantly from D3 to D7 in all but the IV group (not significant increase). In contrast, activity at 24±0.5°C (Figure 3b) for groups CIV, IM and IV decreased from D3 to D7. Group CIM showed exactly the opposite pattern. Significant differences between time points, were observed only in control groups. Apparently, at lower temperature infected groups showed higher levels of hemolytic activity over time. Generally, hemolysis rate ranged at low levels at both experimental temperatures in agreement to the results reported from Key *et al*. (2002) and Houyvet *et al*. (2018) by salivary gland extracts from the *E. cirrhosa* and cuttlefish hemolymph peptides, respectively. Niu *et al*. (2018) suggested that the hemolytic reactions in the hemolymph of bivalve *S. constricta* showed complement-like activity and that both physical and chemical conditions have effects on hemolysis. Interestingly, total proteins levels of infected specimens (Figure 1a and b) show similarities to the changes of hemolytic activity in the same groups (Figure 3a and b), at both temperatures.

As is known, alternative pathway relies on many hemolymph proteins (Nayak *et al*. 2018) and this may be the reason of the observed correlation. The high concentrations of proteins in hemolymph and the low hemolysis rates observed in our study may be related to an earlier than D3 hemolytic activity.
Hopefully, results of this study may contribute to further research on humoral immune defense in cephalopods and specifically in *Octopus vulgaris*.

Acknowledgments

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References

Aguila, J., Cuzon, G., Pascual, C., Domingues, P. M., Gaxiola, G., Sánchez, A., Maldonado, T., Rosas, C., 2007. The effects of fish hydrolysate (P) level on *Octopus maya* (Voss and Solis) diet: digestive enzyme activity, blood metabolites, and energy balance. Aquaculture. 273, 641–655

Bradford M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle ofprotein dye binding. Anal Biochem. 72, 248-54.

Castellanos-Martinez, S., Gestal C., 2013. Pathogens and immune response of cephalopods. Journal of Experimental Marine Biology and Ecology. 447, 14–22.

Castellanos-Martínez, S., Arteta, D., Catarino, S., Gestal, C., 2014. De Novo transcriptome sequencing of the *Octopus vulgaris* hemocytes using Illumina RNASeq. Technology: response to the infection by the gastrointestinal parasite *Aggregata octopiana*, PLoS One. 9 (10),1- 15.

Castillo, M.G., Salazar, K.A., Joffe, N.R., 2015. The immune response of cephalopods from head to foot. Fish & Shellfish Immunology. 46 (1), 145-160.
Ellis, A.E., 1990. Lysozyme assays. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., van Muiswinkel, W.B., (Eds.), 1990. Techniques in fish immunology, SOS Publications, Fair Haven, N.J. pp. 101-103.
Gestal, C., Castellanos-Martínez, S., 2015. Understanding the cephalopod immune system based on functional and molecular evidence. Fish Shellfish Immunol. 46(1), 120-130.
Grimaldi, A.M., Belcari, P., Pagano, E., Cacialli, F., Locatello, L., 2013. Immune responses of *Octopus vulgaris* (Mollusca: Cephalopoda) exposed to titaniumdioxide nanoparticles. J. Exp. Mar. Biol. Ecol. 447, 123–127.
Houyvet, B., Zanuttini, B., Corre, E., Le Corguillé, G., Henry, J., Zatylny-Gaudin, C., 2018. Design of antimicrobial peptides from a cuttlefish database. Amino Acids. 50(11):1573-1582.
Kasumyan, A., Isaeva, O., Zvonareva, S., 2021. Coloration type of two allied cowries (Ovulidae:Gastropoda) tested through palatability evaluation in feeding experiments with fish. Journal of Experimental Marine Biology and Ecology. 538, (151529), 1-7.
Key, L.N, Boyle, P.R., Jaspars, M., 2002. Novel activities of saliva from the octopus *Eledone cirrhosa* (Mollusca; Cephalopoda). Toxicon. 40(6), 677-683.
Lorenzon, S., Martinis, M., Ferrero, E.A., 2011. Ecological Relevance of Hemolymph Total Protein Concentration in Seven Unrelated Crustacean Species from Different Habitats Measured Predictively by a Density-Salinity Refractometer. Hindawi Publishing Corporation Journal of Marine Biology. 153654, 1-7.
Machałowski, T., Jesionowski, T., 2021. Hemolymph of molluscan origin: from biochemistry to modern biomaterials science. Appl. Phys. A. 127(3), 1-22.
Malham, S.K., Runham, N.W., Secombes, C.J., 1998. Lysozyme and antiprotease activity in the lesser octopus *Eledone cirrhosa* (Lam.) (Cephalopoda). Dev. Comp. Immunol. 22, 27–37.
Nayak, S., Portugal, I., Zilberg, D., 2018. Analyzing complement activity in the serum and body homogenates of different fish species, using rabbit and sheep red blood cells. Vet Immunol Immunopathol. 199, 39-42.
Niu, D., Xiong, Y., Peng, M., Meng, X., Lan, T., Li, J., 2018. Hemolytic reactions in the hemolymph of bivalve *Sinonovacula constricta* show complement-like activity. Fish Shellfish Immunol. 79,11-17.
Norusis, M. J., 2004. SPSS 12.0 Guide to Data Analysis. Prentice Hall PTR. pp. 637.
Pascual, C., Cruz-Lopez, H., Mascaró, M., Gallardo, P., Sánchez, A., Domingues, P., Rosas, C., 2020. Changes in Biochemical Composition and Energy Reserves Associated with Sexual Maturation of *Octopus maya*. Frontiers in Physiology. 11, 1-22.
Rögener, W., Renwrantz, L., Uhlenbruck, G. 1987. Analysis of *Octopus vulgaris* hemolymph containing a glycoprotein with blood group A-like properties. Comp. Biochem. Phys. B Comp. Biochem. 86, 347–351.
Sigma-Aldrich, 2021. https://www.sigmaaldrich.com/GR/en/technical-documents/protocol/protein-biology/enzyme-activity-assays/enzymatic-assay-of-lysozyme (accessed 30/07/2021).
Tort, L., Gomez, E., Montero, D., Sunyer, O., 1996. Serum haemolytic and agglutinating activity as indicators of fish immunocompetence: their suitability in stress and dietary studies. Aquaculture International. 4, 31-41.
Zelek, W.M., Harris, C.L., Morgan, B.P., 2018. Extracting the barbs from complement assays: Identification and optimisation of a safe substitute for traditional buffers. Immunobiology. 223(12),744-749.

THE EFFECT OF SALINITY ON THE GROWTH PERFORMANCE AND SURVIVAL RATE OF GILTHEAD SEABREAM (*Sparus aurata*) AND ROCK SAMPHIRE (*Crithmum maritimum*) IN AN EXPERIMENTAL BRACKISH AQUAPONIC SYSTEM

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Abstract
In recent years the design for brackish water aquaponic systems is increasing. The possibility of simultaneous production of halophyte and euryhaline fishes creates huge interest in both commercial aquaponics systems and in research. The aim of the present study was to investigate the effect of three different salinities (20 ppt, 14 ppt and 8 ppt) on the growth performance and survival rate of sea bream (*Sparus aurata*) and rock samphire (*Crithmum maritimum*) in an experimental brackish aquaponic system. A total number of 234 sea bream individuals with an average initial weight of 3.90 ± 0.05 g were used. They were divided into 9 autonomous aquaponic systems. The experiment lasted 45 days. In total, 54 rock samphire individuals were used and distributed into groups of 6 individuals in the hydroponic tanks using the raft method. The results showed that sea bream showed statistically better growth performance (SGR,% / d) in salinity 8 ppt compared to salinities 14 ppt and 20 ppt (ANOVA, $p <0.05$). Also, the best rock samphire growth (height increase and percentage height increase) appeared to be significantly higher in 8 ppt salinity (20.27 ± 1.2 cm) compared to 20 ppt and 14 ppt, which were smaller (ANOVA, $p <0.05$). The results showed that sea bream and rock samphire can be used in brackish aquaponics systems with satisfactory growth performance and have a range of commercial applications which generate interest in their production.
Keywords: *Brackish aquaponic system, growth performance of gilthead seabream and rock samphire, salinity.*
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1. Introduction

Due to the continuous growth of the human population, the need to find effective solutions for the problems regarding the lack of resources makes aquaponics systems an innovative and important solution that can contribute substantially to this direction. Also, the reckless use of freshwater, combined with the vast availability of brackish water and saltwater, is of interest to researchers using brackish water in aquaponics systems.

Previous studies reported that aquaponics is a system of sustainable food production, which combines fish farming and hydroponics, cultivating aquatic organisms (fish, invertebrates) and plants (vegetables, herbs, algae) to thrive in a symbiotic environment (Nelson 2008; Fronte *et al.* 2016; Vlahos *et al*. 2019; Lennard 2021; Stathopoulou *et al*. 2021). Animals, plants and microorganisms coexist in multicultural systems that offer environmentally friendly conditions as well as increased efficiency in the use of resources. The basic operating principle of aquaponics systems is the oxidation of ammonia to nitrite and nitrate ions, thus creating nutrients that are highly exploitable by plants for their growth (Somerville *et al*. 2014).

Ammonia is released as a metabolic product through gill mediators and is described as the process that enables the simultaneous growth of plants, bacteria and fish. The ammonia-oxidizing bacteria (AOB) (most important are those of the genus *Nitrosomonas* sp.), convert toxic ammonia to nitrite ions, and the nitrite-oxidizing bacteria (NOB) (most common are those of the genus *Nitrobacter* sp.) further convert to nitrate ions (Li *et al*. 2018). The operation of aquaponic systems is based on the operation of close recirculating aquaculture systems and the use of the biological filter that achieves control of the water quality. Water is transported from fish tanks to the biological filter by gravity, and from there to plants via pumping. Finally, the water returns from the plants back in the fish aquariums.

In brackish aquaponic systems, the most commonly used species are sea bream, sea bass and tilapia. The co-cultivation of euryhaline species and halophytes (since the latter are more resistant to salt stress) can render greater yields in brackish aquaponics systems. Sea bream (*Sparus aurata)*, is a Mediterranean farmed species with high commercial value and increased adaptation to salinity fluctuations. However, there is little knowledge about its growth performance in aquaponic systems (Vlahos *et al*. 2019). In the present study, the Mediterranean halophyte rock samphire (*Crithmum maritimum)* was used. The aim of the present study was to investigate the effect of different salinities (8 ppt, 14 ppt and 20 ppt) on the growth performance and survival rate of sea bream (*Sparus aurata*) and rock samphire (*Crithmum maritimum*) in a brackish experimental aquaponic system.

2. Material and Methods

The experiment was carried out at the Laboratory of Aquaculture (lab-scale aquaponics section), Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece. Gilthead seabream (*Sparus aurata*) and rock samphire (*Crithimum maritimum*) were provided from a local nursery facility and the Institute of Plant Breeding & Genetic Resources, Hellenic Agricultural Organization Demeter, respectively. They were divided into three treatments under three different salinities 8 ppt, 14 ppt and 20 ppt, respectively. For the experiment nine autonomous small-scale aquaponic systems (135 L) consisting of 9 fish tanks (54 L), 9 hydroponic tanks (54 L) and 9 sump biofilters (27L) were used as described by Vlahos et al. (2019). The experiment lasted for 45 days.

Fish and plants were adapted in the three experimental salinities for 30 days. After the successful adaptation of gilthead seabream and rock samphire in the experimental salinities, 234 gilthead sea bream individuals were divided into 26 individuals per aquarium with an initial average weight of 3.90±0.05 g, and an initial average length of 6.75±0.03 cm. Also, 54 individuals of rock samphire were divided into 6 individuals in each system with an average initial height of 7.90 ±0.05 cm. Gilthead seabream fed with a commercial pellet (1.5 mm), by hand in three meals six days a week, except the 7^{th} day where the fish remained with an empty stomach and the system was allowed to discharge from fish wastes. The nutrition level was determined at 5% and was kept constant throughout the experiment, three times per day. Fish were weighed every fifteen days during the experiment to remeasure their weight and length and to culculate the amount of daily food intake. Fish tanks were cleaned, and non-consumed food was removed every day by siphoning. At the end of the experiment, fish were anaesthetized with an 0.20 mg/L MSS 222, and final fish body weights and lengths were measured.

The measurement of the morphometric characteristics of plants was performed every 15 days during the experimental period. Throughout the experiment, the physicochemical parameters of water were monitored three times a week for dissolved oxygen, temperature and pH, and two times a week for total ammonia, nitrite, nitrate ions, phosphates, calcium, alkalinity and magnesium. The plants were placed in clay pebbles (8-16 mm) substrate of the hydroponic tank at a distance of 7 cm. The photoperiod was set up to be 14 light: 10 h dark (summer photoperiod). The homogeneity of photosynthetically active radiation (PAR) reaching the plant top was maintained at the level of 500-600 μmol m^2 sec^{-1}.

At the end of the experiment, the following indexes were calculated using the equations below as described by Mente et al. (2016), where W_{in} and W_{fin} are the initial and final weight of the fish, respectively, and Δt is the duration of the experiment in days:

SGR (%/day) = $((\ln W_{fin} - \ln W_{in})/\Delta t) \times 100$
WG (gr) = $W_{fin} - W_{in}$
FCR = Food offered (g)/weight gain (g)
DFI (%/d) = 100 × ((food offered/weight gain)/feeding days)
Condition factor (K) = $(W \times L^{-3}) \times 100$ where W is the bodyweight of the fish (gr) and L is the total length of the fish (cm)
S = ((final number of fish − initial number of fish)/initial number of fish) × 100

The growth rate of the plants was calculated according to the equation below as described by Endut *et al.* (2010). Plant Growth (cm/d) = height of plant/day, $DH=H_{fin}-H_{in}$, $G\%=(H_{fin}-H_{in}) \times 100 / H_{in}$, $h/t=H_{fin}/day$

Data were checked for normality (Kolmogorov-Smirnov test), homogeneity (Levene test) and comparisons of means (One way ANOVA). Data were considered as statistically significant at $p<0.05$ (Zar 1999). Statistical analyses were carried out using the software package IBM SPSS Statistics V25.

3. Results

Rearing conditions and the dissolved nutrients in the brackish aquaponic system are presented in Table 1. In all aquaponic systems, there are no significant differences between TAN_{in}, TAN_{out}, $NO_3^-{}_{in}$, $NO_3^-{}_{out}$, $PO_4^={}_{in}$, $PO_4^={}_{out}$, NO_2^-, Ca (ANOVA, $p<0.05$). The mean value of pH_{FT} in fish tanks ranged between 6-6.5 and was within the same range (6.3-6.6) in the hydroponic tanks (pH_{GB}) (Table 1).

Table 1. Rearing conditions (pH_{FT}, pH_{GB}) and dissolved nutrients (TAN_{in}, TAN_{out}, $NO_3^-{}_{in}$, $NO_3^-{}_{out}$, $PO_4^={}_{in}$, $PO_4^={}_{out}$, NO_2^-, and Ca) in the fish and hydroponic tanks of the brackish aquaponic system over the experimental period of 45 days.

	20 ppt	14 ppt	8 ppt
TAN_{in}	$0.51±0.13^a$	$0.50±0.11^a$	$0.70±0.24^a$
TAN_{out}	$0.48±0.09^a$	$0.46±0.08^a$	$0.67±0.22^a$
$NO_3^-{}_{in}$	$114.61±14.73^a$	$122.38±11^a$	$120±11.12^a$
$NO_3^-{}_{out}$	$112.08±16.05^a$	$111.41±17.83^a$	$109.76±16.09^a$
NO_2	$0.17±0.04^a$	$0.14±0.04^a$	$0.18±0.96^a$
$PO_4^-{}_{in}$	$0.51±0.18^a$	$0.52±0.16^a$	$0.52±0.18^a$
$PO_4^-{}_{out}$	$0.42±0.15^a$	$0.47±0.18^a$	$0.43±0.10^a$
Ca	$414±28.20^a$	$468 ±43.99^a$	$464±46.57^a$
pH_{FT}	6.0±0.41	6.1±0.41	6.5±0.38
pH_{GB}	6.3±0.4	6.5±0.34	6.6±0.29

Data were expressed as mean ± S.E.M (n=16). Means in a row followed by the same superscript are not statistically significantly different (p>0.05).

Fish growth performance is presented in Table 2. At the start of the experiment, there were no statistically significant differences in the means of the gilthead seabream initial body weights and lengths (ANOVA, p>0.05) in all salinity treatments tested. At the end of the experiment, there were no significant differences in the means of the weight gain, coefficient factor (K_{in}), bodyweight increase (%) in all treatments (ANOVA, p>0.05). Final mean weight, specific growth rate (SGR) and final coefficient factor were significantly higher at 8 ppt than 14 ppt and 20 ppt treatments (ANOVA, p<0.05). Survival rate for gilthead seabream over 45 days of culture was ranged between 89.8%, 96% and 97.3%, respectively, for aquaponic systems with 20 ppt, 14 ppt and 8 ppt (Table 2).

Mean values of FCR and DFI at the end of the experiment were not significantly different between the salinity treatments (ANOVA, p>0.05) (Table 2). At the start of the experiment, there are no significant differences in the means of rock samphire initial height (ANOVA, p>0.05). At the end of the experiment, rock samphire growth performance (final height, dH, % G and dH/d) was statistically significant higher at 8 ppt than 14 ppt and 20 ppt salinities (Table 2).

4. Discussion

The present study investigated aquaponics in brackish water using a Mediterranean fish species (gilthead seabream) and a halophyte (rock samphire), both associated with a high commercial and nutritional value. According to the results of the present study, the salinity affects the growth performance of gilthead seabream and the rock samphire. The results showed that gilthead sea bream in the brackish aquaponics system had a high growth performance ($SGR8_{ppt}$=$SGR20_{ppt}$ =3.6%/day>$SGR14_{ppt}$=3.1%/d), survival rate ($S8_{ppt}$=96% $S20_{ppt}$ =89,6%, $S14_{ppt}$=97%) and FCR. Previous studies (Vlahos *et al.* 2019) report that gilthead seabream when cultured in a brackish aquaculture system with 20 ppt and 8 ppt salinities show SGR 3.1%/d for both salinities (8 ppt, 20 ppt), which is in agreement to SGR values in the 14 ppt salinity treatment observed in the present study. Laiz-Carrion *et al.* (2005) have reported that 20 g gilthead seabream cultured in a brackish aquaculture system for 100 days have shown better growth performance in 12 ppt (1.99%/d) than 28 ppt (1.91%/d) and 6 ppt (1.79%/d), lower from the SGR values reported in the present study. Regarding rock samphire, our findings showed statistically significant better growth in terms of final height, final height/d and growth (%) in 8 ppt than in 20 ppt and 14 ppt salinities. The results of the present study are in agreement with those reported by Vlahos *et al*. (2019). Tasiou

(2019) reported that rock samphire growth performance was better in salinity 8 ppt than in 14 ppt and 20 ppt in a brackish aquaponic system cultured with seabass. According to Flowers *et al.* (1986), halophytes are capable of surviving and reproducing in salt concentrations around 200 mmol/L NaCl or more (equivalent to >12 ppt).

Table 2. Gilthead seabream and rock samphire growth performances during the 45 days cultivation trials in brackish aquaponics systems.

	20 ppt	14 ppt	8 ppt
	Gilthead seabream *growth performance*		
Initial mean weight (W_{in}, g)	3.89 ± 0.05 ^{a}	3.85 ± 0.04 ^{a}	3.97 ± 0.05 ^{a}
Final mean weight ($W_{fin,}$ g)	19.44 ± 0.31^{a}	18.52 ± 0.28 ^{a}	19.71 ± 0.36 ^{b}
Weight gain (WG,g)	14.91 ± 0.61 ^{a}	14.67 ± 0.28 ^{a}	15.7 ± 0.38 ^{a}
Specific growth rate (SGR, %/ d)	3.60 ± 0.05 ^{b}	3.09 ± 0.14 ^{a}	3.58 ± 0.06 ^{b}
Survival (%)	89.8± 6,74^{a}	97.3 ±1,33^{a}	96 ±2,30^{a}
Body weight icrease (BWI $_{t45}$%)	412.8±11.88 ^{a}	387.3 ± 10.24 ^{a}	408.9 ± 13.70 ^{a}
Initial Initial coefficient factor (Kin)	1.24 ± 0.01^{a}	1.28 ± 0.01^{a}	1.26 ± 0.01^{a}
Final coefficient factor (Kfin) (K_{fin})	1.49 ± 0.01^{a}	1.48 ± 0.01^{a}	1.54 ± 0.01^{b}
Initial mean Length (L_{in}, cm)	6.77 ± 0.03^{a}	6.70 ± 0.03^{a}	6.80 ± 0.03^{a}
Final mean Length (L_{fin}, cm)	10.71 ± 0.18^{a}	10.76 ± 0.05^{a}	10.82 ± 0.06^{a}
FCR (t45)	0.70 ± 0.01^{a}	0.70 ± 0.01^{a}	0.74 ± 0.04^{a}
Daily Food Intake (DFI $_{t45}$ %/d))	1.55 ± 0.02^{a}	1.65 ± 0.04^{a}	1.54 ± 0.04^{a}
	Rock samphire growth performance		
Final height gain/day	2.82 ± 0.42^{a}	2.62 ± 0.45^{a}	8.97 ± 0.92^{b}
Plant Growth (%G)	46.84 ± 8.62^{a}	20.60 ± 3.03^{b}	77.14 ± 6.71^{c}
Final Height/d	0.22 ± 0.01^{a}	0.32 ± 0.01^{b}	0.45 ± 0.02^{c}

Data were expressed as mean ± S.E.M (n_{fish}=78, n_{plants}=54 and $n_{initial}$=234). Means in a row followed by the same superscript are not statistically significantly different (p>0.05).

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References

Endut A., Jusoh A., Ali N., Wan-Nik W., Hassan A., (2010). A Study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresour. Technol. 101: 1511-1517.

Fronte, B., Galliano, G., Bibbiani, C, (2016). *From freshwater to marine aquaponic: new opportunities for marine fish species production*. 4th Conference with International Participation Conference VIVUS-on Agriculture, Environmentalism, Horticulture and Floristics, Food Production and Processing and Nutrition «With Knowledge and Experience to New Entrepreneurial Opportunities» 20th and 21st April 2016, Biotechnical Centre Naklo, Strahinj 99, Naklo. Slovenia.

Laiz-Carrion, P., Sangiao-Alvarellos, S., Guzman, J.M., Martın del Rıo, M.P., Soengas, J.L., Mancera, J.M. (2005). Growth performance of gilthead seabream *Sparus aurata* In different osmotic conditions: Implications for osmoregulation and energy metabolism. Aquaculture 250: 849-861.

Lennard, W., (2021). A comparison of the effect of starting nitrate concentration on fish growth, plant production and water quality within a research-scale, recirculating aquaponics system. *Aquaculture and Fisheries*. 1-10. DOI: 10.1016/j.aaf.2020.11.001

Li, G., Taoa, L., Lia, X., Penga, L., Songa, C., Daia, L., Wub, L., Xieb, L. (2018). Design and performance of a novel rice hydroponic biofilter in a pond-scale aquaponic recirculating system. Ecological Engineering, 125: 1-10.

Mente, E., Solovyev, M.M.,Vlahos, N., Rotllant, G., Gisbert,E (2016). Digestive enzymes activity during initial ontogeny and after feeding diets with different protein sources in zebra cichlid *Archocentrus nigrofasciatus*. Journal of World Aquaculture Society, 48(5): 831-848.

Nelson, R.L.(2008). Aquaponic Food Production: Growing Fish and Vegetables for Food and Profit. Nelson and Pade, Wisconsin.

Stathopoulou, P., Berillis, P., Vlahos, N., Nikouli, E., Kormas, K., Levizou, E., Katsoulas, N. and Mente, E., (2021). Freshwater-adapted sea bass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. *PeerJ*, 9, p.e11522.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., Lovatelli, A. (2014). Small-scale aquaponic food production: Integrated fish and plant farming 16-18, 22-26, 75-81.

Tasiou K. (2019). The production process of sea bass in an aquaponic system. MSc thesis. Department of Ichthyology and Aquatic Environment, University of Thessaly, pp. 85.

Vlahos, N., Levizou, E., Stathopoulou, P., Berillis, P., Antonopoulou, E., Bekiari, V., Krigas, N., Kormas, K ., and Mente, E. (2019). An experimental brackish aquaponic system using juvenile gilthead sea bream (*Sparus aurata*) and rock samphire (*Crithmum maritimum*). Sustainability 11(18). DOI: 10.3390/su11184820.

Zar J. H. Biostatistical Analysis; Prentice-Hall: London,UK., (1999); P. 718.

THE EFFECT OF DIFFERENT OZONE CONCENTRATIONS ON THE OXIDATION RATE OF AMMONIA AND NITRITE ION AT A SEAWATER AQUARIUM

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Abstract
The effect of two different concentrations of ozone in oxidation rate of ammonia and nitrite ion was studied. For this experiment, nine tanks were used with a total capacity of 40 L. Larvae medium 0,92 ± 0,28 cm was used for the filter bed and the filtration speed was 1.53 ± 0,08 cm / min. The aquariums were divided into three groups, of which the ozone concentrations were supplied at 0.2 and 6 mg / L levels, respectively. The results showed that ozone levels improved the aquarium water quality. The total ammonia and nitrite ions were oxidized by 85% and 95% respectively when the ozone concentration was 2 mg/L and when the ozone concentration was 6 mg/L, the total ammonia and nitrite ions were oxidized by 90% and 87 % respectively. As ozone is a well-known oxidizing agent that could be used for the dissolution of organic effluent in aquaculture, the present work shows also its possible use as an oxidation agent for the inorganic contaminants.
Key words: *Ozone, oxidation, ammonia removal, nitrite, saltwater aquarium*
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1. Introduction

The aquarium is a small scale close recirculation system for rearing and preserve fish and invertebrates with commercial value. The intensification of the production process in the aquarium leads to the increase of the organic and nitrogen production load. As a result, there is an increase of nitrogen effluent at the biological filter and at the same time, the oxidation gets weaker or fully stops. This situation can create serious problems for the survival of the fish and the invertebrates in the aquarium, or even kill them eventually (Krumins *et al.* 2001a). The survival of the fish and invertebrates at the aquarium depends on the dissolved oxygen levels, the nitrification rate, the fish waste and the ammonia reduction rate (King 2001). Ozone is one powerful oxidizing agent and could be used for the dissolution of organic effluent at aquaculture systems (Tango & Gagnon 2003; Sharrer & Summerrfelt 2007; Read 2008). Ozone has many different roles at aquariums and recirculating aquaculture systems (RAS). It can remove organic carbon and clean the aquarium's water by deburring one big variety of factors that causes turbidity, odour, colour and taste (Gonçalves & Gagnon 2011). Also, ozone is very effective against pathogens of every type like bacteria, viruses, funguses and parasites (Gonçalves & Gagnon 2011). In addition, ozone improves water quality by reducing biochemical oxygen demand (BOD), ammonia (NH_3) and nitrous ions (NO_2), and is widely used as a disinfectant in fish hatcheries and fish eggs (Crisp & Bland 1990; Grotmol *et al*. 2003; Buchan *et al.* 2005; Ritar *et al*. 2006). The aim of the present study was to investigate the effect of two different ozone concentrations (2 mg/L and 6 mg/L) at the oxidation rate of ammonia and nitrite ion at a seawater aquarium system.

2. Materials and Methods

The experiment was conducted at the experimental facility of the Laboratory of Aquarium, Department of Animal Production, Fisheries and Aquaculture (University of Patras, Greece). For the experimental process, nine aquariums were used with a total volume of 40L (40 cm x 23.5cm x 35cm). They were divided into three treatments with their replicates under three different ozone concentrations 0, 2 and 6 mg/L, respectively. These concentrations were chosen for identifying the optimum ozone concentration for ammonia removal in the aquarium system. The concentrations 2 mg/L and 6 mg/L were chosen as the minimum and maximum ozone concentrations affecting the ammonia oxidation rate. The third treatment was without ozone supply and was used as the control treatment. The aquaria were supported by a filter bed (mechanical and biological filtration) properly fitted in each part and were covered with 4.5 kg of lava grain with a mean diameter of 0.92±0.28 cm to act as filter bed substrate for the biological filtration. The surface area of the filter bed was 940 cm^2. The conditioning of the filter followed the method described by Vlahos *et al.* (2004) and Mente *et al.* (2016). To set up the filter 10 lava grains was added from a conditioned filter bed (Spotte 1992). Two days later 50 × 10^6 cells/aquarium were added. The salinity was 30 ppt in all treatments and the water was continuously recycled through the filter bed using an air-lift pump with an adjusted flow rate of 2.142 cm^3/min creating a filtration speed of 1.53 ± 0.08 cm/min. Oxygen levels were fluctuating and ranged between 80 to 90% of saturation. An amount of 0.2 g solid NH_4Cl was dissolved in each aquarium to serve as the ammonia source (Vlahos *et al*. 2013; Mente *et al.* 2016).

For the addition of ozone to the aquariums, ozone generators were used (Sander-C25). They were adjusted to two different concentrations levels of 2 mg/L and 6 mg/L, respectively. To check the performance of the Ozonator, cylindrical portable dehumidifiers were connected and were filled with silica gel, so the oxygen passes through the silica gel to eliminate air moisture (Tango & Gagnon 2003). Total ammonia nitrogen (T.A.N), nitrite and nitrate were analyzed every two days by using an absorption spectrophotometer (HACH 3800). pH, dissolved oxygen and Oxidation-Reduction Potential (ORP) were monitored twice a week by using a multimeter (HACH 40QH). Residual ozone was monitoring with a spectrophotometer (Spectroquant Pico-Multi colourimeters, Merck). Ionized and non-ionized ammonia were calculated from the below equations described by Spotte (1992): non-ionized ammonia = α * TAN, Ionized ammonia = TAN-Non-ionized ammonia, a: molar fraction of ammonia Values are presented as means ± standard error of the mean (S.E.M.). Data were tested for normality and homogeneity with Kolmogorov–Smirnov and Levene's tests, respectively. To determine any significant differences between different ozone treatments, one-way ANOVA was used, followed by Tukey's post-hoc test. Independent t-tests were considered statistically significant at $p < 0.05$ (Zar 1996). Statistical analyses were carried out using the software package IBM SPSS Statistics V25.

3. Results

The oxidation of total ammonium nitrogen (TAN) and nitrite ion levels was monitored at all aquariums (Figure 1). At the highest ozone concentration (6 mg/L) the oxidation rate of ammonia (TAN) was higher compared with the lower ozone concentration where ammonia was fully oxidized in 22 days. However, there weren't any statistically significant differences between the ozone treatments (ANOVA, $p>0.05$), (Table 1). The oxidation rate of ammonia at the control treatment (zero ozone concentration) showed no statistically significant differences from the highest ozone concentration (6 mg /L) or the lowest (2mg/L) (ANOVA, $p> 0.05$).

The value of nitrite ion was calculated at 0.24±0.11 mg/L for the aquariums with the lowest ozone concentration and 0.16±0.09 mg/L for the aquariums with the highest ozone concentration (Figure 2). For the ozone-free aquariums, the value was 0.88±0.13 mg/L. The nitrite oxidation was faster (9 days) in aquariums where the ozone concentration was higher (6 mg/L), in contrast to the lower concentration (2 mg/L) where the rate of oxidation was higher (12 days), without showing statistically significant differences (ANOVA, $p> 0.05$) (Table 1). Also in the aquariums where ozone was not administered, the rate of oxidation of nitrite ions lasted more days (27 days), compared to the aquariums where ozone was administered, showing statistically significant differences (ANOVA, $p<0.05$) (Table 1). In all aquariums a slow increase of nitrate ion concentration was observed (Figure 3) and was of the order of 35.3 ± 4.01 mg/L for the aquariums where ozone was administered 2 mg/L, 28.19 ± 2.56 mg/L for the aquariums where ozone was administered 6 mg/L and 20.38 ± 1.94 mg/L for aquariums where ozone was not administered, showing statistically significant differences (ANOVA, $p <0.05$) (Table 1).

Table 1. Water Parameters (pH, ammonia, nitrate, nitrite, redox and residual ozone) in experimental aquariums during 30 days

	2 mg/l	**6 mg/l**	**0 mg/l**
pH	$7.01±0.05^a$	$6.75±0.07^b$	$7.65±0.03^c$
Total ammonium nitrogen (TAN) (mg/L)	$1.02±0.28^a$	$1.33±0.41^a$	$2.06±0.32^a$
Nonionized ammonia (NH_3-N) (mg/L)	$0.013±0.00^a$	$0.016±0.01^a$	$0.058±0.01^b$
Ionized ammonia (NH_4^+) (mg/L)	$1.00±0.28^a$	$1.32±0.41^a$	$2.00±0.31^a$
Nitrite ions (NO_2^--N) (mg/L)	$0.24±0.11^a$	$0.16±0.09^a$	$0.88±0.13^b$
Nitrate ions (NO_3^--N) (mg/L)	$35.30±4.01^a$	$28.19±2.56^b$	$20.38±1.94^c$
ORP (mV)	$357±6.37^a$	$659±16.17^b$	$259±3.85^c$
Residual ozone (ORD) (mg/L)	$0.54±0.06^a$	$0.52±0.04^a$	-

Data were expressed as mean ± S.E.M (n=15). Means in a row followed by the same superscript are not significantly different ($p>0.05$).

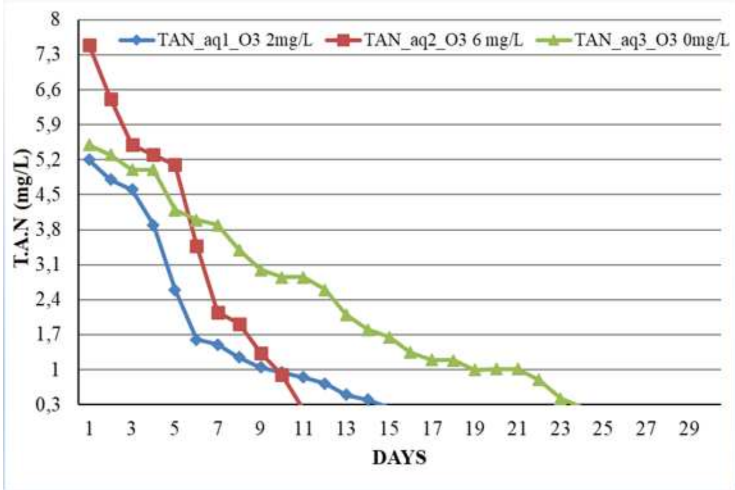

Figure 1: Variation of total ammonia (TAN) in experimental aquariums for 30 days.

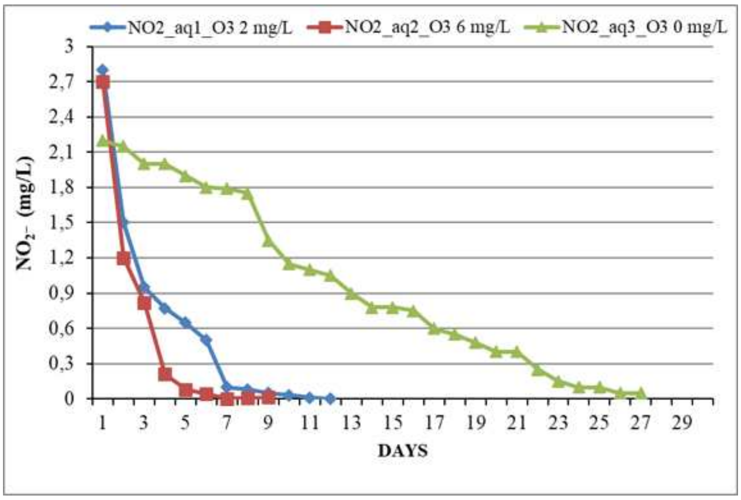

Figure 2: Nitrite ion fluctuations in experimental aquariums for 30 days

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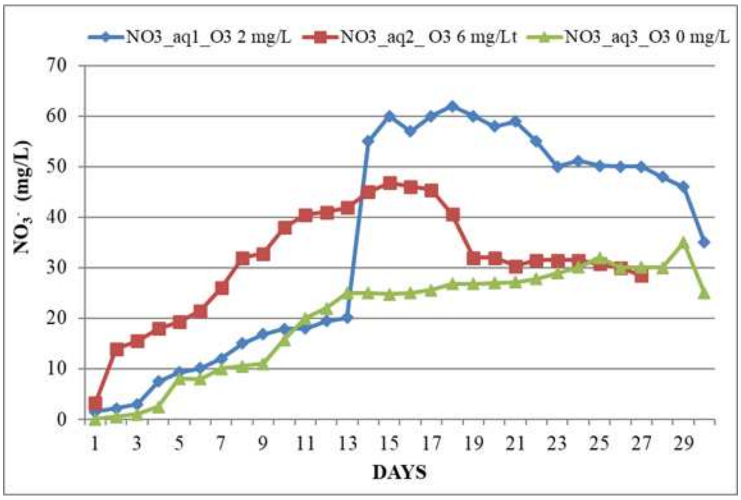

Figure 3: Nitrate ion fluctuations in experimental aquariums for 30 days.

Oxidation-reduction Potential (ORP) showed the highest value (659 ± 16.17 mV) when the ozone concentration was 6 mg/L, compared to the lowest ozone concentration (2 mg/L), where the redox was 357 ± 6.37mV. In aquariums that did not administer ozone, redox received the lowest value (259 ± 3.85mV) showing statistically significant differences (ANOVA, $p<0.05$) (Table 1). Also, there were no statistical differences ($p>0.05$) between the residual values of ozone concentrations in the experimental aquariums (Figure 4).

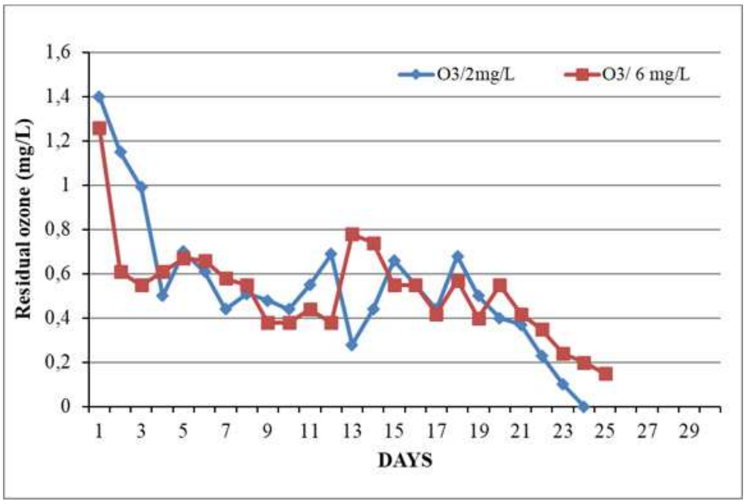

Figure 4. Concentrations of the residual ozone in the experimental aquariums duration of 30 days.

4. Discussion

Ozone is widely used in aquaculture because it improves the quality of the water for rearing fish and inverterbates and acts as a complementary disinfectant in marine aquariums. Moreover, ozone contributes to the inactivate of pathogens, oxidizes organic waste and nitrite ions. The results from the present study showed a higher removal rate of ammonia (85%) when ozone was supplied in the aquariums, compared to non-ozone-treated aquariums. The results are in agreement with those of Sutterlin *et al.* (1984), which reported that the ammonia removal from a freshwater fish farming system with ozone was 26% higher than that of a non-ozone system. In addition, the results of the present study are consistent with those of Rahmadi & Kim (2014) who investigated the effect of five different ozone concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 ppm) on the oxidation process of nitrogen compounds (ammonia and nitrite ions) and reported that higher values (approximately 5 mg/L) of ammonia (NH_4^+-N) and nitrite ions (NO_2^- -N) were fully oxidized in 24 h and 1.5 h, respectively in all treatments. According to the results of the present study, ozone improved significantly the water quality levels in the aquariums by speeding up the conditioning of the biological filter in contrast to the aquariums that did not use ozone (ANOVA, $p<0.05$). The effect of the two different ozone concentrations (2 & 6 mg / L) on the oxidation rate of total ammonia and nitrite ions was evident without statistically significant differences (t-test, $p> 0.05$). Ammonia levels decreased by 85%, at the lowest ozone concentration (2 mg/L) compared to the higher ozone concentration (6 mg/L), where a greater ammonia reduction-oxidation (90%) was induced. Nitrite ions showed a higher decrease (95%) when ozone was 6 mg / L, compared to aquariums that received a lower ozone concentration (2mg / L), where a decrease of 87% was observed. The results of the present work are consistent with those of Suantika *et al*. (2001) who showed that water quality is significantly improved. Their results showed a 67% reduce in ammonia (TAN) levels, nitrite ions (NO_2^-) by 85% and nitrate ions (NO_3^-) by 67%. According to Spotte (1992), ozone does not simply convert nitrite ions (NO_2^-) to nitrates (NO_3^-) but causes the organic bonds to break down, making them easier to be removed from the breakdown filter of organic waste as well as reduces the nitrate ions from the filter. In addition, Adlin *et al.* (2020) showed that auxiliary use of ozone, together with DHS-USB filters, effectively breaks down nitrogenous compounds and removes pigments substances from water. The results were so drastic that in this experiment the carps in the aquariums with the combination of ozone and DHS-USB filters survived for 425 days without any water change. Non-ozone-treated aquariums were ensured to have a stable period of biological filter regulation and bacterial abundance and growth. In addition, the concentration of redox determines the amount of pathogens present in the water (Spotte 1992). In the present study, it was found that high concentrations of ozone (6 mg/L) create a safe environment for fish to live in comparison to lower ozone concentration (2 mg/L) or non-ozone.

Increasing the redox in the aquarium water signals the minimization of pathogens and provides a healthy aquarium system for the rearing of the fish and the invertebrates.

5. References

Adlin N., Hatamoto M., Yamazaki S., Watari T., Yamaguchi T. (2020). A potential zero water exchange system for recirculating aquarium using a DHS-USB system coupled with ozone. Environmental Technology, DOI:10.1080/09593330.2020.1784295

Buchan K.A.H., D.J. Martin-Robichaud, T.J. Benfey. (2005). Measurement of Dissolved Ozone in Sea Water: A Comparison of Methods, Aquacult. Eng., 33(3):225–231

Crisp L.M. &. Bland C.E (1990). Potential Use of Ozone to Disinfect Sea Water of Fungi Causing Diseases of Cultured Marine Crustacean, J. Invert. Pathol., 55:380–386

Gonçalves A. A. & Gagnon, G., A. (2011). Ozone Application in Recirculating Aquaculture System: An Overview, *Ozone: Science & Engineering*, 33: 345–367

Grotmol S. E., Dahl-Paulsen, and Totland G.K. (2003). Hatchability of Eggs from Atlantic Cod, Turbot and Atlantic Halibut after Disinfection with Ozonated Seawater Aquaculture 221:245–254

King R.K.,(2001). The Presence of Bacterial Pathogens in Biofilms of Recirculating Aquaculture Systems and Their Response to Various Sanitizers, PhD Thesis, Faculty of the Virginia Polytechnic Institute and State University, Virginia, Available in http://scholar.lib.vt.edu/theses/ available/etd-042420014/unrestricted/dissertation.pdf

Krumins V., Ebeling J., Wheaton F. (2001a). Part-Day Ozonation for Nitrogen and Organic Carbon Control in Recirculating Aquaculture Systems, Aqua. Eng., 24(3):231–241

Rahmadi P. & Kim Y. R (2014). Effects of different levels of ozone on ammonia, nitrite, nitrate, and dissolved organic carbon in sterilization of seawater, Desalination and Water Treatment, 52(22-24), 4413-4422

Read P., (2008). Ozone in Recirculating Aquaculture, New South Wales Department of Primary Industries – Fishing and Aquaculture. New South Wales, Australia, 2008.

Ritar, A.J., Smith G.G., Thomas C.W. (2006). Ozonation of Seawater Improves the Survival of Larval Southern Rock Lobster, Jasus edwardsii, in Culture from Egg to Juvenile", Aquaculture, 261:1014–1025

Sharrer M.J. & Summerfelt S.T. (2007). Ozonation Followed by Ultraviolet Irradiation Provides Effective Bacteria Inactivation in a Freshwater Recirculating System, Aquacult. Eng., 37:180–191

Spotte, S. (1992). Captive Seawater Fishes. John Wiley & Sons, New York. 942 pp.

Suantika, G., Dhert P., Rombaut G., Vandenberghe J., De Wolf T., Sorgeloos P. (2001).The Use of Ozone in a High Density Recirculation System for Rotifers Aquaculture, 201:35–49

Sutterlin, A.M, Couturier C.Y., Devereaux T. (1984). A recirculating system using ozone for the culture of Atlantic salmon. The Progressive Fish-Culturist 46:239-244.

Tango M.S., & Gagnon G.A.. (2003). Impact of ozonation on water quality in marine recirculation systems. Aquacultural Engineering 29:125– 137

Zar,J.H. (1996).Biostatistical analysis, 3rdedition.Prentice-Hall,Englewood Cliffs,N.J.

Vlahos N., Hotos G, Kapetanios,N. (2004). The effect of temperature on the conditioning of filter bed in aquaria. 2nd International Congress on Aquaculture, Fisheries Technology and Environmental Management. Athens 18-19 June 2004

Mente, E., Solovyev, M.M.,Vlahos, N., Rotllant, G., Gisbert, E. (2016). Digestive enzymes activity during initial ontogeny and after feeding diets with different protein sources in zebra cichlid *Archocentrus nigrofasciatus.* Journal of World Aquaculture Society, 48(5):831-848.

Vlahos, N., Kormas, KA, Pachiadaki, MG., Meziti, A., Hotos G, Mente, E. (2013). Changes in bacterioplankton apparent species richness in two ornamental fish aquaria. *SpringerPlus 2, 66.* doi:10.1186/2193-1801-2-66.

ASSESSMENT OF GILTHEAD SEA BREAM (*Sparus aurata*) BIOMASS IN AQUACULTURE CAGES USING SPLIT-BEAM ECHOSOUNDERS

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Abstract
This study reports on the application of fisheries acoustic methods for monitoring *Sparus aurata* biomass and behavior in the Greek aquaculture. A split-beam SIMRAD EK80 system was used, operating at two frequencies (38 and 120 kHz). A biomass estimate within the range of 9-20% of reference values was produced, making use of *ex-situ* Target Strength measurements of the monitored fish.
Keywords: *Echosounder, aquaculture, target strength, fish biomass, Sparus aurata*
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1. Introduction

The regular acquisition of fish biomass estimates is important for an aquaculture facility, as it allows managers to assess fish growth in a real-time manner and optimize feeding in accordance with the actual needs of the farmed population. To date, the biomass per cage is mostly estimated by combining manual sampling and empirical models that take into account the initial number of sea-transferred fish, the daily feed intake, and the number of fish removed during production. Advancing nonintrusive technologies for the continuous monitoring of fish biomass is of great interest to the fish farming industry (Li *et al*. 2020), and acoustic methods are often considered as a primary means to this end (Haugholt *et al*. 2012; Puig *et al*. 2012; Soliveres *et al*. 2014; 2017).

Acoustic fish biomass estimation is standard practice in pelagic surveys (Misund 1997), however many difficulties are in effect when this technology is applied at the aquaculture setting (Burczynski *et al*. 1990). Acoustic extinction due to the high fish densities maintained in fish cages (Foote 1990), scarce studies on the acoustic properties of fish species that are of interest to the Mediterranean aquaculture (Soliveres *et al*. 2014), platform stability issues (Espinosa *et al*. 2006), and the acquisition of measurements at close range to the transducer and fish (Dawson *et al*. 2000) are all important challenges that hinder the direct applicability of fisheries acoustic methods to aquaculture.

This work presents initial results from an effort that applied fisheries acoustic technologies at the Greek aquaculture in order to estimate the biomass, and monitor the behavior, of farmed gilthead sea bream (*Sparus aurata*) in production conditions.

2. Materials and Methods

2.1 Study area and study duration

Fieldwork was conducted from November 2019 to February 2021 at the facilities of the research sea farm of the Hellenic Center for Marine Research (HCMR) located at Souda bay, northwestern Crete, Greece.

2.2. Exprerimental set up and instrumentation

A production net pen cage sized 6×6×9.5 m (length, width, height) hosting *S. aurata* was acoustically monitored using two SIMRAD EK80 split-beam echosounders operating at 38 and 120 kHz. The transducers were mounted on a stainless steel plate that was installed below the cage (upwards-looking) at a depth of 14 m, and a custom-built Raspberry Pi controller was developed to remotely manage the acoustic system (Figure 1).
The echosounders were calibrated three times (November 2019, July 2020, October 2020) using standardized protocols (Demer *et al*. 2015) and two copper spheres of 60 and 23 mm diameter for the 38 and 120 kHz transducers, respectively. The calibrations were performed in an empty net pen cage that was identical to the production cage, and the transducers were installed at 14 m depth, upwards-looking. For all calibration sessions, the correction factors for both echosounders were within the tolerances specified by the manufacturer.

2.3 Target strength measurements

A key factor for converting the acoustic measurements into fish biomass is the relation between the mean Target Strength (TS) of fish and their mean length L. For most fish species observed with vertical echosounding, TS is expressed as: $TS(L; b_{20}) = 20logL + b_{20}$ (Love 1977), where b_{20} is a constant that depends on the wavelength of the transmitted pulse, the internal physiology of the studied species (e.g. the shape and size of the swimbladder), and the tilt behavior of fish, i.e. their orientation relative to the incident wavefront.

Three acoustic experiments were conducted for measuring the ventral TS of farmed *S. aurata* (22/Jul – 9/Aug/2020, 16/Oct – 19/Oct/2020, and 20/Jan – 1/Feb/2021), using an empty net pen cage that was identical to the monitored production cage. For each experiment, the 38 and 120 kHz transducers were installed below the TS cage at 14 m depth (upwards-looking), and 12 to 15 *S. aurata* individuals of similar length were collected from the production cage and moved into the TS cage. The length and weight of each participating fish was manually measured after completion of the TS experiment.

The acoustic data were analyzed using the Echoview software. A Single Echo Detection (SED) algorithm (Soule 1997) was initially applied in order to produce echograms that contained only echoes from individual fish. The SED echograms were subsequently processed with a fish tracking algorithm (Ehrenberg 1996) that groups together consecutive insonifications of an individual as it swims within the acoustic beam.

Figure 1. (a)–(b) Stainless steel plate for mounting the 38 and 120 kHz split-beam transducers below the fish cage (upwards-looking). (c) Raspberry Pi controller for the remote management of the acoustic system. (d) Operational installation of echosounders at the production cage; the inset image shows the anchored floating platform that hosted the EK80 transceivers and the peripheral electronic equipment.

2.4 Acoustic biomass estimation

During echo-integration over a time window and depth layer, the echosounder outputs the volume backscattering strength S_v (dB), i.e. the contribution of all targets recorded within the acoustic sampling volume, referenced to 1 m^3. The fish biomass B (kg/m^3) is calculated as (Simmonds & MacLennan 2005):

$$B = \frac{s_v}{\sigma_{bs}} W \qquad (1)$$

where W (kg) is the mean weight of insonified fish, and σ_{bs} (m^2) and s_v (m^{-1}) are the base-10 antilogarithms of TS and S_v, respectively. The acoustic biomass estimates reported herein assume a uniform distribution of fish in the production cage, and no corrections for acoustic extinction have been applied.

2.5 Reference data

Sea temperature and salinity was measured in order to compute the sound speed. The acoustic biomass estimates were ground-truthed against a reference time series which empirically reported the biomass of the production cage by taking into account: (a) the number of juveniles initially put into the cage, (b) the quantity of fish feeds used per day, (c) the number of fish removed due to mortalities or harvesting, and (d) weight samples that were collected regularly from the farmed population (Table 1).

Table 1. Example reference data of the production cage biomass.

Date	Number of fish in the cage	Mean fish weight (g)	Total cage biomass (kg)
1 July 2020	6943	263.8	1831.6
2 July 2020	6943	265.1	1840.4
3 July 2020	6943	266.7	1851.6

3. Results

The ventral TS of *S. aurata* measured during the July/August 2020 experiment was -35.16 and -33.17 dB at the 38 and 120 kHz frequencies, respectively (N = 12 individuals, L = 27.2 cm, W = 0.34 kg). The acoustically estimated biomass varied within and across days (Figure 2), reflecting a non-uniform distribution of fish in the sampling volume. Using the top 5% of night-time values and the aforementioned TS, the mean acoustic biomass estimate for 1/Jul/2020 to 3/Jul/2020 underestimated the reference value by 9 to 20% (Table 2).

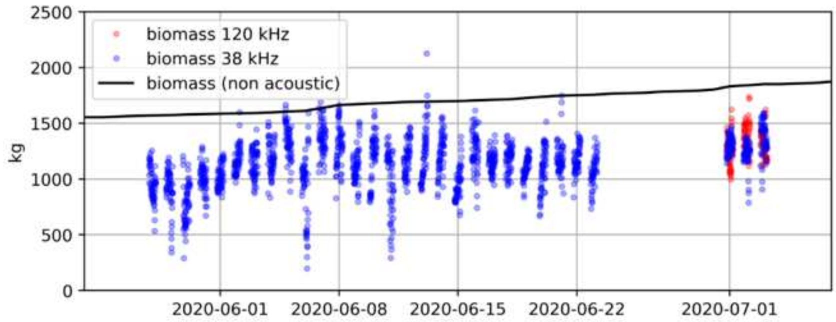

Figure 2. Night-time acoustic biomass estimate by echo-integration over 10 minute intervals, compared to the reference time series.

Table 2. Acoustic biomass estimate of the production cage during 1/Jul/2020 to 3/Jul/2020.

Echo-integration window (minutes)	Frequency (kHz)	Acoustic biomass (kg)	Reference biomass (kg)	Offset (%)
5	38	1487	1851.6	-19%
	120	1679	1851.6	-9%
10	38	1480	1851.6	-20%
	120	1657	1851.6	-10%

The acoustic system can also offer an insight into the vertical distribution of fish within the sampling volume (Figure 3) which reflects their diurnal behavior and responses to stimuli such as feeding.

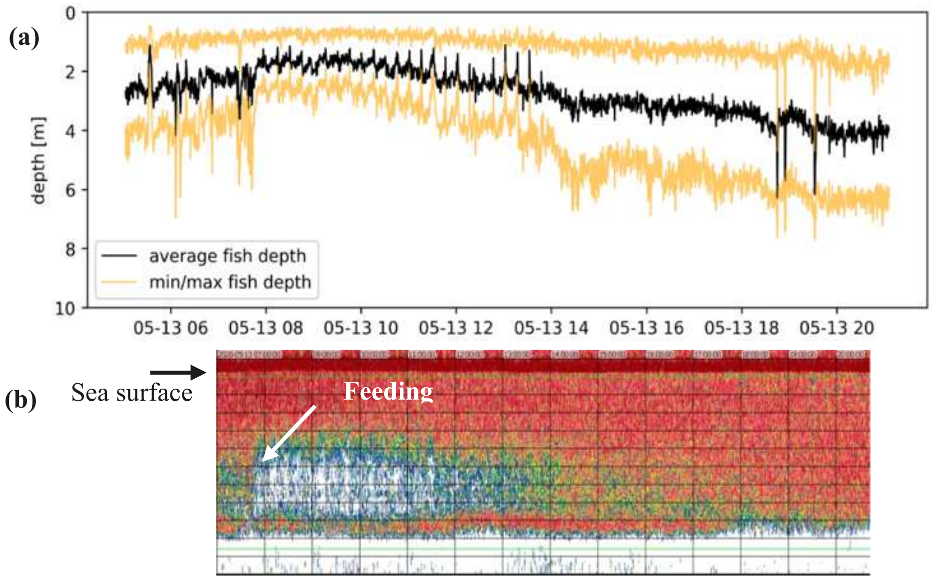

Figure 3. (a) Mean (black) and lower/upper 95% percentiles (yellow) of the biomass-weighted vertical distribution of fish inside the production cage, during a 14 hour window (120 kHz). (b) Corresponding volume backscattering strength (S_v) echogram.

4. Discussion

Using state-of-the-art scientific echosounders and standard fisheries acoustic methods resulted to a biomass estimate of *S. aurata* that was 9-20% below reference values. The central assumptions were the absence of acoustic extinction, the uniform distribution of fish within the cage, and a static rectangular model for the cage. Addressing these simplifications needs much work and will improve the acoustic estimate.

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References

Burczynski J.J., Johnson R.L., Kreiberd H., Kirchner W.B. (1990). Acoustic estimation of dense aggregations of fish in sea cages. Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer 189, 54–64.

Dawson J., Wiggins D., Degan D., Geiger H., Hart D., Adams B. (2000). Point-source violations: split-beam tracking of fish at close range. Aquatic Living Resources 13, 291–295.

Demer D.A., Berger L., Bernasconi M., Bethke E., Boswell K., Chu D., Domokos R., Dunford A., Fässler S., Gauthier S., Hufnagle L.T., Jech J.M., Bouffant N., Lebourges-Dhaussy A., Lurton X., Macalulay G.J., Perrot Y., Ryan T., Parker-Stetter S., Stienssen S., Weber T., Williamson N. (2015). Calibration of acoustic instruments. ICES Cooperative Research Report No. 326, 133 pp.

Ehrenberg J. (1996). Application of dual-beam and split-beam target tracking in fisheries acoustics. ICES Journal of Marine Science 53, 329–334.

Espinosa V., Soliveres E., Estruch V.D., Redondo J., Ardid M., Alba J., Escuder E., Bou M., Vicente D. (2006). Acoustical monitoring of open mediterranean sea fish farms: problems and strategies. Proceedings of the 37th EAA European Symposium on Hydroacoustics, Tecniacústica (2006), Gandia, 5 pp.

Foote K.G. (1983). Linearity of fisheries acoustics, with addition theorems. Journal of the Acoustical Society of America 73, 1932–1940.

Foote K.G. (1990). Correcting acoustic measurements of scatterer density for extinction. Journal of the Acoustical Society of America 88, 1543–1546.

Haugholt K.H., Kavli T., Knappskog V., Løvhaugen O., Pedersen A., Pedersen G. (2012). EXACTUS Technical Report. T1.1 Technology Survey, 37 pp.

Li D., Hao Y., Duan Y. (2020). Nonintrusive methods for biomass estimation in aquaculture with emphasis on fish: a review. Reviews in Aquaculture 12, 1390–1411.

Love R.H. (1977). Target strength of an individual fish at any aspect. Journal of the Acoustical Society of America 62, 1397.

Misund O.A. (1997). Underwater acoustics in marine fisheries and fisheries research. Reviews in Fish Biology and Fisheries 7, 1–34.

Puig V., Espinosa V., Soliveres E., Ortega A., Belmonte A., de la Gándara F. (2012). Biomass estimation of bluefin tuna in sea cages by the combined use of acoustic and optical techniques. Collective Volume of Scientific Papers ICCAT 68(1), 284–290.

Simmonds J., MacLennan D. (2005). Fisheries Acoustics, 2nd edition. Chapman & Hall, London, pp. 472.

Soliveres E., Poveda P., Estruch V.D., Pérez-Arjona I., Puig V., Ordóñez P., Ramiz J., Espinoza V. (2017) Monitoring fish weight using pulse-echo waveform metrics. Aquacultural Engineering 77, 125–131.

Soliveres E., Puig V., Ordónez P., Pérez-Arjona I., Ardid M., Ramis J., Poveda P., Estruch V.D., López M.D, Agraso M., Robles R., Espinosa V. (2014). Acoustical biomass estimation results in Mediterranean sea cages. Proceedings of the 2nd International Conference and Exhibition on Underwater Acoustics (UA2014), pp. 1423–1428.

Soule M. (1997). Performance of a new phase algorithm for discriminating between single and overlapping echoes in a split-beam echosounder. ICES Journal of Marine Science 54, 934–938.

CAN DIETARY INSECT MEAL AFFECT FISH COLOUR AND BODY SHAPE?

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Abstract
Fish external morphology is the most important deciding factor during purchase. In this study, the body shape and skin coloration of gilthead sea bream and European sea bass were studied using machine vision. Fish were fed with a control diet containing 65% fish meal or with one of the experimental diets with 19.5% inclusion of insect meals from the larvae of *Tenebrio molitor* (TM), *Hermetia illucens* (HI) or *Musca domestica* (MD). The body shape was assessed using landmarks and morphological indices. The analysis did not reveal any differences in the transverse measurements or in the measurements of the abdominal area of the fish. Thus, the inclusion of insect meals did not affect the overall shape of the fish. In gilthead sea bream (*Sparus aurata*), fish fed TM and MD had similar skin colour with the control group, while the lower lightness (L*) of the dorsal region of fish fed HI resulted in a visible colour difference compared to the control fish. In European sea bass (*Dicentrarchus labrax*), fish fed TM and HI had similar skin colour with those of the control group. The dorsal region of the fish fed MD had a more yellowish tone (significantly higher b*) compared to the control group, which also affected colour saturation (chroma) and led to a colour difference perceptible to the human eye.
Keywords: *skin pigmentation, colour attributes, CIELab, morphometry, image analysis*
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1. Introduction

The visual quality of fish (size, shape, colour, and texture) can have a direct impact on the price and consumer acceptance, as it plays the most important role in the purchase decision (Alasalvar *et al.* 2011). Consumers have a standard idea of what is a "normal" shape of a fish species and any deviation from this image may render the product undesirable. Moreover, product colour is one of the characteristics that the consumer first notices and tends to associate it with freshness, higher quality, and desirable taste (Hong *et al.* 2014). Dietary ingredients can greatly affect skin coloration (Moutinho *et al.* 2020). Modern sustainable diets incorporating innovative ingrediets are of major interest for reseachers in attempts to minimize the environmental impact of fish meal utilization. In this regard, insect meals are an emerging sustainable candidate for use in aquafeeds and are thus investigated extensively.

The aim of the present study was the analysis of the external morphology of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) to investigate whether the feeding with insect meals could lead to changes in body shape due to different muscle mass deposition or to different fat deposition. These changes could result in different body height or in swelling of the abdominal area. Moreover, the effects of insect meals on skin coloration were assessed.

2. Materials and Methods

Details of the diets and feeding trials have been described by Mastoraki *et al.* (2019; 2020). In brief, four diets for each species were formulated to be grossly isonitrogenous and isoenergetic. The control diet incorporated fish meal as main protein source (65% inclusion), and in the three experimental diets 30% of the fish meal was substituted by insect meals from the larvae of *Tenebrio molitor* (TM), *Hermetia illucens* (HI) or *Musca domestica* (MD) (19.5% inclusion). After three months of feeding with the experimental diets, fish were anaesthetized and photographed using a lightbox and a high-resolution camera (13 MP) mounted on a tripod.

The external morphological analysis was performed using reference points according to Arechavala-Lopez *et al.* (2012). The photos were uploaded to Image J software (51 photos per diet for *S. aurata* and 30 photos per diet for *D. labrax*) and the scale was adjusted using the ruler in each photo, respectively. The reference points were marked on the body of each fish (12 and 13 landmarks for *S. aurata* and *D. labrax,* respectively) and each point was given (x, y) coordinates. The distances between the morphological landmarks were calculated and standardized using the standard length of each fish (Reist 1985).

For the colour analysis, in total 204 photos of *S. aurata* (123.1 ± 13.9 g) and 88 photos of *D. labrax* (23.3 ± 6.1 g) were analyzed. The photos were uploaded to Adobe Photoshop CS5 where colour and white balance corrections were performed using gray and white cards. Six 5x5 pixel points on the left lateral side of each fish (cranial, medial, and caudal points on dorsal and ventral locations) were selected for colour analysis using the colour sampler tool and the CIELab colour model. The L*, a*, and b* values for each point were recorded and the parameters of hue and colour saturation (chroma) were calculated using the following equations:

Chroma = $(a^{*2} + b^{*2})^{1/2}$ and °Hue = $(\arctan(b^*/a^*)) \times 180°) / \pi$

The colour difference index was calculated to compare between the experimental groups and the control group $\Delta E = ((\Delta L^*)^2 (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$.

3. Results and Discussion

The morphological indices of *S. aurata* and *D. labrax* calculated by the distance between the landmarks are presented in Table 1 and Figure 1. A statistically significant difference was observed in four out of the nine markers in *S. aurata* and in three out of the seven markers in *D. labrax* associated with the insertion of the operculum on the profile (landmarks 11 and 12, respectively) as well as with the point of maximum curvature in the head profile curve (landmark 2). Both landmarks were slightly difficult to locate in the photo and their localization depended on the researcher. Since no differences were observed in the transverse measurements and in those involving the abdominal area of the fish (for example the markers 3-10, 4-8, 3-9, 3-8 and 4-9 in *S. aurata* and the markers 3-11, 4-10, 5-9, 4-11, 3-10, 5-10 and 4-9 in *D. labrax*), it was assessed that the dietary insect meals did not affect fish shape.

The effects of dietary insect meals on skin colour of *S. aurata* and *D. labrax* are presented in Table 2. In gilthead sea bream, the perceptual lightness (L*) of the dorsal region was significantly lower in fish fed HI compared to the TM group and the L* of the ventral region of the fish fed HI was lower compared to the control and TM groups. No differences were observed in the colour saturation (Chroma), °hue, red/green (a*) and yellow/blue (b*) values between the different dietary treatments. Skin colour is an indicator of the physiological status of the fish. Under stress conditions, the metabolic changes that occur can be reflected on the external morphology of the fish, with more obvious changes the skin colour alterations (Saberioon *et al.* 2016). In *S. aurata* the significant decrease in lightness of the fish fed HI compared to the control group in the dorsal region makes the colour difference visible to the human eye (ΔE = 3.6), given that the human eye can perceive the colour difference when the colour difference index (ΔE) is greater than 3 (Panini *et al.* 2017). On the other hand, the control, TM, and MD groups had overall similar skin colour. In the growth trial, no differences were observed with the inclusion of the different insect meals (Mastoraki *et al.* 2019), thus the reason for the darker dorsal region of the HI group is still under investigation.

In European sea bass, fish fed with TM and HI had no difference in the colour parameters neither in the dorsal nor the ventral regions of the body compared to the control group. However, the TM group had a duller greyish colour in the dorsal region compared to the HI group as shown by the significant decrease in L*. In the ventral region, fish fed MD had similar colour parameters with the control group, the dorsal part of the MD group had a more yellowish tone (significantly higher b*) compared to the control group, which also affected chroma. An increase in yellowness of the skin or fillet has been observed with the increased inclusion of feed ingredients that are rich in pigments like astaxanthin (Kaya Öztürk *et al.* 2019) or corn gluten (Iaconisi *et al.* 2018). Finke (2013) reported that adult houseflies are rich in yellow pigment substances like riboflavin, in this study housefly larvae (MD) could also contain these substances and give a yellowish colouration to the skin of the MD group. Overall, fish fed TM had a very small colour difference with the control group (ΔE = 1.9), while the dorsal region of the fish fed HI and MD had a colour difference with the control group perceptible to the human eye (3.9 and 4.4, respectively). A sensory panel with trained panellists could be used to assess if the difference in the colour is actually visible.

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Table 1. Effect of dietary insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*) on morphological indexes of *Sparus aurata* and *Dicentrarchus labrax*

Sparus aurata					*Dicentrarchus labrax*				
Marker	Control	TM	HI	MD	Marker	Control	TM	HI	MD
1-2	3.1±0^{b}	3.2±0^{a}	3.2±0^{ab}	3.2±0^{ab}	1-2	1.2±0^{b}	1.2±0^{ab}	1.3±0^{ab}	1.3±0^{a}
2-10	6.4±0.1	6.5±0	6.4±0.1	6.5±0	2-12	2.0±0	1.9±0	2.1±0	1.9±0
10-11	3.6±0	3.8±0.1	3.6±0.1	3.8±0	1-12	2.2±0	2.2±0	2.4±0	2.2±0
1-11	3.4±0	3.4±0	3.5±0	3.5±0	2-3	3.0±0.1	2.9±0	3.1±0.1	2.9±0.1
1-10	6.9±0.1	6.9±0.1	6.9±0.1	7.0±0	3-11	2.9±0.1	2.8±0	3.0±0.1	2.8±0
2-11	4.3±0	4.4±0	4.4±0	4.4±0	11-12	1.8±0.1^{a}	1.6±0^{b}	1.7±0^{b}	1.6±0^{b}
2-3	4.2±0.1	4.1±0.1	4.1±0.1	4.3±0.1	2-11	3.3±0.1^{a}	3.1±0^{b}	3.3±0.1^{a}	3.1±0.1^{b}
3-9	7.8±0.1	7.8±0.1	7.7±0.1	7.9±0.1	3-12	3.4±0.1	3.3±0	3.4±0.1	3.3±0.1
9-10	4.4±0.1	4.4±0	4.3±0.1	4.5±0.1	3-4	2.5±0.1	2.4±0.1	2.7±0.1	2.4±0.1
2-9	9.7±0.1^{ab}	9.8±0.1^{ab}	9.6±0.1^{b}	10±0.1^{a}	4-10	2.9±0.1	2.8±0	3.0±0.1	2.8±0.1
3-10	6.4±0	6.4±0	6.4±0.1	6.5±0	10-11	4.0±0.1	3.9±0.1	4.1±0.1	3.9±0.1
3-11	6.7±0^{b}	6.8±0.1^{ab}	6.7±0.1^{ab}	6.9±0.1^{a}	3-10	4.6±0.1	4.4±0.1	4.7±0.1	4.4±0.1
3-4	7.8±0.1	7.8±0.1	7.7±0.1	7.7±0.1	4-11	4.0±0.1	3.9±0.1	4.2±0.1	3.9±0.1
4-8	2.3±0	2.2±0	2.2±0	2.3±0	4-5	2.1±0	2.0±0	2.1±0	2.0±0.1
8-9	3.2±0	3.2±0.1	3.1±0	3.1±0	5-9	1.6±0	1.6±0	1.7±0	1.6±0
3-8	8.8±0.1	8.8±0.1	8.7±0.1	8.8±0.1	9-10	1.5±0	1.5±0	1.6±0	1.6±0
4-9	4.6±0	4.6±0.1	4.6±0	4.6±0	4-9	3.3±0.1	3.2±0	3.4±0.1	3.2±0.1
4-10	8.1±0.1	8.1±0.1	8.0±0.1	8.2±0.1	5-10	2.3±0	2.3±0	2.4±0	2.3±0
4-5	1.4±0	1.3±0	1.4±0	1.4±0	5-6	2.3±0.1	2.2±0	2.3±0.1	2.2±0
5-7	1.5±0	1.6±0	1.6±0	1.6±0	6-8	1.4±0	1.4±0	1.5±0	1.4±0
7-8	1.3±0	1.3±0	1.3±0	1.3±0	8-9	1.7±0	1.7±0	1.8±0	1.7±0
4-7	2.3±0	2.3±0	2.3±0	2.4±0	5-8	2.7±0.1	2.6±0	2.8±0.1	2.7±0
5-8	2.3±0	2.3±0	2.3±0	2.3±0	6-9	2.2±0.1	2.2±0.1	2.3±0.1	2.2±0.1
5-6	1.6±0	1.6±0	1.6±0	1.7±0	6-7	0.8±0	0.8±0	0.8±0	0.7±0
6-7	1.7±0	1.6±0	1.6±0	1.7±0	7-8	0.8±0	0.8±0	0.8±0	0.8±0
1-12	5.6±0	5.6±0	5.6±0	5.7±0	1-13	3.2±0.1	3.1±0	3.3±0.1	3.1±0.1
11-12	3.3±0^{b}	3.4±0^{a}	3.3±0^{ab}	3.4±0^{ab}	7-13	8.0±0.1	7.7±0.1	8.2±0.1	7.8±0.1
6-12	10.6±0.1	10.6±0.1	10.5±0.1	10.8±0.1	1-7	11.1±0.2	10.8±0.1	11.5±0.2	10.9±0.2
1-6	16.2±0.1	16.2±0.1	16.1±0.1	16.4±0.1					

The measured values (average ± standard error; n = 51 for *S. aurata* and 30 for *D. labrax*) are presented (cm), while the statistical analysis was performed on standardized data (see Materials and Methods). Different letters in each row and each fish species indicate a statistically significant difference (p<0.05).

Table 2. Colour parameters of the skin of *Sparus aurata* and *Dicentrarchus labrax* fed with different insect meals (TM: *Tenebrio molitor*, HI: *Hermetia illucens* or MD: *Musca domestica*).

	S. aurata				*D. labrax*			
Parameters	Control	TM	HI	MD	Control	TM	HI	MD
Dorsal								
L*	35.5±0.7^{ab}	36±0.9^{a}	32.6±1^{b}	33.4±0.8^{ab}	62.6±1.3^{ab}	61.2±1.1^{b}	65.7±1^{a}	60.1±0.9^{b}
a*	0.1±0.2	-0.3±0.2	0.2±0.2	-0.1±0.2	-1.9±0.2	-2.1±0.3	-2.2±0.2	-2.7±0.2
b*	6.7±0.4	6.7±0.3	6.8±0.3	6.9±0.3	5.8±0.7^{b}	7±0.7^{ab}	8.1±0.9^{ab}	9.2±0.7^{a}
Chroma	6.9±0.3	6.8±0.3	6.9±0.3	7±0.3	6.6±0.5^{b}	7.4±0.6^{ab}	8.6±0.9^{ab}	9.6±0.7^{a}
°Hue	5.3±11.3	-15.1±11	20.6±11.1	5.1±11.7	-60.1±8.4	-62.1±7.8	-71.2±2.5	-72.5±1.4

ΔE		0.7	2.9	2.1		1.9	3.9	4.4
Ventral								
L*	64.9±0.5[a]	64.5±0.6[a]	61.3±0.8[b]	63.2±0.7[ab]	76.7±1.4	77.7±0.8	78.7±1.2	78.6±0.9
a*	-0.1±0.1	-0.1±0.1	-0.1±0.1	-0.1±0.1	-1.3±0.1	-1.3±0.1	-1.6±0.1	-1.8±0.1
b*	4.7±0.2	4.7±0.2	5.2±0.2	4.9±0.2	5.7±0.8	5.9±0.7	7.8±0.6	5.7±0.6
Chroma	4.8±0.2	4.8±0.2	5.3±0.2	4.9±0.2	6±0.8	6.1±0.7	8±0.6	6.1±0.5
°Hue	-2.3±11.6	12.7±11.5	11.9±11.7	-8.8±11.7	-66.6±5.5	-72.3±2.9	-68±7.9	-68.2±3
ΔE		0.4	3.6	1.8		1.5	2.9	2.7

Values represent average ± standard error; n = 51 for *S. aurata* and 22 for *D. labrax*. ΔE: colour difference index, a comparison between the overall colour of the control group with each of the TM, HI and MD groups. Different letters in each row and each fish species indicate a statistically significant difference (p<0.05).

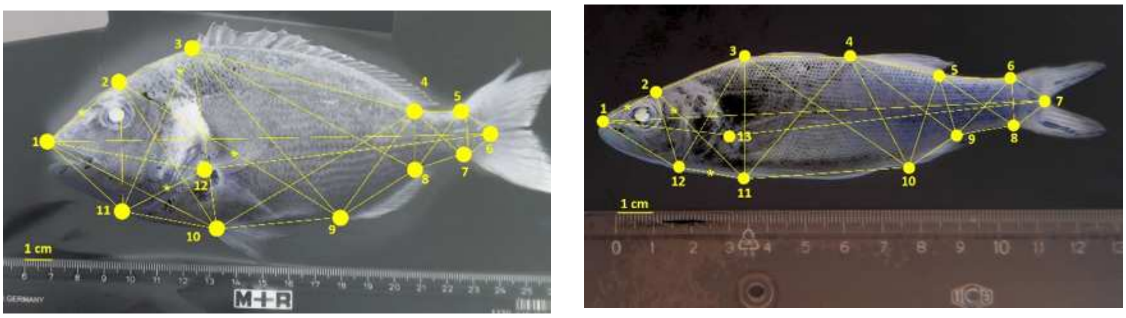

Figure 1. Examples of the landmarks used to determine the morphological indices of fish fed different insect meals. The asterisks indicate the indices which differed significantly. In *Sparus aurata* (a), 12 landmarks were used to measure 29 morphological markers while in *Dicentrarchus labrax* (b) 13 landmarks were used to calculate 28 morphological markers.

References

Alasalvar C., Miyashita K., Shahidi F., Wanasundara U. (2011). Handbook of seafood quality, safety and health applications. John Wiley & Sons, Oxford, UK, pp 576.

Arechavala-Lopez P., Sanchez-Jerez P., Bayle-Sempere J.T., Sfakianakis D.G., Somarakis S. (2012). Morphological differences between wild and farmed Mediterranean fish. Hydrobiologia. 679, 217-231

Finke M.D. (2013). Complete nutrient content of four species of feeder insects. Zoo Biology. 32, 27-36.

Hong H., Yang X., You Z., Cheng F. (2014). Visual quality detection of aquatic products using machine vision. Aquacultural Engineering. 63, 62-71.

Iaconisi V., Bonelli A., Pupino R., Gai F., Parisi G. (2018). Mealworm as dietary protein source for rainbow trout: Body and fillet quality traits. Aquaculture. 484, 197-204.

Kaya Öztürk D., Baki B., Öztürk R., Karayücel S., Uzun Gören G. (2019). Determination of growth performance, meat quality and colour attributes of large rainbow trout (*Oncorhynchus mykiss*) in the southern Black Sea coasts of Turkey. Aquaculture Research. 50, 3763-3775.

Mastoraki M., Katsika L., Antonopoulou E., Gasco L., Chatzifotis S. (2019). The effect of fishmeal substitution with three different insect meals on growth performance, nutrient utilization and digestibility of gilthead sea bream (*Sparus aurata*), Proccedings of Aquaculture Europe 19. Our future growing from water, Berlin, Germany 7-10 October 2019, pp. 884-885.

Mastoraki M., Ferrándiz P.M., Vardali S.C., Kontodimas D.C., Kotzamanis Y.P., Gasco L., Chatzifotis S., Antonopoulou, E. (2020). A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (*Dicentrarchus labrax* L.). Aquaculture. 528, 735511.

Moutinho S., Pedrosa R., Magalhães R., Oliva-Teles A., Parisi G., Peres H. (2020). Black soldier fly (*Hermetia illucens*) pre-pupae larvae meal in diets for European seabass (*Dicentrarchus labrax*) juveniles: Effects on liver oxidative status and fillet quality traits during shelf-life. Aquaculture. 736080.

Panini R.L., Pinto S.S., Nóbrega R.O., Vieira F.N., Fracalossi D.M., Samuels R.I., Prudêncio E.S., Silva C.P., Amboni R.D. (2017). Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp *Litopenaeus vannamei*. Food Research International. 102, 445-450.

Reist J.D. (1985). An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Canadian Journal of Zoology. 63, 1429-1439.

Saberioon M., Gholizadeh A., Cisar P., Pautsina A., Urban J. (2016). Application of machine vision systems in aquaculture with emphasis on fish: state-of-the-art and key issues. Reviews in Aquaculture. 9, 369-387.

INSECT MEAL DIETS MODULATE HSP EXPRESSION AND MAPK ACTIVATION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND GILTHEAD SEA BREAM (*Sparus aurata*)

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Abstract
Novel aquafeed protein sources are of great interest in order to ensure an economic and environmental sustainable aquaculture industry. In this regard, insects are considered as a potential alternative to fish meal protein-rich ingredient with small ecological impact. Dietary changes may stimulate molecular responses including heat shock proteins (HSPs) induction and mitogen-activated protein kinases (MAPKs) phosphorylation. The present study aims to investigate differentiations in the expression of HSP90 and the phosphorylation of p38 MAPK in the intestine of *Dicentrarchus labrax* and *Sparus aurata* following a partial fish meal substitution with insect larvae meals from *Hermetia illucens*, *Tenebrio molitor* and *Musca domestica*. In general, the two teleost species showcased similar differentiation in the HSP90 expression and p38 MAPK phosphorylation. A significant induction in protein expression of HSP90 was observed in both teleosts with *D. labrax* displaying a more pronounced increase. However, *M. domestica* inclusion in *S. aurata* diet led to a reduction in HSP90 expression. Fish meal substitution with insect meals diminished the phosphorylation of p38 MAPK in both teleost species. The present results provide a first insight into the effects of fish meal substitution with insect meals on the cellular responses of the two most important teleost species cultivated in Greek marine aquaculture.
Keywords: *insect meal, protein expression, European sea bass, gilthead sea bream*
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1. Introduction

Aquaculture is among the most critical food-producing sectors to meet the nutritional food demand of a continuously growing population (Deutsch *et al*. 2007). Due to the prolonged use of fish meal as the basal protein source in aquafeed and the subsequent over-exploitation of pelagic fish, aquaculture production is under great pressure for a shift to more sustainable sources (Hardy 2010). Therefore, fish meal inclusion is often limited to the amount that provides the balanced profile of essential amino acids required for optimum growth and flesh quality (Cho & Kim 2011; Gasco *et al*. 2018). Alternative feed ingredients including plants, fishery and animal by-products and microbial biomass have been studied as environment-friendly fish meal substitutes (Hua *et al*. 2019). Utilization of insects as potential alternative substitute of fish meal in aquafeed has recently attracted attention. Insects are an integral part of the diet of many freshwater and marine carnivorous fish, with high essential amino acid content and feed conversion ratio (van Huis 2013; Henry *et al*. 2015). Additionally, in contrast to the high ecological footprint of livestock production, rearing of commercial insect species requires much less water, energy and land and produces lower levels of greenhouse gases (Oonincx & de Boer 2012; van Huis 2013).

According to the existing literature, diet interventions may trigger cellular stress responses including both heat shock proteins (HSPs) induction and mitogen-activated protein kinases (MAPKs) phosphorylation (Feidantsis *et al*. 2014). Heat shock proteins, a family of conserved cellular proteins, are present in numerous organisms, including teleosts, and their expression displays a tissue-specific manner (Basu *et al*. 2002). Several members of the HSPs, including HSP90 and HSP70, function as molecular chaperones and participate in protein folding and maturation, thus ensuring protein homeostasis of cells (Wegele *et al*. 2004). Protein kinases of the MAPK family, including p38 MAPK, are activated by oxidative stress and cytokines and regulate important cellular processes such as proliferation, apoptosis and immune defense (Duan *et al*. 2019; Li *et al*. 2019).

Thus, the aim of the present research is to evaluate the cellular stress responses in the intestine of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) following a partial substitution of fish meal with insect meals derived from larvae of black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*) or common housefly (*Musca domestica*).

2. Material and Methods

The experimental procedure was conducted at the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of Hellenic Centre for Marine Research. Four isoproteic and isoenergetic diets were designed according to the respective nutritional requirements of the two teleost species. Control diet (FM) contained 65% fish meal as the main protein source, while insect meals from larvae of *Tenebrio molitor* (TM), *Hermetia illucens* (HI) and *Musca domestica* (MD) were used as partial (30%) substitution of fish meal in the three experimental diets (Table 1). Juvenile European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) were randomly allocated into twelve indoor 250 l tanks, three for each diet. Throughout the three months of the feeding trial, fish were hand-fed to apparent satiation, three times a day. Following a day of starvation, for each teleost, twelve individuals per dietary treatment (four from each tank) were euthanized by anesthesia overdose and intestine tissue was aseptically extracted.

Prior to the SDS-PAGE and immunoblot analysis, total protein extraction from frozen intestine tissue samples of *D. labrax* and *S. aurata* was carried out using lysis buffer according to established protocols and protein concentration was determined with the Bio-Rad protein assay. Equivalent amounts of protein (50 μg) were separated on 10% (w/v) acrylamide and 0.275% (w/v) bisacrylamide slab gels through electrophoresis and thereafter electrotransferred to nitrocellulose membrane (0.45 μm, Schleicher & Schuell, Keene, NH, USA). Subsequently, membrane blocking was implemented in order to prevent the non-specific binding of antibodies. To bind to the target proteins, primary antibodies were incubated overnight with the membranes at -4 °C. Primary antibodies used in the present study were polyclonal rabbit anti-heat shock protein, 90 kDa (Cat. No. 4874, Cell Signaling, Beverly, MA, USA) and polyclonal rabbit anti-phospho-p38 MAP kinase (Thr180/Tyr182) (Cat. No. 9211, Cell Signaling, Beverly, MA, USA). Afterwards, incubation of membranes with a HRP-conjugated secondary antibody, specific for the respective primary antibody (anti-rabbit IgG, HRP-linked Antibody (Cat. No. 7074, Cell Signaling, Beverly, MA, USA)), was carried out for an hour at room temperature. Quantification of the protein bands density was conducted using the Image Studio Lite software.

Alterations in protein expression among the four dietary treatments were tested for significance at the 5% level through one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using the Tukey test. Statistical analysis was performed using SPSS Statistics software, version 25 (IBM).

Table 1. Ingredient composition and proximate analysis of control (fish meal) diet and insect meal diets of *Dicentrarchus labrax* and *Sparus aurata*.

	Dicentrarchus labrax				*Sparus aurata*			
	FM	HI	TM	MD	FM	HI	TM	MD
Ingredients								
Fishmeal	650	455	455	455	650	455	455	455
Insect-based meal	0	195	195	195	0	195	195	195
Fish oil	100	97	60	62	100	97	51	62
Wheat	175.5	163.5	183.5	184.5	161	149	188	169
Wheat Gluten meal	67	66	82	91	64	64	71	85
Premix	2.5	2.5	2.5	2.5	25	25	25	25
DL-methionine	5	9	10	3	0	4	4	2
Lysine	0	12	12	7	0	11	11	7
Composition								
Crude protein (%)	55	55	55	55	55.0	55.0	55.0	55.0
Crude fat (%)	16.2	15.2	15.2	15.2	16.2	15.2	16.2	15.2
Ash (%)	12.7	10.5	10	10.5	13.3	11.1	10.4	11.1
Carbohydrates (%)	16.1	19.3	19.8	19.3	15.4	18.1	17.8	18.4
GE (Mj/kg)	22.1	22.3	22.4	22.3	22.0	22.2	22.1	22.2
Lysine (%)	5.1	5	5	5	5.2	5.2	5.2	5.2
Methionine (%)	2.6	2.6	2.6	2.6	2.8	2.8	2.8	2.8

Abbreviations: FM, Fish meal; HI, *Hermetia illucens*; TM, *Tenebrio molitor*; MD, *Musca domestica*.

3. Results

The expression levels of HSP90 and phospho-p38 MAPK in response to the fish meal substitution with insect meals are presented in Figures 1 and 2, respectively. In the intestine of European sea bass, the expression of HSP90 (Figure 1A) displayed a significant induction following the fish meal substitution with the insect meals (one-way ANOVA followed by Tukey post hoc test; $p < 0.01$). Specifically, the dietary inclusion of *H. illucens* resulted in an up to six times higher expression level of HSP90. A similar pattern of increased HSP90 expression was observed in gilthead sea bream with the exception of *M. domestica* inclusion, which resulted in a significant reduction (Figure 1B). In both teleosts, significant differences in the expression of HSPs were observed among the three insect meal diets.

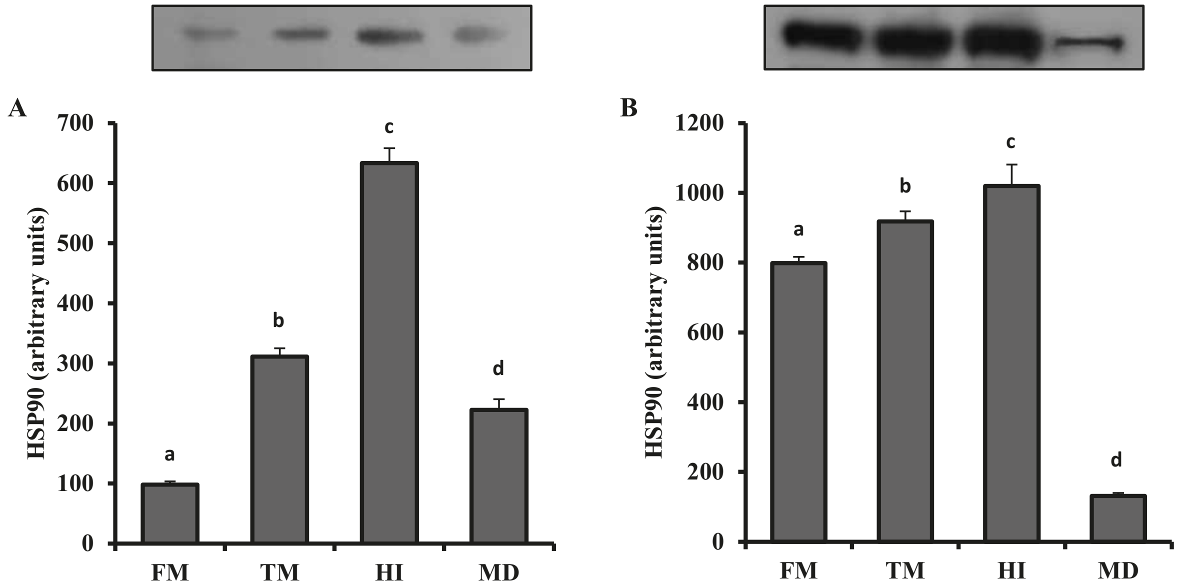

Figure 1. Expression of HSP90 in the intestine of (A) *Dicentrarchus labrax* and (B) *Sparus aurata* fed control (FM) and insect meal diets (TM, HI, MD). Values represent means ± SD; n = 3 replicates. Different letter (a, b, c, d) denotes significant difference between the four experimental diets. Statistical significance assessed at the 0.05 level. Abbreviations according to Table 1.

The phosphorylation of p38 MAPK were significantly reduced in both European sea bass and gilthead sea bream as a response to the substitution of fish meal with insect meal (Figure 2). However, European sea bass fed with the *T. molitor* diet displayed significant lower levels of phospho-p38 MAPK compared to the other two insect meal diets. Similarly to the HSP90 expression pattern, dietary inclusion of *M. domestica* in gilthead sea bream diet resulted in up to six times decreased levels of phosphorylated p38 MAPK.

4. Discussion

Current knowledge regarding the impact of dietary insect inclusion on cellular responses of teleosts is scarce. The results of the present study showcased similar differentiations in the patterns of HSP90 expression and p38 MAPK phosphorylation among the two teleost species, following dietary treatment with insect meal diets. In addition, it was evident that partial substitution of fish meal with meal from different insect species resulted in versatile cellular response in the two teleosts. The expression of HSPs in response to nutritional challenges has been reported in several studies. Functions of the aforementioned proteins seem to have an important role in protein metabolism (Basu *et al*. 2002). Herein, dietary inclusion of insect meals as fish meal substitute elevated the expression levels of HSP90. In accordance with this result, changes in the dietary lipid content induced the hepatic HSP70 and HSP90 expression of *Argyrosomus regius* (Antonopoulou *et al*. 2014).

Similarly, elevated HSP90 levels were reported in the liver of *D. labrax* following feeding with soy-based diets (Feidantsis *et al*. 2014). Variations in the amino acid profiles provided through insect meal diets as well as linkage of proteins in chitin fibres may require the recruitment of more HSPs proteins for several processes in protein metabolism such as translocation of newly synthesized proteins (Nogales-Mérida *et al*. 2019).

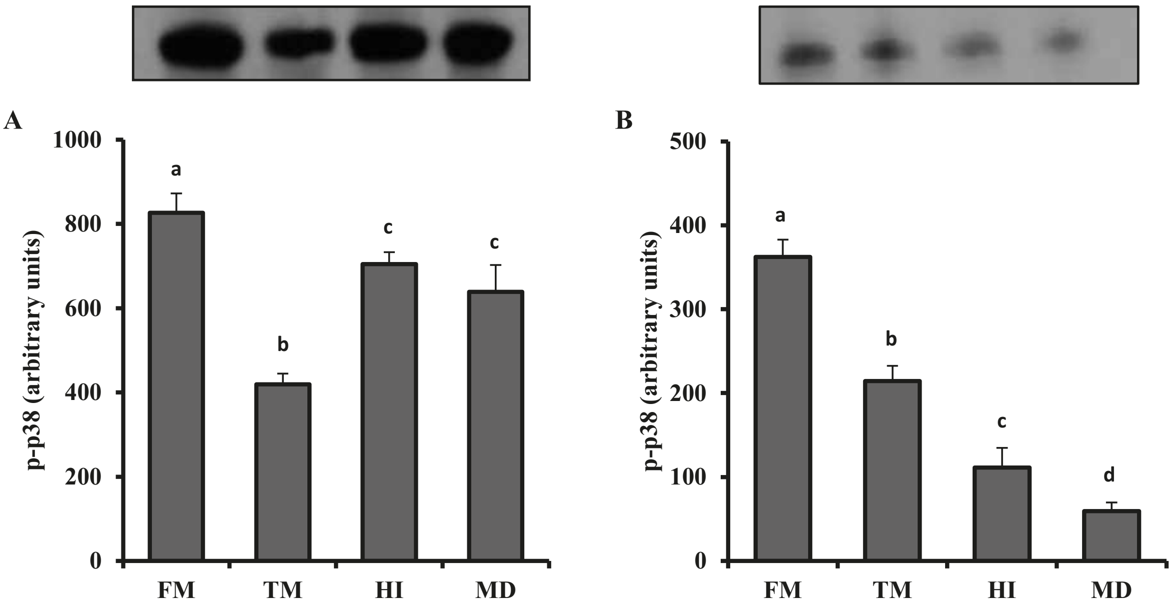

Figure 2. Phosphorylation of p38 MAPK in the intestine of (A) *Dicentrarchus labrax* and (B) *Sparus aurata* fed control (FM) and insect meal diets (TM, HI, MD). Values represent means ± SD; n = 3 replicates. Different letter (a, b, c, d) denotes significant difference between the four experimental diets. Statistical significance assessed at the 0.05 level. Abbreviations according to Table 1.

In contrast to the increased levels of HSP90, insect-fed *D. labrax* and *S. aurata* displayed a reduction in phosphorylated p38 MAPK levels. Similarly, partial substitution of fish meal with soy meal resulted in decreased phosphorylation of p38 MAPK in the intestine of *Dentex dentex* (Antonopoulou *et al*. 2017). However, the present results are contrary to previously reported induction of HSPs via MAPK signaling pathways (Feidantsis *et al*. 2014). Considering that activation of MAPKs is closely linked to a variety of stress stimuli, inhibition of phospho-p38 MAPK indicate that consumed diets with insect meal may not disrupt fish welfare. However, available information concerning MAPK activation in teleosts due to dietary changes is limited. Further research should focus on the molecular and cellular aspects of insects' consumption in order to elucidate their suitability as ingredient in fishfeed.

Acknowledgement

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References

Antonopoulou E., Chouri E., Feidantsis K., Lazou A., Chatzifotis S. (2017). Effects of partial dietary supplementation of fish meal with soymeal on the stress and apoptosis response in the digestive system of common dentex (*Dentex dentex*). Journal of Biological Research-Thessaloniki 24, 1-10.

Antonopoulou E., Kousidou E., Tserga E., Feidantsis K., Chatzifotis S. (2014). Dietary lipid levels in meagre (*Argyrosomus regius*): effects on biochemical and molecular indicators of liver. Aquaculture 428, 265-271.

Basu N., Todgham A.E., Ackerman P.A., Bibeau M.R., Nakano K., Schulte P.M., Iwama G.K. (2002). Heat shock protein genes and their functional significance in fish. Gene 295, 173-183.

Cho J.H., Kim I.H. (2011). Fish meal–nutritive value. Journal of Animal Physiology and Animal Nutrition 95, 685-692.

Deutsch L., Gräslund S., Folke C., Troell M., Huitric M., Kautsky N., Lebel L. (2007). Feeding aquaculture growth through globalization: Exploitation of marine ecosystems for fishmeal. Global Environmental Change 17, 238-249.

Duan X.D., Feng L., Jiang W.D., Wu P., Liu Y., Kuang S.Y., Tang L., Tang W.N., Zhang Y.A., Zhou X.Q. (2019). Dietary soybean β-conglycinin suppresses growth performance and inconsistently triggers apoptosis in the intestine of juvenile grass carp (*Ctenopharyngodon idella)* in association with ROS-mediated MAPK signalling. Aquaculture Nutrition 25, 770-782.

Feidantsis K., Kaitetzidou E., Mavrogiannis N., Michaelidis B., Kotzamanis Y., Antonopoulou E. (2014). Effect of taurine-enriched diets on the Hsp expression, MAPK activation and the antioxidant defence of the European sea bass (*Dicentrarchus labrax*). Aquaculture Nutrition 20, 431-442.

Gasco L., Gai F., Maricchiolo G., Genovese L., Ragonese S., Bottari T., Caruso G. (2018). Fishmeal alternative protein sources for aquaculture feeds. In: "Feeds for the aquaculture sector". Springer, Cham., p. 1-28.

Hardy R.W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41, 770-776.

Henry M., Gasco L., Piccolo G., Fountoulaki E. (2015). Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology 203, 1-22.

Hua K., Cobcroft J.M., Cole A., Condon K., Jerry D.R., Mangott A., Praeger C., Vucko M.J., Zeng C., Zenger K., Strugnell, J.M. (2019). The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1, 316-329.

Li X., Cui K., Fang W., Chen Q., Xu D., Mai K., Zhang Y., Ai Q. (2019). High level of dietary olive oil decreased growth, increased liver lipid deposition and induced inflammation by activating the p38 MAPK and JNK pathways in large yellow croaker (*Larimichthys crocea*). Fish & shellfish immunology 94, 157-165.

Nogales-Mérida S., Gobbi P., Józefiak D., Mazurkiewicz J., Dudek K., Rawski M., Kierończyk B., Józefiak A. (2019). Insect meals in fish nutrition. Reviews in Aquaculture 11, 1080-1103.

Oonincx D.G., De Boer I.J. (2012). Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. PloS one 7, e51145.

van Huis A. (2013). Potential of insects as food and feed in assuring food security. Annual review of entomology 58, 563-583.

Wegele H., Müller L., Buchner J. (2004). Hsp70 and Hsp90—a relay team for protein folding. In: "Reviews of physiology, biochemistry and pharmacology", Amara S.G., Bamberg E., Grunicke H., Jahn R., Lederer W.J., Miyajima A., Murer H., Offermanns S., Schultz G., Schweiger M. (eds). Springer, Berlin, Heidelberg, p. 1-44.

PRELIMINARY RESULTS OF NUTRIENT DISTRIBUTION AT AN AQUACULTURE AREA OF PAGASITIKOS GULF USING GIS

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Abstract
The impact of fish farming on the aquatic environment of Pagasitikos Gulf was investigated on July 2021. The aim of the study is to monitor the aquaculture's effect on the nutrients of the water column.For this reason, twenty-eight (28) stations were selected in the area where the farm is located. At each sampling station, water samples were collected at the surfaceand 10 m depth for nutrient analysis (ammonium, nitrite, nitrate, phosphate, silicate).The results of the present study will be further utilized in order toassess the nutrient distribution in the whole study areaby means of GIS.
Keywords: *Aquaculture, environmental impacts, nutrients, eutrophication, GIS*
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1. Introduction

The expansion of fish farming in the Mediterranean coastal zone during the last 35 years has often resulted in conflicts with other users of the coastal zone, particularly tourism and fisheries (Neofitou *et al*. 2019). The main reason for these conflicts is related to the impact of fish farming on the marine environment and particularly its water quality. The impact of fish farming on the concentration of nutrients in the Eastern Mediterranean has been addressed in a number of studies in the recent past (Klaoudatos *et al*. 2006; Neofitou & Klaoudatos, 2008; Neofitou *et al*. 2019).

Nutrients are elements dissolved in water, which can be used by aquatic plants. Essential nutrients, necessary for the growth of plant organisms, in concentrations above 1000mg/l (macronutrients), are ammonia, nitrite, nitrate and phosphate, while silicates are essential in diatoms and other planktonic species. Therefore, their concentrations can shape the nutritional status of an aquatic environment (oligotrophic, mesotrophic, eutrophic) (Klaoudatos & Klaoudatos 2010).

The aim of the study is to monitor the aquaculture's effect on the nutrients of the water column. Specifically, basic physicochemical parameters were recorded (temperature, salinity, dissolved oxygen, pH, chlorophyll a) and the enrichment of the area in nutrients (ammonium, nitrites, nitrates, phosphates, silicates) was measured.

2. Material and Methods

The present study was carried out on July 2021 at the facilities of a fish farm, which is situated at the south-eastern part of Pagasitikos in Limnionas bay, Milina Magnesias (Figure 1).

In this study, a grid of twenty-eight (28) stations was selected. 9 samplingstationswere the farm stations (FS), 17 werethe complementary stations (S)andanother 2 were the control stations at a distance approximately 600 maway from the fish cages.

Vertical profiles of temperature, salinity, dissolved oxygen, pH and chlorophyll *a* were measured at each sampling station by means of a CTD (Seabird-19plus).In order to determine the concentration of nutrients (ammonium, nitrite, nitrate, phosphate and silicate) in the water column, water samples were collected from each sampling stationby means of a water sampler (Lymnos 1.4 l) from the surface and 10 m depth. Water samples were placed in 500 ml plastic vials where mercury chlorine (2 ml $HgCl_2$ per 500 ml sample) was added in order to neutralize the bacterial and other photosynthetic organisms. Samples were stored at −20 °C until analysis took place with a spectrophotometer (SHIMADZU UV-1800) according to the procedure described by Eaton *et al.* (1995).

Data for statistical analysis were evaluated for normal distribution by employing the Aderson-Darling test for normality and homogeneity of variance by assessing residual plots and employing Bartlett's and Levene's tests and when necessary data transformation was performed.One-way ANOVA was used to analyze the spatial differences of nutrient by means of the MINITAB software.All statistical tests were performed with a significance level of $\alpha = 0.05$.

Figure 1. Location of the study area

3. Results

Table 1 shows the range, average and standard deviation of physicochemical and biological parameters of the water column at the FS, S and C.

Figure 2 shows the range, average and standard deviation of nutrient concentrations at the FS, S and C.The highest ammonium (NH_4) concentration was observed at FS (0.48 μM), while the lowest one at C (0.13μM). In the case of nitrites (NO_2), the highest concentration was recorded at FS (0.001 μM) and the lowest one at C (0.000 μM). Both nitrate (NO_3) and phosphate (PO_4) concentrations showed slight variations amongall sampling stations. Finally, the highest silicate (SiO_2) concentrations were observed at S (0.68 μM) and FS (0.60 μM), while the lowest one at C (0.29 μM).According to the statistical analysis, ammonium (NH_4) and nitrites (NO_2) showed statistically significant differences among sampling stations ($P<0.001$).

4. Discussion

Fish farming release in the aquatic environment wastes, which are characterized by high concentrations of nitrogen (N) and phosphorus (P). Among the nutrients, the most toxic are ammonia (NH_3) and ammonium (NH_4^+), followed by nitrites (NO_2) and nitrates (NO_3).Ammonia's concentrationis measured as Total Ammonia Nitrogen (TAN), and especially as the sum of ammonia (N-NH3) and ammonium (N-NH4) or as Unionized Ammonia Nitrogen (UAN), N-NH3 (Klaoudatos & Klaoudatos 2010).According to Sawyer (1996), negative effects such us growth of plant cells and appearance of eutrophication are created when concentrations of total inorganic nitrogen exceed 0.3 mg/l and inorganic phosphorus 0.1 mg/l. Ammonium is the primary form of nitrogen that realized by fish farm (Neofitou & Klaoudatos 2008).

In this study, the concentration of ammonium, nitrite and silicate was increased at FS comparedto C. The results of Neofitou & Klaoudatos (2008), indicated increased ammonium, nitrite, phosphate and silicate concentrations at farm sites compared with the control sites. The present study showed similar results except for the phosphate.

The effects of fish farming seem to be limited in a short distance from the fish cages, without affecting the wide area of Pagasitikos Gulf. Nutrient concentrations did not exceed eutrophication levels or in any way affected the welfare of the cultured organisms.

Table 1. Physicochemical and biological parameters of water at the FS, S and C.

	FS	S	C
Temperature (°C)			
Range	20.80-27.22	18.28-27.35	14.48-27.22
Mean ± SD	24.61 ± 2.63	22.51 ± 3.16	18.25 ± 4.84

Salinity (psu)			
Range	37.51-38.32	34.12-38.43	37.51-38.78
Mean ± SD	37.90 ± 0.36	37.88 ± 0.99	38.44 ±0.45
Dissolved oxygen (ppm)			
Range	3.94-5.55	4.09-5.67	4.93-6.05
Mean ± SD	4.77 ± 0.67	5.09 ± 0.59	5.57 ±0.37
pH			
Range	8.50-8.63	7.73-8.64	8.34-8.55
Mean ± SD	8.58 ± 0.05	8.48 ± 0.20	8.43 ± 0.06
Chlorophyll *a* (mg/m^3)			
Range	0.45-1.68	0.54-1.81	0.51-4.15
Mean ± SD	0.96 ± 0.49	1.23 ± 0.46	1.73 ±0.90

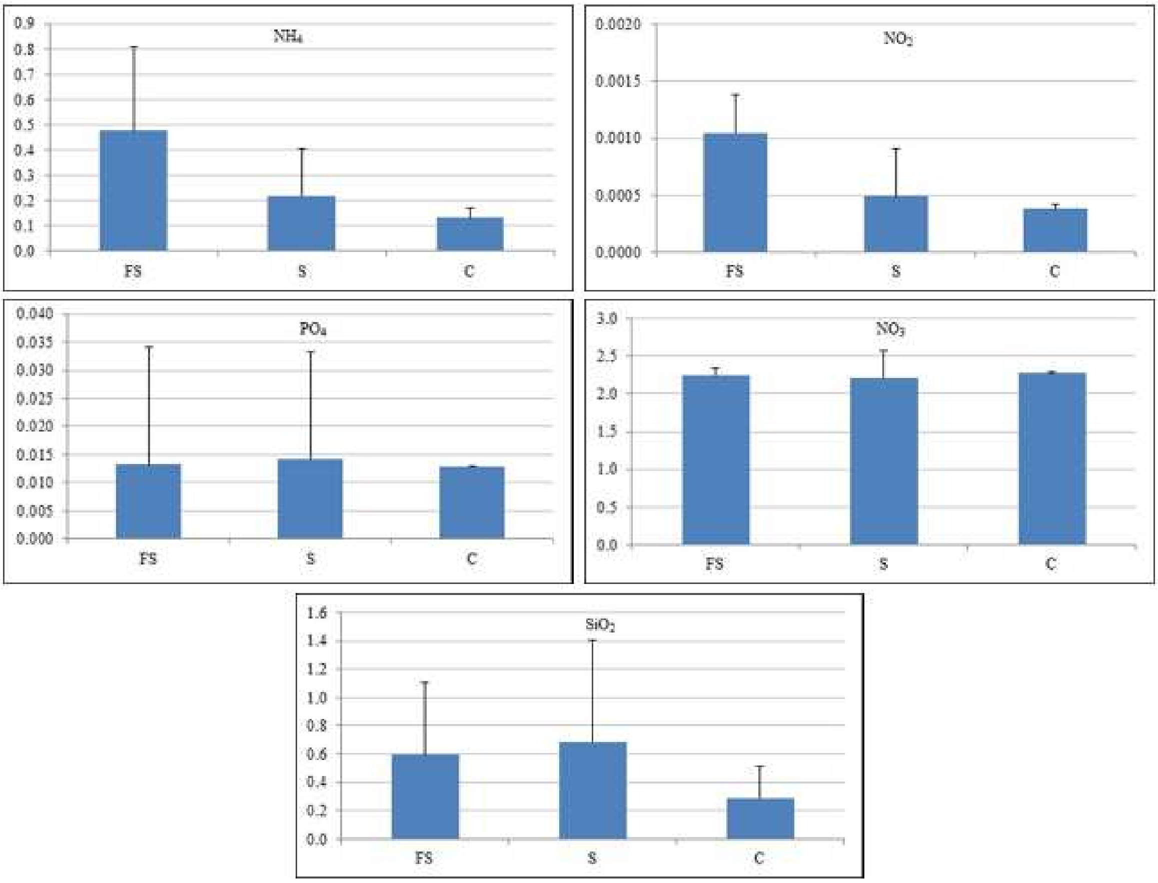

Figure 2. Mean value and standard deviation of nutrients (μM) at FS, S and C.

References

Eaton D.A., Clesceri L.S.& Greenberg A.E.(1995)Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 19th Edition, Washington, USA.

Klaoudatos S., Klaoudatos D. (2010) Constructions of aquaculture systems Marine – Lagoon – Land. Propobos, Athens, p.44-45.

Klaoudatos S.D., Klaoudatos D.S., Smith J., Bogdanos K. & Papageorgiou E. (2006) Assessment of sitespecific benthic impact of floating cage farming in the eastern Hios Island, Eastern Aegean Sea, Greece. Journal of Experimental Marine Biology and Ecology 338: 96– 111.

Neofitou N., Klaoudatos S. (2008) Effect of fish farming on the water column nutrient concentration in a semi-enclosed gulf of the Eastern Mediterranean. Aquaculture Research, 39: 482-490.

Neofitou N., Papadimitriou K., Domenikiotis C., Tziantziou L., Panagiotaki P. (2019) GIS in environmental monitoring and assessment of fish farming impacts on nutrients of Pagasitikos Gulf, Eastern Mediterranean. Aquaculture, 501: 62-75

Sawyer C.N. (1966) Basic concepts of Eutrophication. J. Wat. Pollut. Cont. Fed., 38: 737-744.

LEAPING GREY MULLET *Liza saliens* AS AN ALTERNATIVE AND CANDITATE FISH SPECIES TO BE USED IN A BRACKISH AQUAPONIC SYSTEM CO-CULTIVATED WITH ROCKET PLANT *Eruca sativa*

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Abstract
The aim of the present study was to investigate the feeding rate (as % of b.w/d) for maximum growth of juvenile leaping grey mullet, *Lisa saliens* reared in a brackish aquaponic system co-cultured with rocket plant and its effect on the ammonia production rate. A total number of 180 individuals of leaping grey mullets with an average initial weight of 0.69 ± 0.01 gr, and an average length of 4.23 ± 0.04 cm were used. They were divided into 3 autonomous nutrient film technique (NFT) aquaponic systems (3 tanks/system). Fish were fed by hand with a commercial diet four times per day, with feeding rates of 2% of b.w/d, 5% of b.w/d and 7% of b.w/d. The experiment lasted 45 days. Ammonia production rate was monitored once a week. The results showed that juvenile leaping grey mullets, showed statistically significantly better growth performance (SGR,% / d) when they fed 7% of their b.w/d (SGR=3.0 ± 0.04%/d) compared to the ones that were fed 5% of their b.w (SGR= 2.1 ± 0.05 %/d) and 2% of their b.w (SGR=0.51 ± 0.03%/d) ($p < 0.05$). Ammonia production rate was statistically significant higher (0.03±0.01 mg TAN/h) for leaping grey mullet, *Lisa saliens* fed 7% of their b.w compared to 5% of their b.w and 2% of their b.w. There were no significant differences in ammonia removal efficiency by the plants (NRE) in the NFT hydroponic systems ($p>0.05$). The results showed that *Lisa saliens,* can be used as a new species in brackish aquaponic systems due to their rapid growth performance and survival rate and produce satisfactory nitrogen levels which can be used by the plants for their growth.
Keywords: *brackish aquaponics system, growth performance of L.saliens, salinity, nutrient removal efficiency, ammonia production rate.*
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1. Introduction

Aquaponics is a sustainable farming method for food production all over the world and combines hydroponics and aquaculture in an close recirculating aquaculture system (RAS) where fish and plants grow under the same conditions (Lennard & Goddek 2019; Sommerville *et al.* 2014). Plants for their growth performance absorb nutrients that become available from fish waste and excretion in the water. Natural biochemical processes, especially nitrification, play an important role in the functioning of the aquaponic systems. Fish waste and uneaten food are used as fertilisers and provide valuable nutrients to promote plant growth. Due to the presence of waste and unconsumed food, total ammonia is formed in the water, which is oxidized into nitrite by the autotrophic bacteria *Nitrosomonas sp*. and then nitrite is oxidized into nitrate by *Nitrobacter sp*., bacteria. In RAS, ammonia is one of the crucial parameters that affect water quality criteria during the cultivation time. The rate of ammonia produced within the system as by-product of protein metabolism depends on the feeding rate (as % fish's body weight) (Francis-Floyd 1996).

Consumers interest is focused on the development of aquaponic in brackish or saltwater using fish species that have an important position in Mediterranean aquaculture such as sea bass (Tasiou 2019; Stathopoulou *et al*. 2021), sea bream (Vlahos *et al*. 2019), or mullets (Besbes *et al.* 2020; García-Márquez *et al*. 2021), and in addition salinity-tolerant plants (halophytes) with commercial interests such as Salicornia (*Salicornia europaea*),(Gunning 2016; Fronte *et al.* 2016), opposite-leaved saltwort (*Salsosa soda*) (Pantanella & Bhujel 2015), rock samphire (*Crithmum maritimum*) (Vlahos *et al.* 2019) and quinoa. Alternatively, crops that would usually be classified as glycophytes which are characterized as low salinity tolerant plants such as the common tomato or the cherry tomato (*Lycopersicon esculentum*) and basil (*Ocimum basilicum*), (Joesting *et al.* 2016), rocket (*Eruca sativa*) and capers (*Capparis spinosa*). There are salt-tolerant and can be used as alternatives species in brackish or saltwater aquaponics.

In the present study, the omnivorous leaping grey mullet, *Lisa saliens,* a member of the Mugilidae Family (including the other 4 species: *Mugil cephalus, Chelon labrosus, Liza aurata,* and *Liza ramada)*, inhabiting the Mesolonghi-Etoliko lagoon (Westestern Greece) was selected as a candidate fish species for brackish aquaponics.

It is an euryhaline fish species being tolerant at a wide range of salinities, and suitable for intensive culture due to their fast growth and resistance to environmental changes (Martínez *et al.* 2019). Leaping grey mullet has a high osmoregulatory capacity, allowing them to survive in wide ranges of salinity without affecting their growth (Hotos & Vlahos 1998). Mugilidae species growth performance is sufficient in green water than in clear water (Besbes *et al.* 2020). To the knowledge of the authors', there are limiting data on the feeding rate of Mugilidae species expressed as % of body weight/d and on their growth performance and survival rate in captivity in recirculating aquaculture systems or aquaponic systems. According to Gisbert *et al*. (2016) most of the information on their feeding habits comes from those that they are produced naturally in lagoons. However, the increased interest in intensive aquaculture of Mugilidae species requires more research on their feeding habits with suitably formulated diets. Therefore, the aim of the present study was to estimate the feeding rate (as % of b.w/d) for maximum growth of juvenile leaping grey mullet, *Lisa saliens* in a brackish aquaponic system and its effect on the rate of ammonia production used from rocket plant for its growth.

2. Material and Methods

The experiment was carried out at the Laboratory of aquaculture, Department of Animal Production, Fisheries and Aquaculture, (University of Patras, Mesolonghi, Western Greece). Leaping grey mullet, *Lisa saliens*, fry, was caught with a beach seining net at the coast of the Mesolonghi-Etoliko lagoon during their annual incoming migration. In totally 250 individuals of 0.70 gr in total weight were transported to the aquarium laboratory and acclimated to brackish water (20 ppt) in 250 L aquarium for 20 days. During the acclimation period leaping grey mullets fry fed a mixture of commercial diet (Formula ONE Marine Pellet, Medium-sized-Ocean nutrition) and Instant Baby Brine Shrimp (nauplii of *Artemia salina*-Ocean nutrition) and frozen formula one (Ocean nutrition formula) at a 5% of their body weight, five times per day. Leaping grey mullet adaptation to 1.5 ppt salinity lasted 30 days, where the salinity was gradually decreased of about 5 ppt, by dilution, adding freshwater into the aquarium once or two times per week (Hotos & Vlahos 1998). Upon successful adaptation of the fish to 1.5ppt salinity, 20 individuals were placed in each aquaponics fish tank for 15 days until the conditioning of the aquaponic system and of the filter bed. At the end of the acclimation, period fish were anaesthetized with 0.20 mg/L MSS 222 to measure total weight and length and were randomly distributed to the aquaponic fish tanks to start up the experiment.

For the experiment 3 autonomous recirculation nutrient film technique (NFT) aquaponic systems with a total volume of 180 L were constructed. Each aquaponic system consisted of three rectangular fish tanks (40 x 35 x 26 cm) creating a volume of 36,4 L, 2 stages, upflow and downflow sump filter with a total volume of 53,6 L and partial aeration and a hydroponic bed (grow bed) which consisted of a cylindrical PVC pipe (113 cm height and 10 cm diameter) creating a total volume of 8.9 L. The water keeps flowing through the NFT so plant roots in NFT grow bed are in continuous contact with the nutrient solution (water), creating a thin “film" of depth ranging from 0.08 cm to 0.3 cm deep and thus in contact with aerated water. Water effluent from the fish tank and hydroponic grow bed was dropping through gravity into the mechanical filter (consist of a porous sponge to capture the faeces and uneaten food from fish) then the water through downflow and upflow into the biofilter containing 20 L bio balls media (Φ31,8-38.1 mm) creating a specific surface area (SSA) of 600 cm^2/cm^3 and 20 L ceramic ring media (Φ25 mm), with SSA 1000 cm^2/cm^3. SSA of filter media is affected by feeding rate and protein content in fish feed.

A total number of 180 individuals of juvenile leaping grey mullet, *L. saliens*, with an average weight 0.69 ± 0.01 gr, and an average length 4.23 ± 0.04 cm were placed in the NFT aquaponics system and divided as 20 fish per tank in three treatments. Fish were fed by hand four times per day, with different feeding rates per treatment (2% of b.w/d, 5% of b.w/d and 7%.of b.w/d) with a commercial diet (Ocean nutrition -formula one marine pellets-medium size ±3.1mm) (containing 42.8% protein, 10.1% fat, ash 10% and 1.2% fiber). The experiment lasted for 45 days. Also, 15 individuals of rocket plant were divided into 5 individuals in each system with an average initial height of 7.90 ±0.05 cm. Fish were reweighed every fifteen days of the experiment to remeasure their weight and length and redefine the amount of daily food intake. Fish tanks were cleaned, and uneaten food was removed every day by siphoning. At the end of the experiment (45 days), fish were anaesthetized 0.20 mg/L MSS 222 and final fish body weights and lengths were measured. The water flow rate was set to be constant across the aquaponics systems and was calculated to be equal to 38.0 ± 9.6 $m^{3/}$d and with a constant filtering speed equal to 0.73 cm/min per system. Air was supplied in each aquaponic system by an external air pump (Siemens 3200pw) to maintain dissolved oxygen levels over 70% -80% saturation. During the experimental process, dissolved oxygen, temperature and pH, were monitored once a week and total ammonia, nitrite, nitrate ions, phosphates, calcium, alkalinity and magnesium were monitored two days a week. The diffusion of air for fishes and plants was achieved through a limestone airstone measuring 12 x 25 mm. The airflow was adjusted and it was similar for all aquarium systems and calculated at 16.5±8.81 cm^3/sec. In each system, a 400 watt light (AQUA MEDIC aqua sunlight NG

2 x 150W + 2 x T5 54W) was placed at a distance of 60 cm from the surface of the grow beds to ensure the appropriate exposure of plants to light. The photoperiod was set up to be 14 light: 10 h dark (summer photoperiod).

The homogeneity of photosynthetically active radiation (PAR) reaching the plant top was maintained at the level of 400-500 μmol m^2 sec^{-1}. At the end of the experiment, the following indexes were calculated using the equations below as described by Mente et al. (2016), where W_{in} and W_{fin} are the initial and final weight of the fish, respectively, and Δt is the duration of the experiment in days:

SGR (%/day) = ((ln W_{fin} − lnW_{in})/Δt) × 100, WG (gr) = $W_{fin} - W_{in}$, FCR = Food offered (gr)/weight gain (gr), DFI (%/d) = 100 × ((food offered/weight gain)/feeding days), Condition factor (K) = (W × L^{-3}) × 100 where W is the bodyweight of the fish (gr) and L is the total length of the fish (cm), S = ((final number of fish − initial number of fish)/initial number of fish) × 100.

The rate of ammonia Production (P_{TAN}, mg TAN/h) and Nutrient removal efficiency (NRE, %) was computed according to the following equations:

P_{TAN}= FA* PC*0.092/t, where FA is the amount of feed per feeding, PC, is protein content of the feed, expressed as s decimal fraction, and t is time in which all of the TAN from the given feeding is excreted. (NRE)= (Cin-Ceff)*100/Cin, where Cin and Cenf is ammonia concentration at influence and effluence of the filter bed (Diaz et al. 2012). Data were checked for normality (Kolmogorov-Smirnov), homogeneity (Levene test) and comparisons of means (One way ANOVA). Data were considered statistically significant at p<0.05 (Zar 1999). Statistical analyses were carried out using the software package IBM SPSS Statistics V25.

3. Results

Fish growth performance is presented in Table 1. At the start of the experiment, there were no statistically significant differences in the means of the leaping grey mullets (*L. saliens*) initial body weights and lengths (ANOVA, p>0.05) for all treatments.

Table 1. Leaping grey mullet, *Lisa saliens* growth performance, ammonia production rate (P_{TAN}), and nutrient removal efficiency (NRE) during the 45 days cultivation in brackish aquaponics systems.

	Feeding rate level 2% of b.w	**Feeding rate level 5% of b.w**	**Feeding rate level 7% of b.w**
Initial weight (W_{in})	0.69 ± 0.01^a	0.70 ± 0.02^a	0.70 ± 0.02^a
Final weight (W_{fin})	0.83 ± 0.02^a	1.70 ± 0.05^b	2.48 ± 0.07^c
Survival (S%)	90.0 ± 2.88^a	86.6 ± 4.41^a	73.3 ± 3.33^b
Weight gain (WG_{d45})	0.17 ± 0.01^a	1.04 ± 0.04^b	1.68 ± 0.07^c
Initial coeficient factor (K_{in})	0.91 ± 0.01^a	0.93 ± 0.01^a	0.90 ± 0.02^a
Final coeficient factor (K_{fin})	0.78 ± 0.01^a	0.85 ± 0.01^b	0.85 ± 0.01^b
Specific growth rate (SGR_{fin})	0.51 ± 0.03^a	2.1 ± 0.05^b	3.0 ± 0.04^c
Bodyweight increased (BWI %)	27.0 ± 1.99^a	160.9 ± 5.5^b	289.6 ± 7.9^c
Initial Length (L_{in})	4.23 ± 0.04^a	4.21 ± 0.05^a	4.26 ± 0.03^a
Final Length (L_{fin})	4.73 ± 0.04^a	5.82 ± 0.06^b	6.59 ± 0.06^c
Ammonia production rate P_{TAN} (mg TAN/h)	0.01 ± 0.01^a	0.02 ± 0.01^b	0.03 ± 0.01^c
Nutrient removal efficiency (ammonia) (NRE,%)	28.3 ± 6.98^a	55.8 ± 8.74^a	40.1 ± 11.73^a

Data were expressed as mean ± S.E.M (n=60, n P_{TAN}=7, n $_{NRE}$ =7). Means in a row followed by the same superscript are not statistically significantly different (p>0.05).

At the end of the experiment, there were significant differences in the means of the final weight, final length, weight gain, body weight increase (%) in all treatments (ANOVA, p>0.05). Specific growth rate (SGR) was significantly higher when leaping grey mullets fed 7% of b.w/d than 5% of b.w/d and 2% of b.w/d treatments (ANOVA, p<0.05). The final coefficient factor (K_{fin}) was significantly higher when *L.saliens* fed 5% of b.w/d and 7% of b.w/d than 2% of b.w/d treatment (ANOVA, p<0.05). Survival rate was ranged from 90%, 86.6% and 73.3%, (Table 1) and was significantly higher when leaping grey mullets feeding rate was 2% of b.w/d and 5% of b.w/d than those fed 7% of b.w/d. The rate of ammonia production was statistically significant different in the means for all trials (ANOVA, p<0.05) and was fluctuated from 0.01±0.01 mg/L for leaping grey mullet, fed 2% of b.w/d, 0.02±0.01 mg/L and 5% of b.w/d and 0.03±0.01 mg/L for leaping grey mullet fed 7% of b.w/d. There were no significant differences in ammonia removal efficiency by the plants (NRE) in the NFT hydroponic systems (ANOVA, p>0.05). Specific growth rates of rocket plant shoots were 0.24±0.08 gr DW/d, 0.25±0.08 gr DW/d, and 0.25±0.08 gr DW/d, respectively for both treatments.

4. Discussion

The present study investigated the use of the leaping grey mullet, *L. saliens* as a candidate fish species in brackish aquaponic systems. The results from the present study indicate the ability of leaping grey mullet to adapt to a brackish aquaponics systems and co-cultivated with plants (such as rocket plant). The results of the present study showed that leaping grey mullet, *L. saliens* had a high growth performance when fed 7% of b.W/d and 5% of b.W/d, respectively than those fed with 2% of b.w/d ($SGR_{2\%of\ BW} = 0.51$ %/d < $SGR_{5\%of\ BW} = 2.1$%/d < $SGR_{7\%of\ BW} = 3.0$ %/d). In previous studies (Syama Dayal *et al.* 2017) reported that grey mullet (*M. cephalus*) and gold spot mullet (*L. parsia*) cultured in a brackish polyculture system with 10% salinity showed SGR 2.3 %/d for *M. cephal*us and 1.85%/d for *L. parsia,* respectively. Their growth rate results were lower to the results from the present study especially for the mullets that were fed 7% of their body weight per day. In addition, when leaping grey mullets fed 5% of body weight/d showed SGR (2.1%/d) which is in agreement with grey mullets SGR values (2.3%/d) reported by Syama Dayal *et al.* (2017). Also, a higher growth rate was observed for *M. cephalus* compared to *L. parsia* when reared in a polyculture system with three mullet species *M. cephalus, L. parsia* and *L. tade*, along with tiger shrimp (Biswas *et al.* 2012).

Ammonia production rate was significantly higher (0.03 ± 0.01 mg TAN/h) when juvenile leaping grey mullets, *L. saliens*, fed 7% of body weight/d compared to 2% of body weight/d and 5% of body weight/d, respectively. The results from the present study were lower compared to those reported from Tasiou (2019) in which the TAN ranged from 2.79-3.03 mg TAN/h. The rate of ammonia production within the system depends on the protein level and type of the fish feed, the feeding rate and TAN excretion rate (Francis-Floyd *et al.* 1996, Ebeling & Timmons 2012). In the present study, ammonia removal efficiency (NRE) ranged from 28.3 ± 6.98 %, 55.8 ± 8.74 % and 40.1 ± 11.73% for leaping grey mullets fed 2% of b.w/d, 5% of b.w/d, and 7% of b.w/d, respectively, and there were no significant differences (p>0.05) between the means of all treatments. Gichana *et al.* (2019) reported that pumpkin (*Cucurbita pepo*), sweet wormwood (*Artemisia annua*), and amaranth (*Amaranthus dubius*) have high ammonia removal efficiency (48.8- 50%) when they are cultivated in small scale recirculating aquaponic systems.

References

Besbes, R.; Benseddik, A.B.; Kokokiris, L.; Changeux, T.; Hamza, A.; Kammoun, F.; Missaoui, H (2020). Thicklip (*Chelon labrosus*) and flathead (*Mugil cephalus*) grey mullets fry production in Tunisian aquaculture. Aquac. Rep. 2020, 17, 100380.

Biswas, G., Ananda Raja, R., De, D., Sundaray, J. K., Ghoshal, T. K., Anand, A., Kumar, S., Panigrahi, A., Thirunavukkarasu, A. R. and Ponniah, A. G. (2012). Evaluation of production and economic returns from two brackishwater polyculture systems in tide-fed ponds. J. Appl. Ichthyol., 28: 116-122.

Díaz, V., Ibánez, ˜ R., Gómez, P., Urtiaga, A.M., Ortiz, I., (2012). Kinetics of nitrogen compounds in a commercial marine Recirculating Aquaculture System. Aquac. Eng. 50, 20–27.

Ebeling, J.M., Timmons, M.B., (2012). Recirculating Aquaculture Systems. In: *Aquaculture Production Systems,* Hoboken, NJ, USA: Tidwell, J.H. Ed, John Willey& Sons.

Francis-Floyd R., Watson C., Petty D., Pourder D. B., (1996). Ammonia in aquatic systems.University of Florida, Department of Fisheries and Aquatic Science, Florida Coop,Ext. Serv. FA-16, 4 pp.

Fronte, B., Galliano, G., Bibbiani, C, (2016). *From freshwater to marine aquapoinic: new opportunities for marine fish species production*. 4th Conference with International Participation Conference VIVUS-on Agriculture, Ennvironmentalism, Horticulture and Floristics, Food Production and Processihg and Nutritioh «With Knowledge and Experience to New Entrepreneurial Opportunities» 20th and 21st April 2016, Biotechnical Centre Naklo, Strahinj 99, Naklo. Slovenia.

García-Márquez, J.; Galafat, A.; Alarcón, F.J.; Figueroa, F.L.; Martínez-Manzanares, E.; Arijo, S.; Abdala-Díaz, R.T.(2021) Cultivated and Wild Juvenile Thick-Lipped Grey Mullet, Chelon labrosus: *Animals*, 11, 2112.https://doi.org/10.3390/ani11072112.

Gichana, Z., Liti, D., Wakibia, J. *et al.* Efficiency of pumpkin (*Cucurbita pepo*), sweet wormwood (*Artemisia annua*) and amaranth (*Amaranthus dubius*) in removing nutrients from a smallscale recirculating aquaponic system. *Aquacult Int* 27**,** 1767–1786 (2019). https://doi.org/10.1007/s10499-019-00442-x

Gisbert, E.; Mozanzadeh, M.T.; Kotzamanis, Y.; Estévez, A. (2016). Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution. Aquaculture, 462, 92–100.

Hotos, G., and Vlahos, N. (1998). Salinity tolerance of *Mugil cephalus* and *Chelon labrosus* (Pisces: Mugilidae) fry in experimental conditions, Aquaculture, 167:329-338.

Joesting HM, Blaylock R, Biber P, Ray A (2016) The use of marine aquaculture solid waste for nursery of salt marsh plants *Spartina alterniflora* and *Juncus roemerianus*. Aquac Rep 3:108–114.

Lennard, W and Goddek, S. (2019). Aquaponics: The Basics. In: Aquaponics Food Production, Combined Aquaculture and Hydroponic Production Technologies for the Future Systems. Eds: Goddek, S., Joyce, A., Kotzen, B., Burnell, G. Springer International Publishing. p 619. Doi:10.1007/978-3-030-15943-6.

Mente, E., Solovyev, M.M.,Vlahos,N., Rotllant, G., Gisbert,E (2016). Digestive enzymes activity during initial ontogeny and after feeding diets with different protein sources in zebra cichlid *Archocentrus nigrofasciatus*. Journal of World Aquaculture Society, 48, 5, 831-848.

Pantanella, E., and Bhujel, C.R., (2015). Saline Aquaponics-Potential Player In Food, Energy Production. Global Aquaculture Advocate, pp. 42-43.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., Lovatelli, A. (2014). Small-scale aquaponic food production: Integrated fish and plant farming 16-18, 22-26, 75-81.

Stathopoulou, P., Berillis, P., Vlahos, N., Nikouli, E., Kormas, K., Levizou, E., Katsoulas, N. and Mente, E., (2021). Freshwater-adapted sea bass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. PeerJ, 9, p.e11522.

Syama Dayal,J., Ambasankar, K., Jannathulla, R., Kumaraguruvas.K.P., Kailasam, M., and Vijayan, K.K. (2017). Polyculture of mullets in brackishwater using compounded feed: proximate and mineral profiles in comparision with wild mullets. Indian J. Fish., 64(4): 50-57. DOI: 10.21077/ijf.2017.64.4.69810-07.

Tasiou K. (2019). The production process of sea bass in an aquaponic system. MSc thesis. Department of Ichthyology and Aquatic Environment, University of Thessaly, pp. 85.

Vlahos N., Levizou E., Stathopoulou P., Berillis P., Antonopoulou E., Bekiari V., Krigas N., Kormas K ., and Mente E. (2019). An Experimental Brackish Aquaponic System Using Juvenile Gilthead Sea Bream (*Sparus aurata*) and Rock Samphire (*Crithmum maritimum*). Sustainability 11(18). DOI: 10.3390/su11184820.

Zar J. H. Biostatistical Analysis; Prentice-Hall: London,UK., (1999); P. 718.

CELLULAR RESPONSE TO SOYBEAN MEAL REPLACEMENT WITH NETTLE LEAF (*Urtica dioica*) MEAL IN RED TILAPIA (*Oreochromis* sp.), REARED IN AN AQUAPONIC SYSTEM

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Abstract
Animal protein demand is projected to increase by 52% over the next 30 years due to the growing human population, which is predicted to rise to 9.74 billion by 2050. The continuous environmental and socioeconomic challenges drive the need for novel and innovative approaches in food production and consumption. The emergence and implementation of aquaponics, a promising concurrent fish and vegetable production system, could contribute toward achieving a sustainable production. Rearing of red tilapia (*Oreochromis* sp.) in aquaponic systems has been attracting particular attention as it is a freshwater fish species that can be cultured under various environmental and nutritional conditions. The present research aims to study the hepatic expression of cellular stress biomarkers, heat shock proteins (HSPs) and mitogen-activated protein kinases (MAPKs), in freshwater red tilapia (*Oreochromis* sp.), reared in an aquaponic system, as a response to soybean meal replacement with nettle leaf (*Urtica dioica*) meal. Our results showed differential fluctuations in HSPs expression and MAPKs phosphorylation. In specific, HSP70 showcased a statistically significant increase in nettle-fed tilapia, compared to the non-significant increase of HSP90. Differentiation in the phosphorylation of MAPKs' members was apparent, as phospho-p38 expression levels were significantly decreased in response to nettle diet. The present findings suggest that dietary nettle is not considered as stress stimuli, but induce HSPs expression due to its high protein content. Further research is required for better understanding of the effects of dietary supplementation of stinging nettle on fish physiology.
Key words: *aquaponics, Oreochromis sp., Urtica dioica, stinging nettle, HSPs, MAPKs*
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Introduction

The continuous increase of human population has led to a higher need for water, food and energy resources. Innovative and sustainable approaches in agricultural and fisheries sectors are imperative in order to minimize environmental and climate impact of primary production, while simultaneously ensuring food safety and quality (Deswati *et al*. 2018). Aquaponics, which is a complete multi-trophic system that combines both recirculating aquaculture (fish farming) and hydroponics (production of consumable crops) elements, has recently emerged as an alternative production strategy (Goddek *et al.* 2015). In aquaponic systems, water enriched with nutrients from the fish tanks is used for plants growth, thus ensuring that water and important nutrients, such as phosphorus and nitrogen, can be recovered and reused. In addition, an important outcome of the reduced fertilizers usage is the limitation of lake eutrophication and ocean acidification (Fimbres-Acedo *et al.* 2020).

Nutritional changes have been reported to trigger cellular stress responses including both heat shock proteins (HSPs) induction and mitogen-activated protein kinases (MAPKs) phosphorylation (e.g. Feidantsis *et al.* 2014). HSPs are highly conserved proteins, present in almost every organism and produced in response to a plethora of biotic and abiotic stimuli (Lund *et al.* 2003). Since HSPs activation is universal to all cells and not restricted to heat stress, the specific proteins are widely used as biomarkers (Iwama *et al.* 1998). Mitogen-activated protein kinases (MAPKs), a family of proteins present in all eukaryotic organisms, are also among the most well studied signaling mechanisms (Hamel *et al.* 2012). MAPKs members, such as ERK1/2 and p38 MAPK, sense multiple stress signals-stimuli, such as pathogens, salinity, temperature, food deprivation, and transduce them to several receptors-targets (Berriri *et al.* 2012).

The present study focuses on rearing of red tilapia (*Oreochromis* sp.), a freshwater species of great importance in aquaculture (high availability, fast growth, high density tolerance and environmental adaptation) (Deswati *et al.* 2018) on an aquaponic production of young basil plants (*Ocimum basilicum*). Nettle (*Urtica dioica*) is a herb of great nutritional value, as it contains a high quantity of nutrients and bioactive compounds like vitamins and minerals. Moreover, it has been previously used for its pharmaceutical effects including anti-proliferative, anti-inflammatory, antioxidant, immunostimulatory and anti-infectious activities (Said *et al.* 2015). Previous studies in fish have shown that dietary intake of *U. dioica* reduced several biochemical parameters such as plasma cortisol and glucose, while increased total protein and albumin (Ngugi *et al.* 2015). In addition, nettle has been reported to stimulate fish immunity and diminish inflammatory response, thus leading in proinflammatory cytokines synthesis reduction (Said *et al.* 2015). The aim of the present study is to evaluate the effects of soybean meal replacement with nettle leaf (*Urtica dioica*) meal on the hepatic cellular stress responses of tilapia, reared in an aquaponic system.

Material and Methods

The experiment was carried out in eight autonomous indoor closed loop aquaponic systems (total water volume of 100L each). Each system included a raft hydroponic unit (0.18 m^2) supported by HPS lamp (400W) (14L:10D), a fish tank and a filter for mechanical and biological support (SSA 429 cm^2). The nutrient solution was applied from the filter to hydroponic unit by a water pump (Flow 2963 cm^3 /min) and then returned to the fish tank via gravity. Aeration pumps and water heaters (23°C) were installed to maintain stable conditions for fish and plants. All aquaponic systems received chelated iron DTPA at concentration of 2 mg/L and plants were sprayed with K_2O and CaO (2 ml/L) once a week to avoid iron, potassium, and calcium deficiencies. Red tilapias (*Oreochromis* sp.) with mean initial weight 11.97±0.36 g (10 individuals/system) and young basil plants (*O. basilicum*) (5 individuals/system) were cultivated for sixty days. The aquaponic systems were divided into two treatments, based on different fish food: control diet (fish feed in which 30% w/w was soybean meal) and nettle diet which contained nettle leaf meal as soybean meal replacement. Fish were fed twice a day *ad libitum* (10.00 / 16.00) with the corresponding fish feed.

For the protein immunoblotting procedure, total protein extraction from *O. niloticus* liver tissue samples was carried out using lysis buffer. Protein concentration was determined with the Bio-Rad protein assay. Equivalent protein amounts were separated on 10% (w/v) acrylamide and 0.275% (w/v) bisacrylamide slab gels based on the SDS-PAGE method. The separated proteins were then electrotransferred from within the gel to a nitrocellulose membrane (0.45μm, Schleicher & Schuell, Keene, NH, USA). Afterwards, the unreacted sites on the membrane were blocked using non-fat dry milk (NFDM) as blocking buffer, to prevent non-specific binding of the antibodies. Primary antibodies specific to bind to the target proteins were incubated overnight with the membrane at 4°C. The antibodies used herein were: polyclonal rabbit anti-heat shock protein, 70 kDa (Cat. No. 4872, Cell Signaling, Beverly, MA, USA), polyclonal rabbit anti-heat shock protein, 90 kDa (Cat. No. 4874, Cell Signaling, Beverly, MA, USA), monoclonal rabbit anti-phospho p44/42 MAPK (Thr202/Tyr204) (Cat. No. 4370, Cell Signaling, Beverly, MA, USA) and polyclonal rabbit anti-phospho-p38 MAPkinase (Thr180/Tyr182) (Cat. No. 9211, Cell Signaling, Beverly, MA, USA). After rinsing the membrane to remove unbound primary antibody, membranes were exposed for one hour at room temperature to an HRP-conjugated secondary antibody, specific to the primary antibody (anti-rabbit IgG, HRP-linked Antibody (Cat. No. 7074, Cell Signaling, Beverly, MA, USA)). The pattern of the separated proteins was imprinted into a chemiluminescence film (Fuji Medical, X-ray Films), using 20X LumiGLO Reagent and 20X Peroxide. Proteins density was quantified using the Image Studio Lite Software (LI-COR Biosciences).

Alterations in protein expression among the two different dietary treatments were tested for any statistically significant differences at the 5% level using one-way analysis of variance (ANOVA). Statistical analysis was performed using SPSS Statistics software (IBM).

Results

During the feeding trial, the survival was 97.5% and 100% in control and nettle diet groups, respectively. The hepatic expression levels of HSP70 and HSP90 in *Oreochromis* sp. following soybean meal replacement with nettle leaf meal are depicted in Figure 1. Compared to the control diet, nettle diet resulted in significant induction of HSP70 (one-way ANOVA, $p < 0.05$), while no significant differences were observed in the expression levels of HSP90 (one-way ANOVA, $p > 0.05$). The phosphorylation of p38 and ERK-1/2 MAPKs are presented in Figure 2.

In specific, incorporation of nettle meal into red tilapia's diet increased significantly the levels of phosphorylated ERK-1/2 MAPK, while no significant change in the phosphorylation of p38 MAPK was observed.

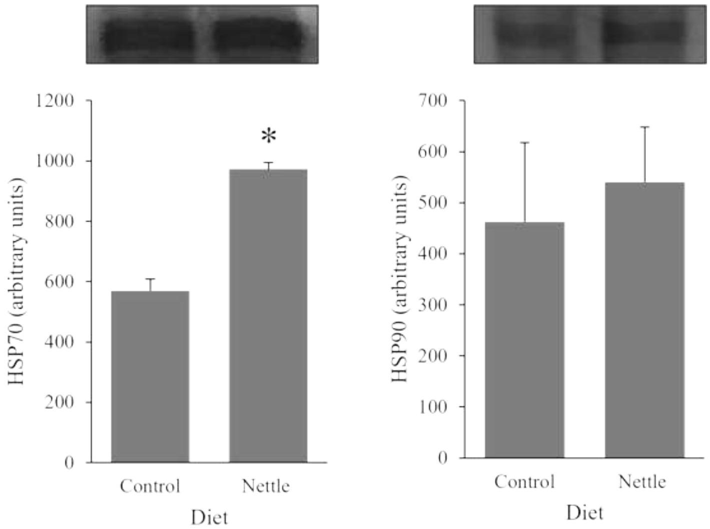

Figure 1. Expression levels of (A) HSP70 and (B) HSP90 in *Oreochromis* sp. liver, following two dietary treatments in an aquaponic system, control diet and nettle diet which contained nettle leaf meal as soybean meal replacement. Statistically significant differences (p < 0.05) between the dietary treatments are symbolized with (*).

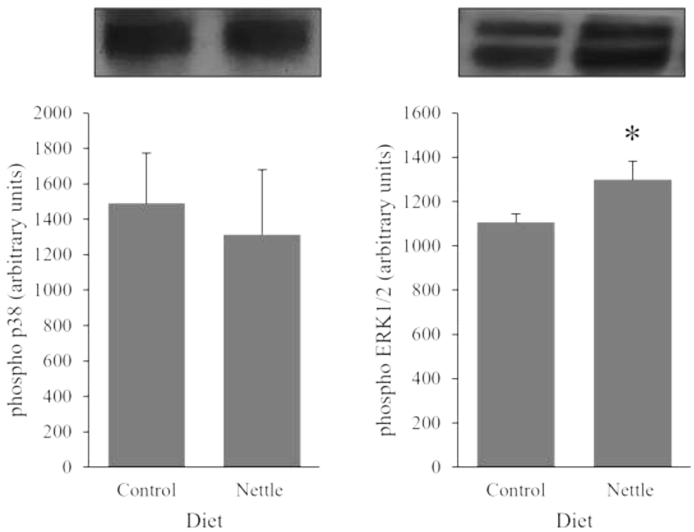

Figure 2. Phosphorylation of (A) p38 MAPK and (B) ERK-1/2 MAPK in *Oreochromis* sp. liver, following two dietary treatments in an aquaponic system, control diet and nettle diet which contained nettle leaf meal as soybean meal replacement. Statistically significant differences (p < 0.05) between the dietary treatments are symbolized with (*).

Discussion

A limited number of studies have highlighted the potential usage of *Urtica dioica* in fish feed. Nettle supplementation had an ameliorative effect on rainbow trout (*Oncorhynchus mykiss*) immune system and growth rate

(De Vico *et al.* 2018). However, changes in the dietary treatment of aquatic animals may alter cellular responses. To the best of our knowledge, effects of stinging nettle on stress inducible proteins in fish have yet to be examined.

Herein, nettle leaf meal as soybean meal replacement induced the expression levels of HSP70 in the liver of red tilapia reared in an aquaponic system. In accordance with these findings, several studies indicated induction of HSPs as a response to numerous nutritional challenges. Elevated HSP70 and HSP90 levels were observed in the liver of meagre (*Argyrosomus regius*) fed with diets of higher lipid content (Antonopoulou *et al.* 2014). Similarly, European sea bass (*Dicentrarchus labrax*) fed with taurine-enriched soy-based diets displayed an increase in the hepatic levels of the aforementioned HSPs (Feidantsis *et al.* 2014). The induction of HSPs in the hepatic tissue of red tilapia may attributed to the increased essential amino acids derived from the dietary incorporation of *U. dioica*, thus resulting in an increase of protein synthesis and metabolism. Consequently, an increase in HSPs proteins is required as they involved in various protein metabolism processes (Ronnestad *et al.* 1999; Hendrick & Hartl 1993).

Regarding mitogen-activated protein kinases (MAPKs), hepatic levels of phosphorylated ERK-1/2 were increased following dietary incorporation of *U. dioica*. In contrast, phospho-p38 showcased a decreasing trend, although no significant difference was detected. The present results are consistent with numerous studies indicating that HSPs are activated through MAPK signaling pathways (Sheikh-Hamad *et al.* 1998; Uehara *et al.* 1999; Rafiee *et al.* 2003; Feidantsis *et al.* 2012). Despite the increase in phospho-ERK-1/2, the non-change in phospho-p38 levels indicates that dietary nettle may not be regarded as stress stimuli for red tilapia. Further research is required in order to elucidate the potential beneficial contribution of stinging nettle in fish growth rate, physiology and disease resistance.

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References

Antonopoulou E., Kousidou E., Tserga E., Feidantsis K., Chatzifotis, S. (2014). Dietary lipid levels in meagre (*Argyrosomus regius*): effects on biochemical and molecular indicators of liver. Aquaculture 428, 265-271.

Berriri S., Garcia A.V., Frei dit Frey N., Rozhon W., Pateyron S., Leonhardt N., Montillet J.L., Leung J., Hirt H., Colcombet J. (2012). Constitutively Active Mitogen-Activated Protein Kinase Versions Reveal Functions of Arabidopsis MPK4 in Pathogen Defense Signaling. The Plant Cell 24, 4281–4293.

De Vico G., Guida V., Carella F. (2018). *Urtica dioica* (Stinging Nettle): A Neglected Plant With Emerging Growth Promoter/Immunostimulant Properties for Farmed Fish. Frontiers in Physiology 9, 285.

Deswati D., Febriani N., Pardi H., Yusuf Y., Suyani H. (2018). Applications of aquaponics on pakcoy (*Brassica rapa* L) and nila fish (*Oreochromis niloticus*) to the concentration of ammonia nitrite and nitrate. Oriental Journal of Chemistry 34, 2447.

Feidantsis K., Kaitetzidou E., Mavrogiannis N., Michaelidis B., Kotzamanis Y., Antonopoulou E. (2014). Effect of taurine-enriched diets on the Hsp expression, MAPK activation and the antioxidant defense of the European sea bass (*Dicentrarchus labrax*). Aquaculture Nutrition 20, 431-442.

Feidantsis K., Pörtner H.O., Markou T., Lazou A., Michaelidis B. (2012). Involvement of p38 MAPK in the induction of Hsp70 during acute thermal stress in red blood cells of the gilthead sea bream, *Sparus aurata*. Journal of Experimental Zoology 317, 303–310.

Fimbres-Acedo Y.E., Servín-Villegas R., Garza-Torres R., Endo M., Fitzsimmons K.M., Emerenciano M.G.C., Magallón-Barajas F.J. (2020). Hydroponic horticulture using residual waters from *Oreochromis niloticus* aquaculture with biofloc technology in photoautotrophic conditions with *Chlorella* microalgae. Aquaculture Research 51, 4340– 4360.

Goddek S., Delaide B., Mankasingh U., Ragnarsdottir K., Jijakli H., Thorarinsdottir R. (2015). Challenges of Sustainable and Commercial Aquaponics. Sustainability 7, 4199–4224.

Hamel L.P., Nicole M.C., Duplessis S., Ellis B.E. (2012). Mitogen-Activated Protein Kinase Signaling in Plant-Interacting Fungi: Distinct Messages from Conserved Messengers, The Plant Cell 24, 1327–1351.

Hendrick J.P., Hartl F.U. (1993). Molecular chaperone functions of heat shock proteins. Annual Review of Biochemistry 62, 349–384.

Iwama G.K., Thomas P.T., Forsyth R.B., Vijayan M.M. (1998). Heat shock protein expression in fish. Reviews in Fish Biology and Fisheries 8, 35–56.

Lund S.G., Lund M.E., Tufts B.L. (2003). Red blood cell Hsp70 mRNA and protein as bio-indicators of temperature stress in the brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 60, 460–470.

Ngugi C.C., Oyoo-Okoth E., Mugo-Bundi J., Orina P.S., Chemoiwa E.J., Aloo P.A. (2015). Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with Aeromonas hydrophila. Fish & Shellfish Immunology 44, 533–541.

Rafiee P., Shi Y., Pritchard Jr K.A., Ogawa H., Eis A.L.W., Komorowski R.A., Fitzpatrick C.M., Tweddell J.S., Litwin S.B., Mussatto K., Jaquiss R.D., Baker J.E. (2003). Cellular redistribution of inducible Hsp70 protein in human and rabbit heart in response to the stress of chronic hypoxia. Journal of Biological Chemistry 278, 43636–43644.

Ronnestad I., Conceicao L.E., Aragao C., Dinis, M.T. (1999). Free amino acids are absorbed faster and assimilated more efficiently than protein in post larval Senegal sole (*Solea senegalensis*). Journal of Nutrition 130, 2809–2812.

Said A.A.H., Otmani I.S.E., Derfoufi S., Benmoussa A. (2015). Highlights on nutritional and therapeutic value of stinging nettle (*Urtica dioica*). International Journal of Pharmacy and Pharmaceutical Sciences, 7, 8-14.

Sheikh-Hamad D., Di Mari J., Suki W.N., Safirstein R., Watts B.A., Rouse D. (1998). p38 kinase activity is essential for osmotic induction of mRNAs for HSP70 and transporter for organic solute betaine in Madin-Darby canine kidney cells. Journal of Biological Chemistry 273, 1832–1837.

Uehara T., Kaneko M., Tanaka S., Okuma Y., Nomura Y. (1999). Possible involvement of p38 MAP kinase in HSP70 expression induced by hypoxia in rat primary astrocytes. Brain Research 823, 226–230.

POTENTIAL ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS AGAINST *Vibrio anguillarum*

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Abstract
Medicinal and aromatic plants and their extracts are increasingly used in disease prevention and treatment (although not strictly recommended) all over the world. *Vibrio anguillarum* is a patogen both in sea and freshwater species. The *Vibrio anguillarum* isolates we used, were isolated form rainbow trout farm in summertime. All isolates were identified with MALDI-TOF. In this study we investigate the 24 hours antimicrobial activities of four different plant extract (rosemary leaf extract, dandelion (taraxacum) extract, grape seed extract and turmeric extract) which also known as plant sap, against four *Vibrio anguillarum* isolate.
Key words: Antimicrobial activity, *Vibrio anguillarum*, Plant Liquid Extract, MALDI-TOF
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1. Introduction

In the traditional plant extraction, plants needed to be dry before extraction. The chemical solvents are used in the traditional extraction methods/protocols. The extract we used were the juice from fresh plants. In this method the extraction does not require the use of solvents and prevents the loss of useful compounds (minerals, vitamins, polyphenols) in the sap of the plant. in the solvent extraction system, the final product can contain a low amount of brix and condensation is needed. In condensation process certain amounts of injured components are subject to thermal degradation. So in the extracts we used in our study were the freshly taken plants and the plant sap was extracted without drying and as a whole in their fresh state, without using any solvent (including pure water), with the juice contained in it (Anonymous 2020).

Alternative medicine applications in infectious diseases have been increasing in recent years. Plants and their products play role as an alternative medicine, in both human and animal health. The effects of herbals and their extracts; on bacteria, virus, fungi, and parasites are investigated both in vitro and in vivo. In those researches, the plant products sometimes are given directly to the animal as a feed additive, sometimes applied directly to the pathogen, and sometimes used in cell cultures, to detect antimicrobial properties (Imran *et al*. 2017; Shen & Su 2017; Tan *et al*. 2017; Valenzuela *et al*. 2018; Aminzare *et al*. 2018; Batmany *et al*. 2019; Memar *et al*. 2019; Huang *et al*. 2020; Jiang *et al*. 2020; Tan et al. 2020; Shan *et al*. 2021; Rahmaningsih *et al*. 2021)

Fish disease also increased as the production increase (Buller 2004). There are uses of allowed antibiotics all around the world. Instead of using antibiotics, the plant base therapeutic agents are used since last two decade. It is known that bacterial pathogens occurs resistance due to antibiotic use. And it is not possible to break down this resistance. That's why plant base therapeutic agents have gained importance. To break down the disease, caused by agents, these plant base therapeutic researches have an important role.

The aim of this study to detect potential antimicrobial effects of rosemary leaf (*Rosmarinus officinalis*), dandelion (taraxacum) (*Taraxacium officinale*), grape seed (*Vitis vinifera*) and turmeric (*Curcuma longa*) extract against four different field isolate of *Vibrio anguillarum*.

2. Material and Methods

Extracts of Rosemary Leaf (*Rosmarinus officinalis*), Dandelion (Taraxacum) (*Taraxacium officinale*), Grape Seed (*Vitis vinifera*) and Turmeric (*Curcuma longa*) were bought in national store in liquid form. The extracts were diluted in Mueller Hinton Broth (MHB) before testing and the starting dose of the dilution was 100 µl/ml.

The fractions were screened in vitro for their potential antibacterial activities against 4 different field isolates of *Vibrio anguillarum* which were isolated form trout farm in Turkey between (June-August 2020). The isolates were named according to the isolation time (Isolate 1 was isolated in May, Isolate 2 was isolated in June, Isolate 3 was isolated in July and Isolate 4 was isolated in August). The isolates were identified with Burker Daltonik MALDI Biotyper. After identified they kept in -20ºC in TSB+Glyserol medium). For determination of the MIC concentrations done micro broth dilution method with 96-well u-plate was used. *Vibrio anguillarum* culture were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) supplemented without NaCl, for overnight.

Serial two-fold dilutions of extracts ranging from 100 to 0,0488 µl/ml were prepared in medium. The density of overnight bacterial growth were set to McFarland 0.5. before transferring the bacterial isolates, the final concentration diluted 10 times, then transferred to 96-well u-plate. A set of wells containing only inoculated broth were used as control. After incubation for 24 at 25±1°C, the last well with no microbial growth was recorded to represent MIC value (µl/ml) (Wayne 2008, Wayne 2009).

3. Results and Discussion

MIC values (mg/ml) of 4 isolate of *Vibrio anguillarum* for Rosemary Leaf, Dandelion (Taraxacum), Grape Seed and Turmeric extract was shown in Table 1.

Table 1. MIC values (µl/ml) of rosemary leaf, dandelion (taraxacum), grape seed and turmeric extract on 4 isolate of *Vibrio anguillarum*

	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Rosemary leaf liquid extract (*Rosmarinus officinalis*)	12,5	12,5	25	50
Dandelion (Taraxacum) liquid extract (*Taraxacium officinale*)	12,5	100	50	50
Grape seed liquid extract (*Vitis vinifera*)	6,25	12,5	12,5	6,25
Turmeric liquid extract (*Curcuma longa*)	12,5	100	12,5	50

All liquid extracts were tested on 4 different *Vibrio anguillarum* isolates. After 24 hour later the MIC values were read. The MIC values were changes according to the isolates. All isolates were isolated from the same river and same trout farms but in different times (May to August). The numbering of the bacteria was numbered according to isolation time. In the present study, different MIC values of isolates may be due to both seasonal differences and the unconscious use of antibiotics in fish farm. Grape Seed Liquid Extract was observed to give the lowest MIC values (6.25, 12.5, 6.25 mg/ml) when compared to all liquid extracts. It was also observed that the MIC values of the liquid extract of the rosemary leaf gave similar results. We are planning to conduct in-vivo studies on the use of plant extracts used in this study against *V. anguillarum* as alternative medicine applications.

References

Aminzare M., Hashemi M., Abbasi Z., Mohseni, M., & Amiri, E. (2018). Vibriosis Phytotherapy: A Review on the Most Important World Medicinal Plants Effective on *Vibrio* spp. Journal of Applied Pharmaceutical Science, 8(01), 170-177.

Anonymous (2020). http://www.kalenaturel.com/

Batmany, Y., Zoriehzahra, S. J., & Kakoolaki, S. (2019). Evaluating the effect of using turmeric (*Curcuma longa*) on growth performance and hematological parameters of the ornamental fish, Green Terror (*Andinocara rivulatus*). Survey in Fisheries Sciences, 5(2), 37-47.

Buller, N. B. (2004). Bacteria from fish and other aquatic animals: A Practical Identification Manual. CABI. pp. 361.

Huang, H. T., Lee, P. T., Liao, Z. H., Chen, H. Y., & Nan, F. H. (2020). Effects of *Phyllanthus amarus* extract on nonspecific immune responses, growth, and resistance to *Vibrio alginolyticus* in white shrimp *Litopenaeus vannamei*. Fish & Shellfish Immunology, 107, 1-8.

Imran, M., Nadeem, M., Saeed, F., Imran, A., Khan, M. R., Khan, M. A., Rauf, A. (2017). Immunomodulatory perspectives of potential biological spices with special reference to cancer and diabetes. Food and Agricultural Immunology, 28(4), 543-572.

Jiang, F., Huang, H., Yang, N., Feng, H., Li, Y., & Han, B. (2020). Isolation, identification, and biological control in vitro of tail rot pathogen strain from *Hippocampus kuda*. PloS one, 15(4), e0232162.

Memar, M. Y., Adibkia, K., Farajnia, S., Kafil, H. S., Yekani, M., Alizadeh, N., & Ghotaslou, R. (2019). The grape seed extract: a natural antimicrobial agent against different pathogens. Reviews in Medical Microbiology, 30(3), 173-182

Rahmaningsih, S., Andriani, R., & Pujiastutik, H. (2021). Effect of Majapahit (*Crescentia cujete* L.) fruit powder on the immune profile of *Litopenaeus vannamei* after infection with *Vibrio* spp. Veterinary World, 14(6), 1480.

Shan, Z., Guan, N., Yang, Y., Jin, T., Xia, X., & Liu, W. (2021). Elucidation of the potential mechanism of punicalagin for conferring disease resistance in Yesso scallops against *Vibrio anguillarum* infection. Aquaculture, 533, 736109.

Shen, X., & Su, Y. C. (2017). Application of grape seed extract in depuration for decontaminating *Vibrio* parahaemolyticus in Pacific oysters (*Crassostrea gigas*). Food Control, 73, 601-605.

Silva-Jara, J., Angulo, C., Macias, M. E., Velazquez, C., Guluarte, C., & Reyes-Becerril, M. (2020). First screening report of immune and protective effect of non-toxic *Jatropha vernicosa* stem bark against Vibrio parahaemolyticus in Longfin yellowtail *Seriola rivoliana* leukocytes. Fish & Shellfish immunology, 101, 106-114.

Tan, X., & Sun, Z. (2020). Dietary dandelion extract improved growth performance, immunity, intestinal morphology and microbiota composition of golden pompano *Trachinotus ovatus*. Aquaculture Reports, 18, 100491

Tan, X., Sun, Z., Chen, S., Chen, S., Huang, Z., Zhou, C., ... & Wang, A. (2017). Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano *Trachinotus ovatus*. Fish & Shellfish Immunology, 66, 198-206.

Valenzuela, M. E. M., Peralta, K. D., Martínez, L. J., & Maggi, R. C. (2018). Taraxacum Genus: Potential Antibacterial and Antifungal Activity. Herbal Medicine.

Wayne, P. A. (2008). "Reference method for broth dilution antifungal susceptibility testing of yeasts", Clinical and Laboratory Standards Institute, Approved standard-third edition, M27-A3

Wayne, P. A. (2009). "Method for antifungal disk diffusion susceptibility testing of yeasts", Clinical and Laboratory Standards Institute, Approved guideline-second edition, M44-A2.

INNOVATIVE APPROACHES TO REDUCE THE USE OF ANTIBIOTICS IN AQUACULTURE: CONTRIBUTION OF MICROALGAE TO THE IMMUNE SYSTEM

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Abstract
The increasing food demand in the world in recent years has increased the interest in aquaculture. However, uncontrolled resource consumption has created the problem of the decrease and disappearance of natural stocks, and the need for aquaculture has paved the way for rapid growth and development in the sector. In this rapid growth process, an increase in bacterial diseases is an inevitable reality along with environmental interactions. Today, the problems caused by diseases have a significant negative impact on the quality and quantity of fish produced in Europe and the world. The resulting economic losses have increased the demand for the use of antibiotics, pesticides and other chemicals in order to fight diseases and support the healthy growth of living things. While increasing use of antibiotics causes the development of microorganisms resistant to antibiotics, their residues in the aquatic ecosystem also cause harmful consequences for human health. It has been demonstrated by many studies that feed additives made from some extracts obtained from microalgae have immune system enhancing and antibacterial properties that reduce the negative effects that may occur as a result of the use of artificial antibiotics and chemicals. Feed additives produced by utilizing the immune effects of microalgae are a valuable alternative application that should be focused on because of their effects on reducing the use of antibiotics and other chemicals used in the fight against bacterial diseases in fish and other species in aquaculture.
Keywords: M*icroalgae, immune system, feed additive, microalgal biotechnology*
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1. Examples of treatment bacterial diseases in aquaculture

Today, losses due to diseases have a significant impact on the quality and quantity of fish produced in Europe and the world. There is an intense increase in the use of antibiotics, pesticides and other chemical substances for the protection and control of diseases and at the same time to support the growth of aquatic organisms. This intensive use of antibiotics can cause harmful consequences for the development of microorganisms resistant to antibiotics, the aquatic environment and human health. The aquaculture sector is trying to control diseases and minimize the use of antibiotics by protecting the aquatic ecosystem. Therefore, studies on disease control by stimulating the humoral and cellular immune systems continue intensively. Salmonid Rickettsial Septicemia (SRS), caused by Piscirickettsia salmonis, has been the leading cause of death in Silide farmed Coho salmon, resulting in an annual economic loss of 100 million (Bravo & Midtlyng 2007).

There is an intense increase in the use of antibiotics, pesticides and other chemicals to support the growth of living things, as well as to prevent and control diseases. This intensive use of antibiotics can cause harmful consequences for the development of antibiotic-resistant microorganisms, the aquatic environment and human health (Barug *et al.* 2006). In a study conducted by the European Animal Health Federation, the amount of antibiotics used for animal production in 1999 was stated as 4700 tons and human consumption as 8500 tons. 3,900 tons of antibiotics used in animal production were used in the treatment of diseases, and 786 tons were used as growth promoters. The aquaculture sector is trying to control diseases and minimize the use of antibiotics by protecting the aquatic ecosystem.

2. Evaluation of microalgae in terms of immune and antioxidant

Microalgae, which are rich in fatty acids and immunants, are important in terms of being the primary food source for aquaculture. When microalgae, which stand out with their high nutritional value in fish nutrition, are examined, Chlorella, Dunaliella and Haematococcus can be counted among the species that can accumulate a high antioxidant carotenoid. They contain α-carotene, lutein and lycopene, especially the important carotenoid group β-carotene. Since fat-soluble pigments such as β-carotene are lipophilic, they reduce the oxidative stress of free radicals on intracellular membrane structures. It also provides repair of liver enzymes such as catalase, peroxidase and super oxide dismutase (Gökpınar *et al.* 2006). In this context, feed additives that benefit from the immune effects of algae create antimicrobial effects on gram-negative and gram-positive bacteria. These products offer an innovative, organic and cost-effective solution.

3. An Alternative and Innovative Solution to Fight Diseases in Aquaculture: Microalgae

Artificial antibiotics are used extensively in the fight against bacteria in aquaculture. Although artificial antibiotics are effective in the treatment of bacterial diseases, they can also cause significant harm. With the continuous use of antibiotics in research conducted at Stanford, Washington and Illinois Universities; It has been determined that beneficial bacteria in living things change and become harmful, hormonal imbalance and digestive problems can occur, and the immune system is adversely affected. In another study conducted in Norway with oxytetracycline and oxolinic acid, it was stated that these antibiotics remained in the sediment under the cages without losing their form for up to seven weeks after administration (Genç 1997).

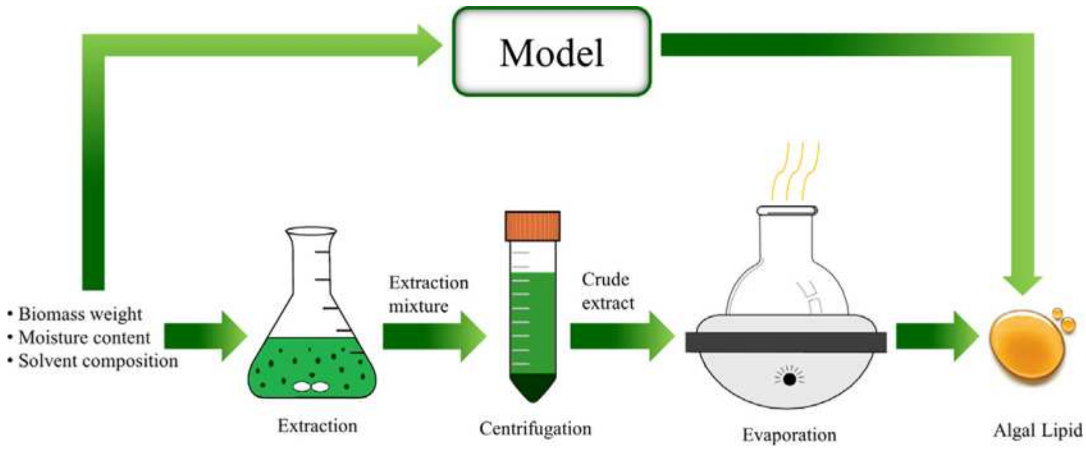
In the cultivation of Salmonidae species, most of the antimicrobials are excreted through urine and faeces after they are integrated into the feed. Accumulation of uneaten fish food on the seabed leads to the formation of resistant bacteria, especially pathogenic bacteria. There is also a risk of the beneficial bacteria being destroyed. Therefore, antimicrobials spread into the aquatic environment through uneaten medicated feed and have the potential to degrade the quality of the aquatic environment.

Salmon in Germany and the Netherlands, sand mussels and black mussels in Australia, shrimp in the Equator are produced in environmental-friendly conditions where the production cycle is provided in an environment free from chemicals and antibiotics, and they are usually offered to consumers in large markets. Aquaculture products produced in standards that support human health create awareness in consumer preferences. In countries such as Germany, UK and the USA, studies on legal regulation and standardization are still ongoing.

It aims to offer an innovative and organic solution in the fight against diseases in aquaculture, due to the high level of natural antibacterial active substances secreted by microalgae in their cells. In this way, it will contribute to its development and economic growth by giving a new impetus to the aquaculture sector.

Antibiotics used for the treatment of diseases in the aquaculture sector both create high costs for companies and slow down the development of living things. Companies are trying to produce alternative solutions for this time and cost spent on treatment. Therefore, studies on disease control by stimulating the humoral and cellular immune systems continue intensively. For example, 18220 kg of oxytetracycline was used for salmon in Norway in 1988, which corresponds to 210 grams per tonne produced. In Finland, it is 115gr/ton. When antibiotics are given with the feed, 20-30% are kept in the fish body and 70-80% are transferred to the environment. A significant amount of antibiotic accumulation was observed in fish caught near the cages and 400 miles away, and in mussels taken from 80 m away, 13 days after the treatment with oxytetracycline, a substance frequently used in bacterial fish diseases in the past (Çelikkale *et al.* 1999).

In laboratory studies, it has been determined that some algae contain antimicrobial properties and are effective against some bacteria, fungi and protozoa. İf microalgae species such as Chlorella grown under various stress conditions (high light intensity, nitrogen deficiency, high salinity, etc), substances with strong antimicrobial and immune properties such as chlorellin, beta carotene, astaxanthin, zeaxanthin, lutein can be accumulated. Thus, various physiological stresses can be created within the scope of microalgal biotechnology. In this way, it can be ensured that the desired antimicrobial substance is produced more from the cultured cells. Aqualg Biotechnology company operating in Turkey has conducted trials on black fish (*Clarias gariepinus*) of the immune system strengthening feed additive obtained from Chlorella microalgae in its research and development studies. In these tests, it was observed that there was a 16% increase in white blood cells compared to the experimental groups. In this study, Chlorella microalgae were grown in specially designed photobioreactor systems by creating various stress conditions, and more active substance in the content of living cells was accumulated. Then, the walls of the algae cells, which were harvested in a concentrated manner, were broken by physical methods and then subjected to extraction and fractionation processes. The values of supernants and active substances in algal cells were obtained in a purified form. Then, they were converted it into feed additive form and carried out his studies on fish. Within the scope of this study, Clarias gariepinus type fish (between 70 gr and 100 gr) were transported to pre-prepared tanks in order to test the immune system strengthening feed additive obtained from microalgae on fish. It was prepared by adding the feed additive to the fish feeds in 3 different ratios (o1%, o3%, o5%). Feeds without additives were prepared for the fish in the control group. Feeding was done by giving 3% of the total weight of the fish twice a day, in the morning and in the evening. In order to determine the activity of the additive given with the baits in the immune system, blood collection was performed from the ventral region between the caudal fins and the anal fins of the fish by injection method. Feeding to fish was stopped 1 day before bloodletting. The blood taken from 20 fish in 12 tanks was put into blood tubes and stored at +4 ℃ until analysis. The blood samples were analyzed and the white blood cells were counted. The white blood cells in the blood taken from the fish in the control group fed with no additives and the white blood cells in the blood taken from the fish in the experimental group fed with additives were compared by counting. and a 16% increase in the number of white blood cells was observed in fish given the feed additive at a rate of 5%.



(Malekzadeh *et al.* 2016)

Feed additives produced by utilizing the immune effects of microalgae reduce the expense items by reducing the use of antibiotics and chemicals against bacterial diseases, which are one of the most important problems of aquaculture companies. Feeds containing microalgae and their extracts; It is expected that it will create an innovative solution in the fight against diseases, which is the most important problem of aquaculture, in environmentally friendly ways, thus making great contributions to the progress and development of the sector.

4. Conclusion

In aquaculture, microalgae, which have various usage areas, are used significantly. Today, studies continue to develop microalgae production systems that can be achieved with large-scale and advanced quality control. In order to ensure sustainability in the use of microalgae, innovative production methods are developed with a systems-based approach in which different areas such as biotechnology, bioprocessing and management procedures are integrated. In addition, in recent studies, microalgae grown and processed with various special methods have begun to be produced in commercial products and studies in which the effect of microalgae on the immune system in living things grown in aquaculture is emphasized. For example, Aqualg Biotechnology company established in Turkey has developed special production methods and processing techniques for the use of microalgae in this field, and the product it produces has achieved successful results on aquatic organisms.
Microalgae have gained an important place in the aquaculture sector with their ease of cultivation in the culture environment and their effectiveness. Microalgae's high protein, vitamin, mineral and fatty acid values, as well as antibacterial properties, support the strengthening of aquatic organisms' immune systems and reducing the rate of bacterial diseases. As a result of this, the production performance of aquaculture farms will increase, work time loss will be reduced and costs will be reduced.

It is clearly seen in the studies that the immune system enhancing feed additives obtained from microalgae will reduce the chemicals used in aquaculture with their natural and antibacterial effects instead of artificial antibiotics. In this way, the negative effects of the antibiotics mentioned above will be minimized. As a result, while supporting sustainable aquaculture will accelerate the development of aquaculture, an environmentally friendly approach will come to the fore.

References

Barug, D., de Jong, J., Kies, A. K., & Verstegen, M. W. A. (Eds.). (2006). *Antimicrobial growth promoters: where do we go from here?*. Wageningen Academic Publishers.

Bravo, S., & Midtlyng, P. J. (2007). The use of fish vaccines in the Chilean salmon industry 1999-2003. *Aquaculture*, *270*(1-4), 36-42.

Burgos-Leon A., Perez-Castaneda R., Defeo O. (2009). Discards from the artisanal shrimp fishery in a tropical coastal lagoon of Mexico: spatiotemporal patterns and fishing gear effects. Fisheries Management and Ecology 16, 130–138.

Çelikkale, M. S., Düzgüneş, E., Okumuş, İ. 1999. Fisheries Sector in Turkey: Potential, Current State,Constraints and Recommendations (in Turkish), İstanbul Ticaret Odası, Yayın No:1999(2): 414, Lebib A.S., İstanbul.

Dimitriou E., Katselis G., Moutopoulos D.K., Akovitiotis C., Koutsikopoulos C. (2007). Possible influence of reared gilthead sea bream (Sparus aurata, L.) on wild stocks in the area of the Messolonghi lagoon (Ionian Sea, Greece). Aquaculture Research 38(4), 398-408.

Franco A., Fiorin R., Zucchetta M., Torricelli P., Franzoi P. (2010). Flounder growth and production as indicators of the nursery value of marsh habitats in a Mediterranean lagoon. Journal of Sea Research 64, 457–464.

Genc, K., Dona, D. L., & Reder, A. T. (1997). Increased CD80 (+) B cells in active multiple sclerosis and reversal by interferon beta-1b therapy. *The Journal of clinical investigation*, *99*(11), 2664-2671.

Gobert B. (1998). Density-dependent size selectivity in Antillean fish traps. Fisheries Research 38, 159-167

Gökpınar, Ş., Koray, T., Akçiçek, E., Göksan, T., & Durmaz, Y. (2006). Algal Antioksidanlar. *Su Ürünleri Dergisi*, *23*(1), 85-89.

Malekzadeh, M., Najafabadi, H. A., Hakim, M., Feilizadeh, M., Vossoughi, M., & Rashtchian, D. (2016). Experimental study and thermodynamic modeling for determining the effect of non-polar solvent (hexane)/polar solvent (methanol) ratio and moisture content on the lipid extraction efficiency from Chlorella vulgaris. *Bioresource technology*, *201*, 304-311.

PRELIMINARY RESULTS OF AN IMTA IN GREECE: CO-CULTURE FISHES, MUSSELS AND OYSTERS

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Abstract
Integrated Multitrophic Aquaculture (IMTA) is an innovate marine fish culture methodology that reduces environmental footprint and increases profit. It is a method that combines cultivation of species belonging to different trophic levels simulating a food web. In this study, three Mediterranean species *were* co-cultured (Sea bream *Sparus aurata,* Mediterranean mussel *Mytilus galloprovincialis* and pearl oyster *Pinctada imbricate radiata),* in three operating fish farms of Aegean (Mediterranean Sea) with different trophic conditions. Sea bream and mussels were cultivated according to the standard growing methods (fish cages and longlines), while the pearl oysters in baskets. Both mussels and oysters were grown near the fish cages in 2-4 m depth. The oysters showed a positive growth rate in all 3 fish farms. In contrast, mussels could not benefit from the fish farm effluents in all areas and their growth reflects on the physicochemical characteristics of the area. Thus, the highest growth rate of the mussels was observed in the mesotrophic fish farm. The above preliminary results are very promising and may became the motivation for experimental IMTA cultures with more and different species.
Keywords: Integrated Multitrophic Aquaculture (IMTA), oysters, mussels, growth, Mediterranean Sea
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Introduction

Integrated Multi-trophic Aquaculture (IMTA) has been proposed as an innovative method of aquaculture development that ensures sustainable development, in agreement with EU guidelines, with actions for Blue Growth and Blue Economy. It is defined as the cultivation of two or more aquatic species from different trophic levels in the same area, in order to simulate the energy flow of natural ecosystems (Chopin *et al.* 2004). As a result, IMTA can maximize the productivity and cost-effectiveness of marine aquaculture through the exploitation of soluble and insoluble substances that have so far been lost by a conventional monoculture. It is an innovative technique that has been tested in aquaculture facilities outside the EU, but their development is not as widespread as in Europe and especially in Greece (Angel & Freeman 2009).

The selection of co-cultivated species targets the greatest possible "representation" of natural ecosystem processes by balancing chemical and biological interactions of cultivated organisms and the environment (Angel & Freeman 2009). Species that can grow in a multi-trophic aquaculture depend largely on environmental characteristics, but the most common practice includes fish farming as a central crop and one species in the water column (usually mussels or algae) and one species in the depths (mainly shellfish or echinoderms) (Hughes *et al.* 2016).

Mussels and oysters are filter-feeding organisms that feed on particles by filtering them from the water column (Bayne 2002). Both have been shown to help reduce the organic load in the water column without significantly burdening the benthic ecosystem with by-products of their metabolism (Gren *et al.* 2008; Dimitriou *et al.* 2015). So, these organisms are ideal for IMTA and a lot of trials have been made to co-cultivate them with fishes (Soto, 2009), while there are some operating IMTA farms with mussels and fishes in other countries (e.g Canada, Scotland; Chopin *et al.* 2013).

This study was designed to examine the survival, robustness and growth of species *Mytilus galloprovincialis* and *Pinctada imbricate radiata* in IMTA systems in three Greek fish farms with different trophic conditions. The results of the project aim to be a first step towards a more sustainable aquaculture industry and offer the Greek aquaculture sector the possibility of commercial exploitation of new species through processes that do not burden the marine environment.

Material and Methods

The IMTA was implemented in 3 different fish farms in the Aegean, Mediterranean Sea. These fish farms were chosen because of their different trophic and physicochemical conditions. The first fish farm is in an off shore, oligotrophic area (LAMAR Fish Farm) in Rhodes, the second is located in mesotrophic waters (Malesina

Fish Farm) in North Evoikos gulf and the last one is sited in eutrophic waters (Ampotis Fish Farm) in Korinthiakos gulf. The same cultivation methodology was used in all farms.

For IMTA mussel cultivation the traditional technique was used: The mussels were grown in long narrow nets placed in rows (longline culture). Initially, the offspring of the mussels were collected either from ropes hanging near cages (spat-collectors) or from already existing ropes in the fish farm area. The mussel offspring were then put in elongated nets with 6 m length, and were submerged between the fish cages for 2 months. Then and for every two months it was necessary to harvest the mussels from the longlines and space them out in more nets to facilitate their growing. To estimate the growth of the mussels, 10 individuals from different parts of the longline were measured every 2 months. Their total weight as well as shell and flesh separately, was measured using a two-decimal scale. These measurements were used to calculate the condition index of the mussels (Bayne 2002). Also, measurements were made regarding their morphometrics, more specifically, their shell length, width as well as thickness to estimate if the mussels are growing properly. When the mussels reached a marketable shell size (5-6 cm) they were removed from the strings and the final samples were collected.

P. i. radiata cultivation was made in oyster baskets made by SEAPA©, a company that designs products specifically for oyster growers. These baskets are a highly productive system for longline oyster farming, however, with appropriate modifications, the baskets can be adapted to suit a range of alternative farming systems and methods.

For the *P. i. radiata* cultivation in baskets, offspring of oysters were collected from natural populations in the area of the fish farm. Before putting them in the IMTA system the oysters were divided into 3 different size classes and placed in separate baskets with increasing diameter holes. The smallest oyster size class (mean length: 4.1) was grown in baskets with 3 mm size, the medium (mean length: 6.7 cm) in 6 mm and the largest (mean length: 8.1 cm) in 12 mm size basket. Oyster baskets were submerged next to the fish cages at depth of 3 m. Every two months the growth of the oysters was recorded by measuring their total weight and the weight of shell and flesh. Their morphometric characteristics were also measured, more specifically, their total length, width and thickness.

Results

In oligotrophic conditions survival of *M. galloprovincialis* was 0%, but in mesotrophic and eutrophic conditions it was 100%. Mussels with initial total mean weight 0.5 g grew to 16.02 g in 11 months in mesotrophic waters and from mean 6.5 g to 21 g in 8 months in eutrophic waters (Figure 1a). From the results it looks like mussels are growing in mesotrophic conditions with a growth rate of 3.116%, while in eutrophic waters the growth rate is 227% (Table 1).

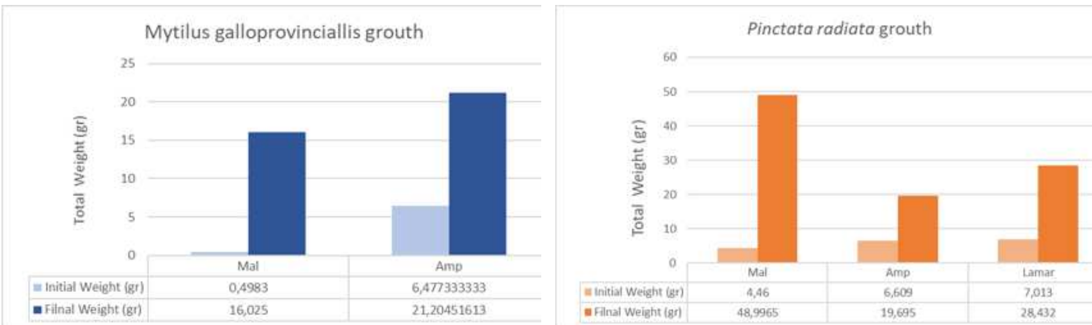

Figure 1: (a) Total mean weight of species *Mytilus galloprovinciallis* in two diffrerent fish farms. Malesina Fish Farm (Mal) is in mesotrophic waters and Ampotis Fish Farm (Amp) is in eytrophic waters and (b) Total mean weight of species *Pinctata i. radiata* in three diffrerent fish farms. Malecina Fish Farm (Mal) is in mesotrophic waters, Ampotis Fish Farm (Amp) is in eytrophic waters and Lamar Fish Farm (Lamar) is in oligotrophic waters.

P. i. radiata can grow in different waters with the growth rate in mesotrophic waters to be 997%, in eutrophic 198% and in oligotrophic 75% (Table 1). In mesotrophic waters oysters can grow in 11 months from 4.5 g to 49 g, in eutrophic from 6 g to 19 g in 8 months and in oligotrophic from 7 g to 28 g in 14 months (Figure 1b). Based on the results it seems that mesotrophic waters are more suitable for the coculture of mussels, oysters and fishes.

Table 1: Presentation of grouth and survival of co-cultivated species in 3 diffrerend fish farms.

Aquaculture	Farmed spieces	Culture Duration (months)	Initial Mean Weight	Final Mean Weight	Growth
Malecina (mesotrophic waters)	*Mytilus galloprovinciallis*	11	0,4983 ± 0,3	16,025 ± 5,5	3115,93%
	Pictata radiata	11	4,465 ± 0,82	48,996 ± 5,2	997%
Ampotis (eytrophic waters)	*Mytilus galloprovinciallis*	8	6,47	21,2 ± 5,18	227%
	Pictata radiata	8	6,609 ± 2,62	19,695 ± 2,37	198%
Lamar (oligitrophic waters)	*Mytilus galloprovinciallis*	-			
	Pictata radiata	14	7,013 ± 1,23	28,432 ± 1,18	75%

Discussion

The results of the study show that the species *P. i. radiata* and *M. galloprovincialis* can survive and grow normally in IMTA systems using traditional methods such as longlines and oyster baskets reaching market size of the Eastern Mediterranean. Although, Mediterranean Sea is mainly oligotrophic, areas near fish farms can be an exception, because a significant amount of nutrients are released from the cultures (Pitta *et al.* 2009). Therefore, fish farms are an ideal cultivating environment for mussels and oysters that need more eutrophic conditions to grow properly. From the results of this study, it can be seen that mussels and oysters can be cultivated in IMTA conditions without any problem on their development despite the high requirements on nutrition and phytoplankton. Unless the fish farm is located in offshore oligotrophic deep waters like LAMAR fish farm, where the environment is not suitable for the growth of *M. galloprovincialis.* On the other hand, the oyster *P. i.. radiata* seems to grow properly in all trophic conditions although with different speed. Thus, oligotrophic areas are not suitable for co-cultivate mussels and fishes, because fish farm effluents are not adequate to replace the lack of dissolved nutrients in the water column. In a different case, *P.i. radiata* can survive and grow commercial size in all examined trophic conditions, but it can be concluded that the best area to cultivate them is in Malecina fish farm, where the waters are naturally mesotrophic. The high growth rate that is shown in that oyster cultivation trial can be due to the high nutrition state of the area.

So, in conclusion depending of the water parameters, IMTA can consist with different types of organisms that can be cultivated near fish cages. That results are very promising and became the motivation for further trials in co-culture different organisms.

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References

Angel D.L. & Freeman S. (2009). Integrated aquaculture (INTAQ) as a tool for an ecosystem approach to the marine farming sector in the Mediterranean Sea. *FAO Fisheries and Aquaculture Technical Paper. No. 529*, 133–183.

Bayne B.L. (2002). A physiological comparison between Pacific oysters *Crassostrea gigas* and Sydney Rock oysters *Saccostrea glomerata*: Food, feeding and growth in a shared estuarine habitat. *Marine Ecology Progress Series*, *232*, 163–178.

Chopin T., MacDonald B., Robinson S., Cross S., Pearce C., Knowler D., Noce A., Reid G., Cooper A., Speare D., Burridge L., Crawford C., Sawhney M., Ang K.P., Backman C. & Hutchinson M. (2013). La red de acuicultura multi-trófica integrada en Canadá (RAMTIC) - La red para una nueva era de acuicultura ecológicamente responsable. *Fisheries*, *38*(7), 297–308.

Chopin T., Robinson S., Sawhney M., Bastarache S., Belyea E., Shea R., Armstrong W., Stewart I. & Fitzgerald P. (2004). The AquaNet Integrated Multi-Trophic Aquaculture Project: Rationale of the Project and Development of Kelp Cultivation as the Inorganic Extractive Component of the System. *Bulletin of the Aquaculture Association of Canada*, *104*: 11–18.

Dimitriou P.D., Karakassis I., Pitta P., Tsagaraki T.M., Apostolaki E.T., Magiopoulos I., Nikolioudakis N., Diliberto S., Theodorou J.A., Tzovenis I., Kagalou I., Beza P. & Tsapakis M. (2015). Mussel farming in Maliakos Gulf and quality indicators of the marine environment: Good benthic below poor pelagic ecological status. *Marine Pollution Bulletin*, *101*(2), 784–793.

Gren I.M., Jonzon Y. & Lindqvist M. (2008). *Costs of nutrient reductions to the Baltic Sea- technical*. 1–64 p.

Hughes A.D., Corner R.A., Cocchi M., Alexander K.A., Freeman S., Angel D., Chiantore M., Gunning D., Maguire J., Mendoza Beltran A., Guinée J., Ferreira J., Ferreira R., Rebours C. & Kletou D. (2016). *Beyond Fish Monoculture - Developing Integrated Multi-trophic Aquaculture in Europe*. 43 p.

Pitta P., Tsapakis M., Apostolaki E.T., Tsagaraki T., Holmer M. & Karakassis I. (2009). "Ghost nutrients" from fish farms are transferred up the food web by phytoplankton grazers. *Marine Ecology Progress Series*, *374*: 1–6.

Soto D. (2009). *Integrated Mariculture A Global Review. FAO Fisheries and Aquaculture Technical Paper. No. 529*.

AMMONIA AND NITRITE TOLERANCE OF *Palaemon adspersus* JUVENILE IN EXPERIMENTAL CONDITIONS

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Abstract
The aim of the present study was to investigate ammonia and nitrite tolerance of the prawn *Palaemon adspersus,* in captivity. LC_{50} experiments were conducted in small scale aquaria with volume 3 L. They were filled with saltwater 30 ppt. During the experiment, pH ranged from 7.7 to 7.9 and the temperature was 22° C. In total, 250 juvenile prawns of *P. adspersus* with an average weight of 0.40±0.02 gr, and a total length of 3.2±0.01 cm were distributed and per treatment 30 individuals (10 prawns/dose) were used. The mortality was recorded and the 24 h LC_{50} of juvenile *P.adspersus*, was calculated, using 0.3 g/L, 0.4 g/L, 0.5 gr/L, 1 gr/L, 1.25 mg/L, 1.5 gr/L, 1.75 g/L, 2gr/L, 2.5 gr/L, 2.75 gr/L, 3 gr/L and 3.25 gr/L concentrations of ammonium chloride (NH_4Cl) and 0.01 g/L, 0.02 g/L, 0.03gr/L, 0.04 gr/L, 0.05 gr/L, 0.07 gr/L, 0.08 gr/L, 0.1 gr/L, 0.15 gr/L, 0.2 gr/L, 0.3 gr/L, 0.9 gr/L, 1 gr/L and 1.5 gr/L concentrations of sodium nitrite ($NaNO_2$). The results showed that the 24 h-LC_{50} values for ammonia and nitrite tolerance was 1.87 mg/L and 7.96 mg/L, respectively.
Keywords: *Ammonia tolerance, nitrite tolerance, Palaemon adspersus, LC_{50}, lethal dose, TAN, nitrite ions, probit method.*
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1. Introduction

Ammonia is the principal metabolic product of aquatic organisms, coming from the catabolism of proteins and amino acids, purines, and pyrimidines (Wajsbort *et al.* 1991). Ammonia levels in intensive culture systems can reach highly hazardous levels for animal survival and growth due to high fish stocking density, decomposition of unconsumed food, waste, and insufficient water refreshment. Ammonia exists in two forms in aqueous solutions: unionized ammonia (NH_3) and ionized ammonia (NH_4^+) (Emerson *et al.* 1975). The $NH_3 : NH_4^+$ ratio increases with increasing water temperature, pH, and decreasing salinity (Trussell *et al.* 1972). Unionized ammonia is toxic to aquatic organisms since it is lipophilic and easily diffuses into the respiratory membranes of organisms (Armstrong *et al.* 1978). Ammonia toxicity has been extensively studied by Alabaster & Lloyd (1980), Chen & Lei (1990), and Schuler *et al.* (2010). The mean lethal concentration (LC_{50}) of ammonia for *P. monodon* larvae reported is 6.00 mg / L (Chin & Chen 1987), for juveniles is 1.76 mg / L (Chen & Lei 1990) and for growing shrimp individuals is 1.76 mg / L. (Chen & Lei 1990). The oxidation of ammonia (NH_4^+) to nitrite is carried out by the action of the bacteria *Nitrosomonas* and *Nitrobacter*. Any factors affecting the action and metabolism of these bacteria can lead to the accumulation of nitrites, which are also characterized by toxic action. These are mainly factors concerning the chemical conditions of the water such as pH, temperature, DO as well as factors concerning the size and age/growth stage of the organism.

The prawn, *Palaemon adspersus,* is widely distributed in Baltic Sea and on the Mediterranean coast, in lagoons and estuaries indicating a wide range of tolerance of the species in different temperatures and salinity (Berglund 1985). The species is a common inhabitant of Messolonghi lagoon in Western Greece and are used for human consumption, as live food for aquaculture (Janas *et al.* 2013), but mainly as live bait for commercial, fisheries, and sport fishing (Glamuzina *et al.* 2014). *P. adspersus* is an omnivorous species, feeding on small crustaceans, polychaetes, algae, and detritus (Bilgin *et al.* 2009) and can be fed with commercial feed pellets. However, to the knowledge of the authors, there are limiting data on the toxicity and lethal concentration mechanisms of ammonia and nitrite in *P. adspersus* prawn, (Chen & Lei 1990) and their farming in captivity in recirculation aquaculture systems. Vlahos *et al.* (2016), reported the survival rate and growth performance of the prawn *P.adspersus*, affecting by temperature in a small recirculation system for 60 days, and were significantly higher in 20°C and 27°C, than those that grew at 10°C. Also, Mastoraki *et al.* (2019), reported that *P. adpsesus* larvae fed formulated diets consist of insect meal, fish meal, and plant meal showed significantly higher growth performance and survival rate when prawn consumed the diet, which includes the inclusion of fish meal and diets incest meal than the plant meal. The aim of this study was to investigate the effect of ammonia and nitrite tolerance on the survival of *P. adspersus* juveniles.

2. Material and Methods

The experiment was carried out at the Laboratory of aquaculture, Department of Animal Production, Fisheries and Aquaculture, (University of Patras, Mesolonghi, Western Greece). Juvenile prawn *Palaemon adspersus* were collected with a hand net (1 mm mesh size) from 0.5-1 m depth at the coast of the Mesolognhi lagoon during spring.

In total, 250 individuals of 0.40±0.02 gr in total weight and 3.2±0.01 cm in total length were transported to the laboratory and acclimatized and maintained in three aquariums of 80 L. The aquarium was filled with brackish water (25 ppt salinity) for 20 days. To avoid prawns jumping over the aquarium, a net was used to cover the aquarium. During the acclimatization period, prawns were hand-fed a commercial diet (nova prawn JBL) with a feeding ratio level 5% of their body mass per day four times per day (Gopal & Raj 1990, Deering *et al.* 1997). Experiments tolerance was conducted by testing the response of various concentrations of ammonia and nitrite and how it affects the mortality of the prawn *P. adspersus.* In LC_{50} experiments the mortality was recorded and the 24 h LC_{50} of juvenile *P. adspersus*, was calculated, using 12 concentrations of ammonium chloride (NH_4Cl) and 14 concentrations of sodium nitrite ($NaNO_2$) using the probit analysis method (Wardlaw 1985). The experimental set-up and conditions were described previously by Hotos & Vlahos (1998). Both experiments (ammonia and nitrite tolerance) were conducted in 5 L glass aquaria which were filled with 1 L, aerated saltwater (30 ppt) without containing any traces of ammonia and nitrite ions properly adjusting for salinity. Before each experiment the shrimps were starved for 24 hours to avoid faeces, ammonia and nitrite in the experimental aquaria. Ammonium chloride tested were 0.3 g/L, 0.4 g/L, 0.5 gr/L, 1 gr/L, 1.25 mg/L, 1.5 gr/L, 1.75 g/L, 2gr/L, 2.5 gr/L, 2.75 gr/L, 3 gr/L and 3.25 gr/L. Sodium nitrite tested were at 0.01 g/L, 0.02 g/L, 0.03gr/L, 0.04 gr/L, 0.05 gr/L, 0.07 gr/L, 0.08 gr/L, 0.1 gr/L, 0.15 gr/L, 0.2 gr/L, 0.3 gr/L, 0.9 gr/L, 1 gr/L and 1.5 gr/L levels.

The above concentrations of ammonium chloride and sodium nitrite were preweighed the day before in a precision balance (AND-HR2000) and then kept in a glass drying cabinet until their use in the experiment (24 h). For its concentration 3 replicate aquarium were used with 10 prawn *P. adspersus* in each (in total 30 prawns), in order to minimize crowding and eliminate stress and cannibalism. An air pump was adjusting in each aquarium to maintain dissolved oxygen up to 70% saturation. The prawns were gradually, smoothly and directly transferred in aquaria with the pre-defined concentrations of ammonium chloride and sodium nitrite. Monitoring and observation of the prawns' behaviour were achieved immediately and started 5-10 min after their introduction to the experimental concentrations, and then every 1 h was monitored its behaviour. Dead prawns were immediately removed from the tanks in order to, affect water quality. At the end of the ammonia and nitrite exposed, a water sample was taken to analyze TAN and nitrite. In the experimental aquaria, pH ranged from 7.7 to 7.9 and the water temperature was maintained at 22ºC. The probit analysis was described previously by Wardlaw (1985). The LC_{50} was calculated by replacing the value 5 for probit of mortality, in the regression equations of probit of mortality vs metabolites (ammonia and nitrite), and 95% confidence limits estimated by the equation LC_{50} (95% CL)= LC_{50} ± 1.96 *(SE LC_{50}), and SE (LC_{50}) $= \frac{1}{b\sqrt{p*n*}\,\overline{w}}$, where: b is the slope of the ammonia/nitrite probit response (regression line), p is the number of concentrations used, n is the number of prawns in each treatment and w is the mean weight of the observation (Table 1). Probit analysis was carried out using the software package IBM SPSS Statistics V25.

3. Results

The results of the mortality of the *P. adspersus* tolerance in ammonium chloride and sodium nitrite concentrations are illustrated in Table 1. Figure 1 represents the linear regression between mortality and the lethal dose of ammonium chloride and sodium nitrite for juvenile *P. adspersus*. It is observed that mortality occurs over a narrow range of shrimp exposure ranging from 1.5 -3.25 g/L for ammonium chloride (Table 1, Fig.1a) and 0.05 - 1.5 g/L for sodium nitrite (Table 1, Fig.1b). Concentrations of ammonium chloride higher than 2.5 g/L increase the mortality rate at 80% to 100%. Similarly, when sodium nitrite concentrations were higher than 0.9 g/L, mortality was more than 90%. 24 h- LC_{50} of NH_4Cl was estimated at 2.04 g/L, corresponding to a TAN concentration that 50% of the shrimps died and was 1.87 mg/L. Regarding 24-h LC_{50} of $NaNO_2$ was estimated at 0.47 gr/L, corresponding to a nitrite ion concentration of 7,96 mg/L. 95% confidence limits were 2.04±0.182 g/L NH_4Cl and 0.47±0.076 g/L $NaNO_2$, respectively.

Table 1. Records of *P. adspersus* juvenile in the ammonium chloride and sodium nitrite tested and probit analysis values used for calculation LC_{50}

Mortality ratio		Dose		Percentage mortality		Probit of observed mortality		Expected probit		W*	
NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})	NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})	NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})	NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})	NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})	NH_4Cl (g L^{-1})	$NaNO_2$ (g L^{-1})
0/30	0/30	0.3	0.01	0		-	-	2	3.75	0.015	0.34
0/30	0/30	0.4	0.02	0		-	-	2	3.78	0.015	0.368
0/30	3/30	0.5	0.03	0		-	-	2	3.80	0.015	0.37
0/30	0/30	1	0.04	0		-	-	2.97	3.83	0.13	0.373
0/30	3/30	1.25	0.05	0	10	-	3.72	3.46	3.86	0.266	0.401
3/30	3/30	1.5	0.07	10	10	3.72	3.72	3.94	3.91	0.409	0.406
9/30	3/30	1.75	0.08	30	10	4.48	3.72	4.43	3.94	0.562	0.409
18/30	6/30	2	0.1	60	20	5.25	4.16	4.92	4.00	0.636	0.439
24/30	9/30	2.5	0.15	80	30	5.84	4.48	5.89	4.13	0.47	0.474
27/30	6/30	2.75	0.2	90	20	6.28	4.16	6.38	4.27	0.301	0.528
30/30	12/30	3	0.3	100	40	-	4.75	6.87	4.54	0.153	0.586
30/30	27/30	3.25	0.9	100	90	-	6.28	7.35	6.17	0.076	0.368
	27/30		1		90		6.28		6.45		0.304
	30/30		1.5		100		-		7.81		0.025
									Σw	3.05	9.12
									$\overline{w}$	0.25	0.36

W*: weight associated with expected probit

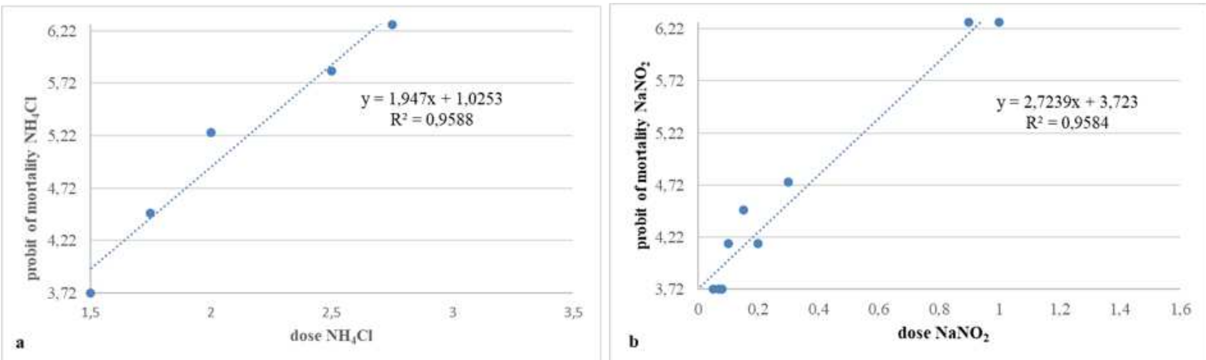

Figure 1. Probit of mortality versus NH_4Cl (a) and $NaNO_2$ (b) for *P.adspersus* juvenile.

4. Discussion

The effect of ammonia and nitrite toxicity on the survival of *Palaemon adspersus* shrimp is being studied for the first time. According to the results obtained from the current study, the shrimp *Palaemon adspersus* appears to be more tolerant to nitrite than to ammonia. The results of the present study showed that the 24h - LC_{50} value for ammonia was found to be 1.87 mg / L and are in agreement with those of Chen & Lei (1990) who studied the shrimp *P. monodon* and found that the 24h lethal concentration for ammonia is 1.76 mg / L. The findings are also similar to those of Chin & Chen (1987) who also investigated *P. monodon* in the mysis stage and found that the 24h - LC_{50} value for ammonia was 2.17 mg / L. In terms of nitrites, the 24h - LC_{50} value was found to be 7.96 mg / L for the *P. adspersus*. Chen & Lei (1990) correspondingly found that the 24h - LC_{50} value for nitrite of the *P. monodon* is 2.18 mg / L. The higher value of 24h - LC_{50} for the shrimp in the current study, compared to the value found by Chen & Lei (1990), may be justified due to the lower temperature and higher pH, conditions that mitigate the toxicity of nitrites. The mortality rate of shrimp depends on the levels of the metabolite (ammonia and nitrite) concentration and time of exposure to it. These results correspond with the findings of Witeska & Jezierska (2003) who reported a positive relationship between mortality and concentration levels, as concentration levels grew, so did the mortality rate. The mortality time and concentration level, on the other hand, had a negative connection; as the concentration level grew, the mortality time dropped. They also found that environmental conditions such as oxygen, temperature, alkalinity, ammonia, nitrite, and the presence of other metals in water affect the levels of toxicity in organisms. In conclusion, ammonia and nitrite accumulation is extremely harmful to crustaceans, despite the fact that a direct comparison of their toxicity, as demonstrated by numerous researches, is not feasible due to the complicated effect of various environmental and biological factors on ammonia and nitrite toxicity. The results of this study will be beneficial for potential farming of the species for sustainable aquaculture development.

References

Alabaster, J.S . LIoyd. R. (1980). Ammonia. In : J.S Alabaster and R. Lloyd (editors). Water quality Criteria for Freshwater Fish. Food and Agriculture Organization of the United Nations , Butterworths, London , pp. 85-102.

Armstrong, D.A..D. Chippendale & A.W. knight & J.E .Colt. (1978). Interaction of ionized and unionized ammonia on short-term survival and growth of prawn larvae , *Macrobrachium rasenbergii. Biol.bull*. 154 :15-31

Berglund A., 1985, Different reproductive success at low salinity determines the estuarine distribution of two *Palaemon* prawn species. Holarctic Ecology 8, 49-52.

Bilgin S., Samsun O. Ozen O, 2009, Seasonal growth and reproduction biology of the Baltic prawn, *Palaemon adspersus* (Decapoda: *Palaemonidae*), in the southern Black Sea. *Journal of the Marine Biological Association of the United Kingdom*, 89(3), 509–519.

Chen ,J.C. & Lei,S.C. (1990).Toxicities of ammonia and nitrite to *Penaeus monodon* juveniles*. J. World Aquacult. Soc* ,21 (4):300-306.

Chin, T.S & Chen ,J.C. (1987) . Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*, Aquaculture,66 :247-253.

Deering M.J., Fielder D.R., Hewitt D.R., 1997, Growth and fatty acids composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. Aquaculture, 151, 131-141.

Emerson, K. &Russo, R.C. &Lund, E.R. &Thurston, R.V. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Bd. Can. 32, 2379–2383.

Glamuzina L., Conides A., Prusina I., Cukteras M., Klaoudatos D., Zacharaki P., Glamuzina B., (2014). Population Structure, growth, Mortality and Fecundity of *Palaemon adspesus* (Rathke 1837; Decapoda: *Palaemonidae*) in Parila Lagoon (Croatia, SE Adriatic Sea) with Notes on the Population Management. Turkish Journal of Fisheries and Aquatic Science 14, 677-687, doi: 10.4194/1303-2712-v14_3_10.

Gopal C., & Raj R.P., 1990, Protein requirement of juvenile *Penaeus indicus*. 1. Food consumption and growth. Proceeding of the National Academy of Science, India, 1990, 99 (5), 401-409.

Hotos, G., and Vlahos, N. (1998). Salinity tolerance of *Mugil cephalus* and *Chelon labrosus* (Pisces: Mugilidae) fry in experimental conditions, Aquaculture, 167:329-338.

Janas U., Pilka M., & Lipinska D., 2013, Temperature and salinity requitements of *Palaemon adspersus* (Rathke 1873) and *Palaemon elegans* (Rathke 1873). Do they explain the occurrence and expansion of prawns in the Baltic Sea. Marine Biology Research, 9, 293-300.

Mastoraki M, Vlahos N, Patsea E, Chatzifotis S, Mente E, Antonopoulou E (2019). The effect of insect meal as a feed ingredient on survival, growth, and metabolic and antioxidant response of juvenile prawn *Palaemon adspersus* (Rathke, 1837). *Aquac Res*. 2020;00:1–12. ht tps:// doi.org/10.1111/are.14692

Schuler D.J., Boardman G.B., Kuhn D.D. & Flick. J.G. (2010). Acute toxicity of ammonia and Nitrite to Pacific White Shrimp, *Litopenaeus vannamei*,at Low Jalinities. J. World Aquacult. Soc. 41 (3), 438-446.

Trussel , R.P. (1972). The percent un-ionised ammonia in aqueous ammonia solutions at different PH levels and temperatures. J. Fish. Res. Board Can , Ottawa , Canada 310 pp.

Vlahos, N., Akrivouli, A., Mente, E., Exadactylos, A., Nystas, T., Patsea, E., Hotos, G. (2016). The effect of three different temperatures on the growth and survival rate of *Palaemon adspersus*. In M.Chatziefstathiou (Ed.), Book of proceedings of the 2nd International Congress on Applied Ichthyology & Aquatic Environment (HYDROMEDIT 2016) (pp. 60–63). Messolonghi, Greece.

Wajsbort, N., &. Gasith, A., &. Krom, M.D. & Popper, D.M., (1991).Acute toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* under reduced oxygen levels. Aquacultur*e* 92, 277-288.

Wardlaw., C.A. (1989). Practical Statistics for Experimental Biologists. 3rd Edition by John Willey & Sons Bublications. Pp. 107-110.

Witeska M, Jezierska B (2003). The effect of environmental factors on metal toxicity of fish. Fresen. Environ. Bull, 12:824-9.

MORPHOMETRIC MEASUREMENT AND CA/P LEVELS OF SEA BASS (*Dicentrarchus labrax*) VERTEBRAE.

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Abstract
The sea bass is one of the most important species of the Mediterranean, and especially of Greece. Individuals with different number of vertebrae have been reported. This number ranges from 24, 25 to 26 vertebrae. In this study a sample of 73 individual of sea bass were collected from fish farms and divided into three age groups. The first group included individuals belonging to the Fingerlings age group, the second group included individuals belonging to the Juveniles age group, and the third group included individuals belonging to the age group of Adults. From radiographs measured the number and the length of their vertebrae. The individuals were divided into subgroups according to their vertebrae number and from each individual was taken the 10th vertebra. Ca and P levels (%) as the Ca/P ratio of all 10th vertebrae were measured by X-ray spectroscopy (EDS). The vertebrae length, and the percentages of Ca, P and the ratio of Ca/P of their 10th vertebrate were compared between the age classes and among the individuals with a different number of vertebrae.
Keywords: *sea bass, number of vertebrae, vertebrae length, Ca and P levels*
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1. Introduction

Since the late 1980s the sea bass has become increasingly important for Europe, particular for the Mediterranean region, with a steady increase in demand. In 2017 Europe produced 84,319 t of sea bass. Greece produced 48,000 t of sea bass, more than the half of the total Europe production, which worth 251.5 million € with an average price of 5.24 € per kg. Of these 48,000 t the 39,000 t. were exported. In 2018 the production of sea bass in Greece reached 41,500 t (Federation of Greek Maricultures 2019). The sea bass is a superior Actinopterygii, more specifically is a Neoteleost fish. Its skeletal system includes bones, cartilage, scales, teeth, connective tissue, fin rays, tendons, ligaments and all the associated stem cells (Ferguson 2007; Papadopoulos 2008). The skeletal system is divided into three parts, the axial skeleton or vertebral column, the cranial skeleton and the zonal skeleton (Moutou 2015). The axial skeleton is made up of bones and cartilage, acts as a support for their body muscles, as a protection for their organs and plays an important role in the metabolism of calcium as a source of stored ions (Papadopoulos 2008). The axial skeleton is structurally varied from a cartilage that encloses the myxins' notochord, to a fully ossified vertebral column in the teleosts. The vertebal column is formed of vertebrae, each of which consists of the vertebral body, the neural and hemal arch. The vertebrae number in the sea bass range from 24 to 26 and the medial vertebral spaces range from 23 to 25 respectively (Darias *et al*. 2011). The axial skeleton of the fish is functionally different from that of the terrestrial vertebrate. The two front vertebrae, which are the atlas and the axis, are differentiated for head adhesion. At the other end of the vertebral column, the last vertebrae that are the undertail, the ontail and the urostil have specially flattened bows for symmetrical adhesion to the tail fin rays. The torso includes vertebrae that support ribs for the adhesion to the muscles, vertebrae of the visceral cavity and vertebrae that forms the hemal canal at the back of the body (Moutou 2015). Bone tissue consists of two types, the cellular bone tissue and the acellular bone tissue. The acellular bone tissue has no osteocytes because the osteoblasts weaken during the calcification stage and thus are not contained within the bones. These bones are therefore 'dead' even though osteoclasts enter the bones (Kranenbarg *et al.* 2005; Papadopoulos 2008). Acellular bones in the superior Osteichthyes, such as sea bass, are surrounded by a two-layer periosteum, the fibrous connective tissue and the osteogenic layer. The latter includes osteoblasts responsible for secretion of the collagen fundamental substance of acellular bones (Groman 1982; Papadopoulos 2008). The center of the vertebrae in the sea bass is composed of acellular bone while in juvenile individuals their skeleton is mostly cartilaginous (Kuzir *et al.* 2004).

Fish consume calcium directly from water and phosphorus through their diet because phosphorus concentration in seawater is low (Lall & Lewis-McCrea 2007). Calcium deficiency is not common in fish as opposed to phosphorus where its signs of deficiency are the slow growth of the fish, slow bone calcification and the appearance of skeletal malformations (Lall 2002). 90% of the organic part of their bones consists of collagen with the main type be type I. 65% of the dry mass of the inorganic part of fish bones is composed of calcium and phosphorus (Berillis & Panagiotopoulos 2015). The calcium to phosphorus ratio (Ca/P) in fish bones ranges from 0.7 to 1.6 (Lall 2002). The combination of collagen fibers, phosphorus and calcium contribute to bone stiffness and hardness. Abnormal regulation of collagen fibers and abnormalities in the fiber structure it can lead to bone pathology (Berillis & Panagiotopoulos 2015).

The purpose of the present study was to measure morphological characteristics as well as to measure and compare the levels of calcium (Ca) phosphorus (P) and Ca/P ratio in sea bass vertebrae between individuals with different age classes and individuals with different vertebrate numbers.

2. Materials and Methods

73 individuals of *Dincentrarchus labrax* were obtained from local fish farms and divided into three age groups. Fingerlings (45 fish, mean weight 5.27 ± 0.06 g, mean length 94.96 ± 0.9 mm), juveniles (18 fish, mean weight 25,25 ± 1,05 g, mean length 143,79±1,98 mm) and adults (10 fish, mean weight 291,9± 29,68 g, mean length 319,43 ±5 mm).

Each individual fish was examined by X-ray through syngofastView program. Their total length, the number of vertebrae and vertebrae length was measured (fig. 1). In fingerlings and juveniles, the number of vertebrae varied to 24, 25 and 26 and in Adults ranged from 24 to 25 vertebrae.

The 10th vertebra, which it considered as a representative sample, was taken from each individual fish, weighed and incinerated in order to measure calcium (Ca) and phosphorus (P) levels (%). Three measurements were taken per vertebra. A scanning electron microscope (Jeol JSM-6510 LV, Ltd, Tokyo, Japan) equipped with an X-ray analyzer (x-act Oxford, Abingdon, United Kingdom) was used for the stoichiometric analysis of the vertebrae by energy dispersive spectroscopy (EDS).

For the statistical analysis the OriginPro8 program was used. All values are given as the means ±standard errors or medians± interquartile ranges. Shapiro-Wilk test or the Kolmogorov- Smirnov test, depending on the data number, was used for normality checking. All the differences presented at the 0.05 level were considered significant.

3. Results and Discussion

Vertebrae length and stoichiometric analysis are given at tables 1 & 2, respectively.

Table 1. Length of the 10th vertebra (mm) of sea bass individuals grouped by age and number of vertebrae. The results are presented as mean ± standard error. Numbers in brackets show the number of measurements.

	Fingerlings	Juveniles	Adults
Individuals with 24 vertebrae	$1.69±0.05^{a,a}$ (17)	$2.7±0.22^{b,a}$ (4)	$7.65±0.27^{c,a}$ (7)
Individuals with 25 vertebrae	$1.67±0.06^{a,a}$ (22)	$2.9±0.17^{b,a}$ (8)	$6.13±1.27^{c,a}$ (3)
Individuals with 26 vertebrae	$1.68±0.09^{a,a}$ (6)	$3.01±0.15^{b,a}$ (6)	-

Note: The means having a different first superscript per row are significantly different (P <0.05). The means having a different second superscript per column are significantly different (P <0.05).

There is a difference in the number of vertebrae that individuals of sea bass have. The number of vertebrae is not related to the presence of skeletal malformations while nutrition factors contribute to both vertebrate number and skeletal malformation (Darias *et al*. 2011). According to Zouiten *et al*. (2011) in the intensively reared sea bass the loss of a vertebra is more frequently observed than the appearance of an additional vertebra, which is similar to our results. Only Juveniles show a slight difference. In fingerlings the length of the 10th vertebra varies from 1.67-1.69 mm, in juveniles from 2.7-3.01 mm and in adults from 6.13-7.65 mm. No significant differences were detected between individuals from the same age but with different vertebrae number. The average length of the torso's vertebrae of adult sea bream varied from 6.7 mm to 7.7 mm (Berills *et. al* 2015; Boursiaki *et al*. 2019). In Atlantic salmon the torso's vertebrae mean length is 8.56 mm. Age is related with Ca/P rate because individuals with incomplete development do not have high Ca/P rate (Guerreiro *et al.* 2001). The Ca/P rate in sea bass individuals is 0.7-1.6 (Lall 2002) and in sea beam individuals is 1.79-2.36 (Ozawa & Suzuki 2002; Berills *et. al* 2015; Boursiaki *et al*. 2019).

Table 2. Ca (%) and P (%) and Ca / P ratio of the 10th vertebra of the sea bass grouped by age and number of vertebrae. The results are presented as mean ± standard error. Numbers in brackets show the number of measurements.

	Fingerlings			Juveniles			Adults		
	Ca	P	Ca/P	Ca	P	Ca/P	Ca	P	Ca/P
Individuals with 24 vertebrae	$19.99 \pm 1.60^{a,a}$ (51)	$11.48 \pm 0.95^{a,a}$ (51)	$2.08 \pm 0.16^{a,a}$ (51)	$23.69 \pm 1.57^{a,a}$ (12)	$14.18 \pm 3.27^{a,a}$ (12)	$2.78 \pm 0.50^{a,a}$ (12)	$52.71 \pm 0.91^{b,a}$ (21)	$27.90 \pm 0.70^{b,a}$ (21)	$1.91 \pm 0.06^{a,a}$ (21)
Individuals with 25 vertebrae	$19.24 \pm 1.34^{a,a}$ (66)	$13.41 \pm 1.19^{a,a}$ (66)	$2.26 \pm 0.28^{a,a}$ (66)	$22.67 \pm 2.35^{a,a}$ (24)	$14.42 \pm 1.39^{a,a}$ (24)	$1.82 \pm 0.24^{a,a}$ (24)	$34.21 \pm 5.84^{b,b}$ (9)	$16.59 \pm 4.27^{a,b}$ (9)	$2.61 \pm 0.39^{a,b}$ (9)
Individuals with 26 vertebrae	$14.83 \pm 2.02^{a,a}$ (18)	$22.72 \pm 3.10^{a,b}$ (18)	$0.82 \pm 0.14^{a,b}$ (18)	$24.00 \pm 2.19^{b,a}$ (18)	$14.38 \pm 1.37^{b,a}$ (18)	$1.90 \pm 0.23^{b,a}$ (18)	-	-	-

Note: The means having a different first superscript per row are significantly different ($P < 0.05$). The means having a different second superscript per column are significantly different ($P < 0.05$)

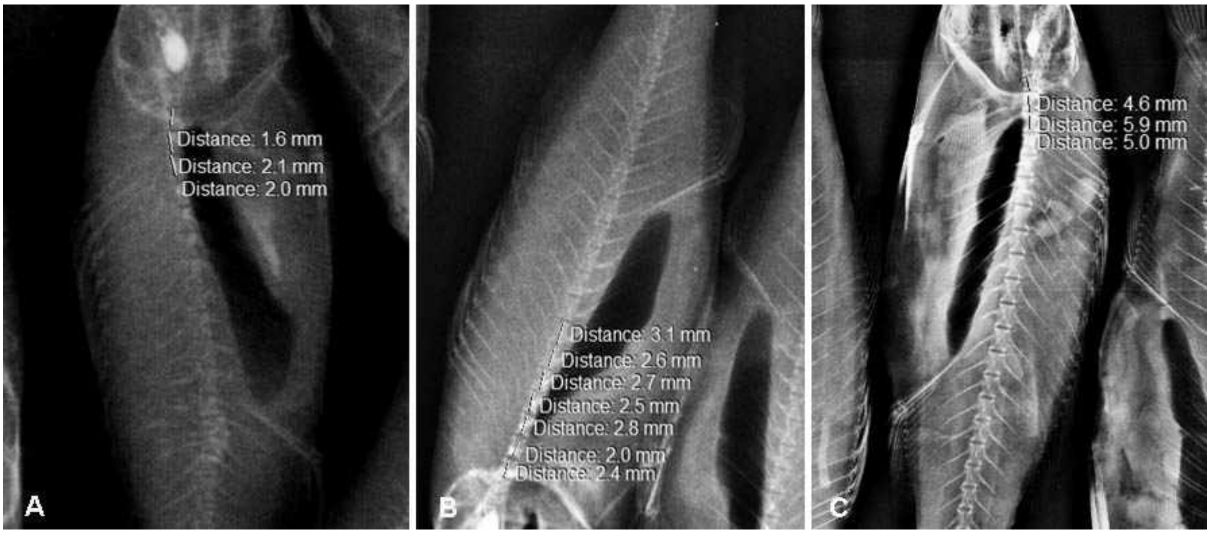

Figure 1: X-ray and vertebrae length measurements of A) Fingerling sea bass, B) Juvenile sea bass and C) Adult sea bass.

Our results vary from 0.82-2.08 for Fingerlings, 1.82-2.78 for Juveniles and 1.91-2.61 for Adults. Fingerlings with 26 vertebrae had the lowest (0.82±0.14) Ca/P ratio. Nutrition is responsible for the P ratio and the appearance of the one additional vertebra (Lall & Lewis- McCrea 2007; Darias *et al*. 2011). In the present research in Fingerlings with 24 vertebrae the P ratio was lower than the P ratio of the Fingerlings with 26 vertebrae. In Adults with 24 vertebrae the Ca ratio was lower than the Ca ratio of the Adults with 25 vertebrae.

4. References

Berillis, P., Panagiotopoulos, N., Boursiaki, V., Karapanagiotidis, I.T., Mente, E. (2015). Vertebrae length and ultra-structure measurements of collagen fibrils and mineral content in the vertebrae of lordotic gilthead seabreams (*Sparus aurata*). Micron 75, 27–33.

Boursiaki, V., Theochari, C., Zaoutsos, S. P., Mente, E., Vafidis, D., Apostologamvrou, C., Berillis, P. (2019). Skeletal Deformity of Scoliosis in Gilthead Seabreams (*Sparus aurata*): Association with Changes to Calcium-Phosphor Hydroxyapatite Salts and Collagen Fibers. Water 11(2), 257.

Darias, M. J., Mazurais, D., Koumoundouros, G., Le Gall, M. M., Huelvan, C., Desbruyeres, E., Quazuguel P., Cahu C. I., Zambonino-Infante, J. L. (2011). Imbalanced dietary ascorbic acid alters molecular pathways involved in skeletogenesis of developing European sea bass (*Dicentrarchus labrax*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 159(1), 46-55.

Federation of Greek Maricultures (2019). https://www.fgm.com.gr/

Ferguson H. W. (2007). Systemic Pathology of Fish: a text and atlas of normal tissues in teleosts and their responses in disease. Journal of Fish Diseases 30(6), 381-382

Groman D. (1982). Histology of the Striped Bass. American Fisheries Society. 5410 Grosvenor Lane, Bethesda, Maryland 20814

Guerreiro P.M., Fuentes J., Power D.M., Ingleton P.M., Flik G., Canario A.V.M. (2001). Parathyroid hormone-related protein: a calcium regulatory factor in sea bream (*Sparus aurata* L.) larvae. American Journal of Physiology- Regulatory, Integrative and Comparative Physiology 281(3).

Kranenbarg S., Cleynenbreugel T., Schipper H., Leeuwe J. (2005). Adaptive bone formation in acellular vertebrae of sea bass (*Dicentrarchus labrax* L.). The Journal of Experimental Biology 208,3493-3502.

Kuzir S., Kozaric Z., Nejedli S. (2004). Development of mandibular arch in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) from the "Cenmar" hatchery, Croatia. Veterinarski Arhiv 74 (5), 321-330.

Papadopoulos P. E. (2008). Atlas of sea bass histology (*Dicentrarchus labrax*). Master's Thesis, University of Thessaly, p. 148

Lall S.P., Lewis- McCrea L.M. (2007). Role of nutrients in skeletal metabolism and pathology in fish- An overview. Aquaculture 267, 3-19.

Lall, S.P. The minerals. In Fish Nutrition, 3rd ed.; Halver, J.E., Hardy, R.W., Eds.; Academic Press Inc.: San Diego, CA, USA, 2002; pp. 259–308.

Moutou A. (2015). Fish physiology. University of Thessaly.

Ozawa, M., Suzuki, S. (2002). Microstructural development of natural hydroxyapatite originated from fish-bone waste through heat treatment. J. Am. Ceram. Soc. 85, 1315–1317.
Zouiten, D., Ben Khemis, I., Slaheddin Masmoudi, A., Huelvan, C., Cahu, C. (2011). Comparison of growth, digestive system maturation and skeletal development in sea bass larvae reared in an intensive or a mesocosm system. *Aquaculture Research 42*(11), 1723-1736.

FFECTS OF HEAT HARDENING ON OXIDATIVE DEFENCE CAPACITY IN THE MANTLE OF THERMALLY STRESSES *MYTILLUS GALLOPROVINCIALIS*

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Abstract
Ectotherms are exposed to a range of environmental temperatures and often face extremes beyond their upper thermal limits. Such temperature extremes can stimulate aerobic metabolism toward its maximum, a decline in aerobic substrate oxidation, and a parallel increase of anaerobic metabolism, combined with ROS generation and oxidative stress. Under these stressful conditions, marine organisms recruit several defensive strategies for their maintenance and survival. However, thermal tolerance of ectothermic organisms may be increased after a brief exposure to sublethal temperatures, a process known as "hardening". In our study, we examined the ability of *M. galloprovincialis* to increase its thermal tolerance under the effect of elevated temperatures (24, 26 and 28 °C) through the "hardening" process. Our results demonstrate that hardening can increase the heat tolerance and antioxidant defense of heat hardened mussels when exposed to temperatures beyond 24 °C, compared to non-hardened individuals. Enhanced cell protection is reflected in better adaptive strategies of heat hardened mussels, and thus decreased mortality. Although further investigation of the molecular and cellular mechanisms regulating mussels' heat resistance is required.
Keywords: *hardening, heat stress, antioxidant defence, Mytillus galloprovincialis*
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1.Introduction

Climate change leading to an increase in sea surface temperatures affects marine organisms at all levels of biological organization, including molecular, biochemical and physiological (Hochachka & Somero 2002; Pörtner 2021). According to the hypothesis of oxygen and capacity limited thermal tolerance (OCLTT), temperature increase beyond organism's optimum limits (at pejus temperatures, Tp) leads to mismatch between energy demand and energy supply from aerobic pathways, a compensatory shift to partial anaerobiosis, and energy deficiency (Pörtner & Farrell 2008; Pörtner 2010; Pörtner 2021). Furthermore, oxidative stress caused by excessive ROS production can lead to cellular damage and eventually cell death, so that mitigation of the oxidative stress via upregulation of antioxidants plays a key role in survival and contributes to the costs of cellular homeostasis under heat stress (Sokolova 2018; 2021).

Thermal tolerance of organisms is a plastic trait and can be modified by brief exposures to sub-lethal temperatures known as heat hardening (Bilyk *et al*. 2012). Heat hardening is defined as a transient response that improves the heat tolerance immediately after the initial heat-stress bout for up to 32 h (Bowler 2005; Bilyk *et al.* 2012). Precht (1973), has defined "*hardening*" as a rapid beneficial response occurring within a few hours in contrast to acclimation that may take days. The mechanisms of heat hardening are not yet fully understood but involve co-activation of multiple stress signaling pathways that lead to phenotypes with increased resistance (Bruce *et al*. 2007; Jaskiewicz *et al*. 2011).

Previous studies showed that *M. galloprovincialis* is thermally stressed when exposed to 26°C-27°C which results in elevated mussels' mortality (Anestis *et al*. 2007; Feidantsis *et al*. 2020). The aim of the present study was to investigate whether heat hardening enhances thermal tolerance of *M. galloprovincialis* and whether transcriptional and/or post-transcriptional regulation of the pathways involved in antioxidant defense are implicated in this phenomenon. To determine the potential involvement of oxidative stress and redox signaling in heat hardening, we determined mRNA expression of enzymatic [Cu Superoxide Dismutase (*CuSOD*), Mn Superoxide Dismutase (*MnSOD*), Glutathione *S*-transferase (*GST*), catalase) and measured activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR) and catalase.

2. Materials and Methods

2.1 Animals

The mussels *M. galloprovincialis* with a total mass of 25.82 ± 4.62 g (mean ± SD), shell length 6.42 ± 0.47 cm and shell width 3.2 ± 0.15 cm, were collected from a mussel farm located in Thermaikos Gulf, Greece (Dramouslis LTD) in late April, when the ambient sea water temperature was approximately 18 °C. Mussels were transferred to the Laboratory of Animal Physiology, Department of Zoology, School of Biology of the Aristotle University of Thessaloniki and kept in 1000 l tanks with recirculating aerated natural seawater for 1 week. Water temperature was controlled at 18 °C ± 0.5, while salinity was kept at 34‰ ± 2.85 and pH at 8.12 ± 0.05, respectively. Mussels were fed daily with 0.5% dry weight cultured microalgae *Tisochrysis lutea* (CCAP 927/14)/gr total weight of mussels. 60% of water was replaced every 2 days with filtered seawater.

2.2 Experimental procedure

The experimental exposures were conducted in two phases (heat hardening and acclimation phases).
Heat-hardening phase. Experimental design was based on Hutchison's (1961) "Repeated-Critical Thermal Maxima (CTM)" method, with minor modifications. Specifically, ~200 randomly selected mussels were divided and conditioned in three aquaria each containing 100 l aerated sea water at 18°C. The sea water was recirculated via pipes connected to a 500 l tank maintained at 18°C. Mussels were kept at 18°C for one week. These aquaria were connected with 2 other tanks, one with increased temperature of 32oC and one with low temperature of 18oC. First the water temperature of the 3 aquaria was increased with a rate of 1,5oC/min at 27ºC through the high temperature tank and mussels maintained in this temperature for 2,5h. Then the water temperature was decreased again to 18oC through the low temperature tank and recovered for 24h. This heat-stress bout (including the thermal shock and recovery phase) was repeated four times. The mussels exposed to this treatment are later called heat-hardened mussels. Control mussels were maintained at 18°C during this time.

Acclimation phase. After the completion of the heat-hardening treatments, both control (non-hardened) and hardened mussels were transferred in four 500 l tanks (50-60 individuals from each group per tank) with recirculating aerated natural seawater at 18°C and left to recover for four days. Thereafter, water temperature of the three tanks was increased (1°C/hour) to 24, 26 and 28°C, respectively. Mussels maintained in the fourth tank were kept at 18°C and used as controls. All mussels were kept at each temperature for 10 days and were fed daily prior to the 15-day experimentation period. However, all four groups were fasted throughout the experimentation period. Individuals (n=8 at each time point) from hardened and non-hardened groups were collected from each tank at 12 h, 1, 3, 5, and 10 days after the target temperature (24, 26 or 28°C) was reached. The mantle tissue was selected and removed, immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

2.3 Analytical procedures

Determination of activities of antioxidant enzymes in the tissue homogenates
Homogenization of mantle samples for the measurement the activities of the cytosolic and mitochondrial enzymes were performed according to well established protocols: (Vmax) of total SOD - mitochondrial Mn- and cytosolic Cu/Zn-superoxide dismutase (EC 1.15.1.1.), CAT (EC 1.11.1.6.) and GR (EC 1.8.1.7.) were assayed.
Determination of mRNA expression. For estimation of mRNA expression profiles

A quantitative real-time PCR was performed. Total RNA was isolated from homogenized mantle tissue using Tri-Reagent (Sigma, St. Louis, MO, USA) following the manufacturer's instructions and the mRNA levels of the target genes were determined including *GST*, Mn superoxide dismutase (*Mn-SOD*), Cu/Zn superoxide dismutase (*Cu/Zn-SOD*), and *CAT* (*cat*). The *actin* gene, used as reference, was amplified using the primers actin-F and actin-R (Accession No: AF157491). Relative quantification was achieved by comparing the cycle threshold values (CT) of the target genes with the CT values of the *actin* using the ΔΔCT quantification algorithm.

2.4 Statistical analysis

Changes in antioxidant enzymes activities and relative mRNA expressions were tested for significance at the 5% level ($p < 0.05$) by using one-way analysis of variance (ANOVA) (GraphPad Instat 3.0) for statistically significant differences between examined groups and two-way (GraphPadPrism 5.0) analysis of variance (ANOVA) for the significance of factors tested each time, with sampling days and treatment as fixed factors. Post-hoc comparisons were performed using the Bonferroni test. Values are presented as means ± S.D.

3. Results

At 24 °C, both hardened and non-hardened mussels exhibited no mortality (Fig. 1). In Temperature increase to 26°C while non-hardened mussels exhibited 55% mortality after 10 days of exposure, the cumulative mortality of hardened mussels was lower, reaching 35% by day 10. At the highest tested temperature (28 °C), mortality of non-hardened mussels was 85% in the first week and 100% by day 10. On the other hand, the mortality of hardened mussels at 28 °C was 30% in the first 7 days and reached 60% by day 10 (Fig. 1). The findings of our research demonstrate enhanced thermotolerance in heat hardened *M. galloprovincialis* as shown by their improved survival during heat exposure (particularly at the lethal temperature of 28°C) compared to the non-hardened mussels.

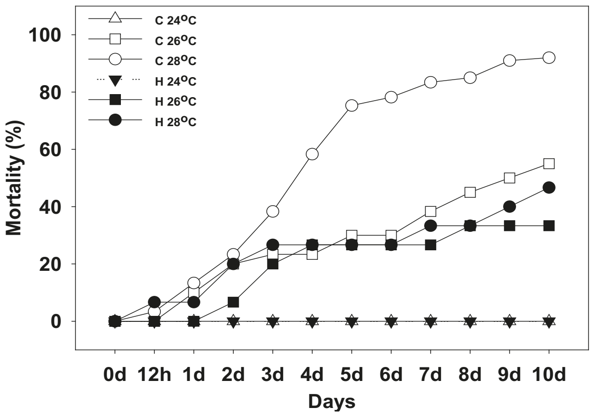

Figure 1. Effect of water temperature (24, 26 and 28 °C) on the mortality of heat hardened (H) and non-hardened (C) *Mytilus galloprovincialis* compared to control at 18 °C.

Both *CuSOD* and *MnSOD* mRNA expression of hardened mussels (H) are increased in all elevated temperatures (24, 26 and 28°C) having peak in the first 12h and 1day and then dropping until 10 day but remaining statistically higher compared to non-hardened (C) mussels and those kept at 18 °C (Fig 2a-f). Although nonhardened mussels depict an increase of *CuSOD* and *MnSOD* mRNA expression, the transcript levels remained considerably lower than in the hardened mussels. On the other hand, a faster and higher SOD enzymatic activity is represented in hardened mussels compared to nonhardened mussels in all elevated temperatures (Fig 2g-i).

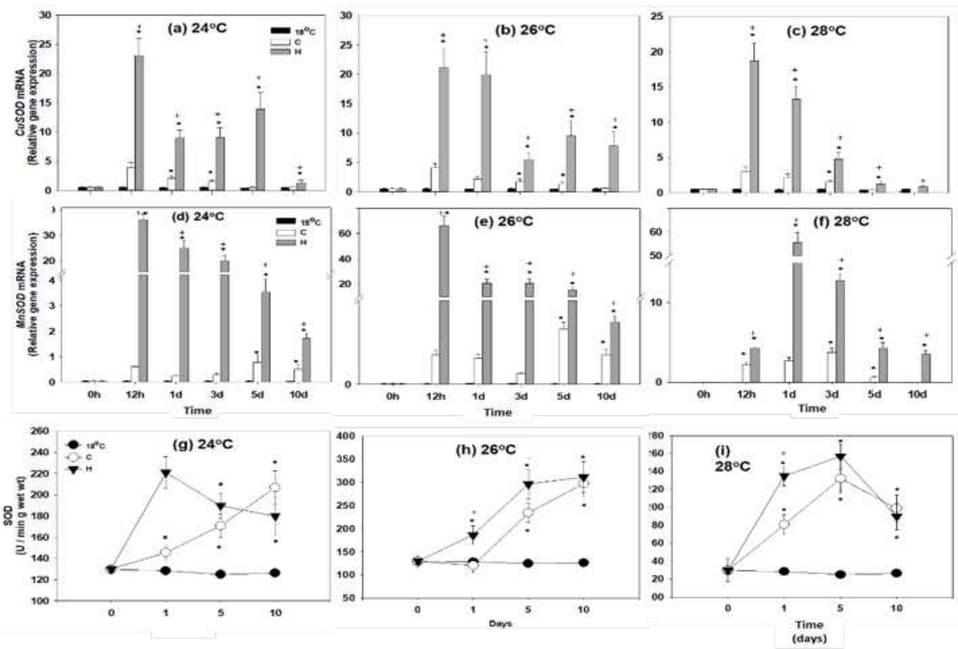

Figure 2. Changes in the relative *CuSOD* and *MnSOD* mRNA levels and SOD enzymatic activity in the mantle of heat hardened (H) and non-hardened (C) *Mytilus galloprovincialis* when exposed to 24 °C (a,d,g),

26 °C (b,e,h) and 28 °C (c,f,i) compared to control at 18 °C. Values are means ± SD, n = 8 preparations from different animals. *p < 0.05 compared to day 0, +p < 0.05 compared to non-hardened (C) mussels.

In the hardened (H) mussels, *CAT* mRNA expression increased significantly above the baseline levels after 12 h of exposure at temperatures beyond 24 ºC and remained strongly elevated until day 10 (Fig. 3a–c), while in the non-hardened (C) mussels, transcriptional upregulation of *CAT* occurred slower and/or to a lesser degree than the hardened individuals. Parallel with the mRNA expression, in both hardened and non-hardened mussels, CAT activity during heat exposure was higher than the baseline measured in the mussels at 18 °C, with higher CAT activity in the hardened compared to the non-hardened mussels (Fig. 4d–f).

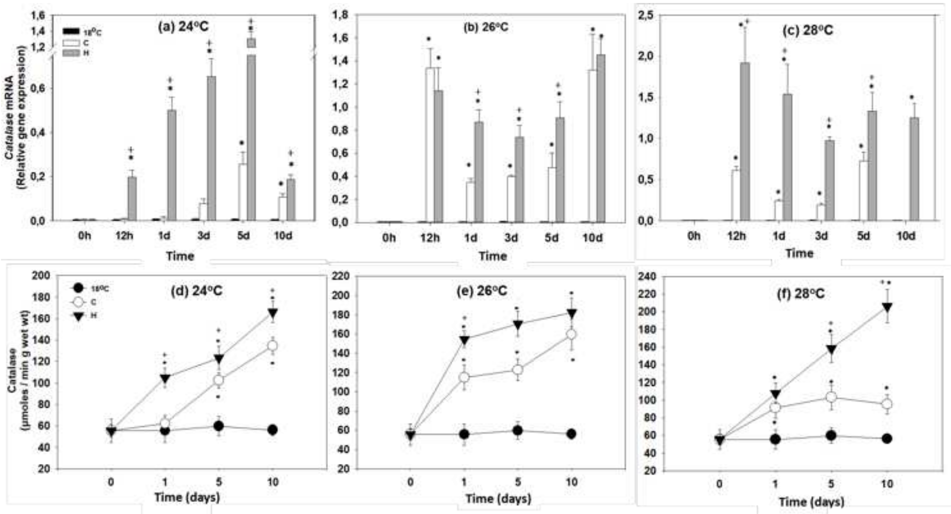

Figure 3. Changes in the relative *CAT* mRNA levels and CAT enzymatic activity in the mantle of heat hardened (H) and non-hardened (C) *Mytilus galloprovincialis* when exposed to 24 °C (a,d), 26 °C (b,e) and 28 °C (c,f) compared to control at 18 °C. Values are means ± SD, n = 8 preparations from different animals. *p < 0.05 compared to day 0, +p < 0.05 compared to non-hardened (C) mussels.

In Fig. 4a-c is depicted that *GST* mRNA expression of hardened (H) mussels strongly increased after 12 h of exposure to elevated temperatures. In the non-hardened mussels. Also, a significant transcriptional upregulation of *GST* above the baseline levels was observed in nonhardened mussels, but they remain significant lower compered to hardened ones. Like the other antioxidant enzymes, GST activity increased in hardened (H) and non-hardened (C) mussels during exposure to elevated temperatures compared with baseline levels with the first ones having higher activity during the exposure (Fig. 4d-f).

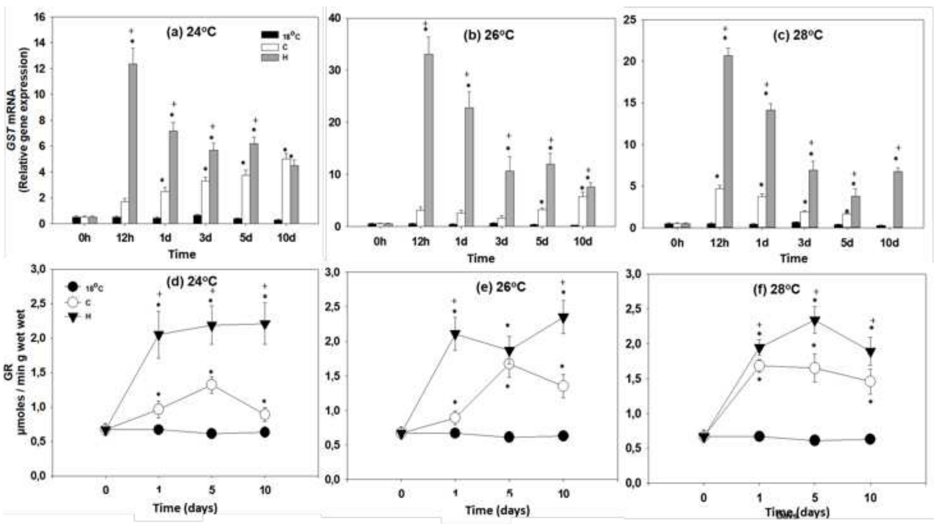

Figure 4. Changes in the relative *GST* mRNA levels and GR enzymatic activity in the mantle of heat hardened (H) and non-hardened (C) *Mytilus galloprovincialis* when exposed to 24 °C (a,d), 26 °C (b,e) and

28 °C (c,f) compared to control at 18 °C. Values are means ± SD, n = 8 preparations from different animals. *p < 0.05 compared to day 0, +p < 0.05 compared to non-hardened (C) mussels.

4. Discussion

Mitochondrial thermal stress commonly suppresses OXPHOS and ETS activity and elevates electron leak resulting in ROS production. These changes can result in energy deficiency due to the mismatch between cellular ATP demand and mitochondrial ATP generation, and lead to oxidative injury if the antioxidant system cannot cope with the increased ROS production (Abele *et al*. 2002; Sokolova 2018). In hardening the increase of expression and activity of antioxidants may reflect thermal acclimation. The early gene expression of antioxidant enzymes may contribute to the dynamic equilibrium between ROS production and detoxification. The expression of both *CuSOD* and *MnSOD* genes indicates that both forms are involved in the mitochondrial reduction of superoxide ($O^{2.-}$) to hydrogen peroxide (H_2O_2), which is then detoxified by CAT, while GR detoxifies endogenous compounds, including peroxidized lipids. Furthermore, we should point out that the beneficial effects of hardening seem to take place after the third day of exposure to sublethal temperatures, a fact coinciding well with the deceleration in the mortality rate of mussels. Consequently, the question raised is which cellular mechanisms are involved in the increase of thermal tolerance in the hardened mussels when exposed to subthelal temperatures beyond 26 °C. First, the more efficient (faster and higher) antioxidant defense against increased ROS production in heat hardened compared to non-hardened mussels could be partly the answer to the above question. In both cases (non-hardened and hardened mussels), all genes for antioxidant enzymes indicate a strong response.

However, the enzymatic activities seem to exhibit different kinds of responses. Specifically, SOD total enzymatic activity exhibited a faster but not stronger response. On the contrary, CAT and GR exhibited faster and stronger responses, indicating a higher capacity in hardened mussels for scavenging redundant ROS compared to nonhardened.

5. References

Abele, D., Heise, K., Portner, H.O., Puntarulo, S. (2002). Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. Journal of Experimental Biology. 205(13), 1831–1841.

Anestis A., Lazou A., Portner H.O., Michaelidis B. (2007). Behavioral and metabolic, and molecular stress responses of marine bivalve Mytilus galloprovincialis during long-term acclimation at increasing ambient temperature. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 293, R911–R921

Bilyk K.T., Clive, W.E., DeVries, A.L. (2012). Heat hardening in Antarctic notothenioid fishes. Polar Biology. 35, 1447-1451.

Bowler, K. (2005). Acclimation, heat shock and hardening. Journal of Thermal Biology. 30, 125-130.

Bruce, T.J., Matthes, M.C., Napier, J.A., Pickett, J.A. (2007). Stressful "memories" of plants: evidence and possible mechanisms. Plant Scinces. 173(6), 603-608.

Feidantsis, K., Giantsis, I.A., Vratsistas, A., Makri, S., Pappa, A.Z., Drosopoulou, E., Anestis, A., Mavridou, E., Exadactylos, A., Vafidis, D., Michaelidis, B. (2020). Correlation between intermediary metabolism, Hspgene expression, and oxidative stress-related proteins in long-term thermal-stressed Mytilus galloprovincialis. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. l319, R264–R281

Hochachka, P.W., Somero, G.N. (2002). Biochemical adaptation: mechanism and process in physiological evolution. Oxford, New York. Oxford University Press

Jaskiewicz, M., Conrath, U., Peterhänsel, C. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Reports. 12(1), 50-55

Pörtner, H.O., Farrell, A.P. (2008). Ecology: Physiology and climate change. Science 322, 690–692

Pörtner, HO (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology. 213, 881–893.

Portner, H.O. (2021). Climate impacts on organisms, ecosystems and human societies: Integrating OCLTT into a wider context. Journal of Experimental Biology. 224, jeb238360.

Precht, H. (1973). In: Precht H, Christophersen J, Hensel H, Larcher W, (Eds), Temperature and Life. pp 302–348.

Sokolova, I.M. (2018). Mitochondrial Adaptations to Variable Environments and Their Role in Animals' Stress Tolerance. Integrative and Comparative Biology. 58(3), 519-531.

Sokolova, I.M. (2021). Bioenergetics in environmental adaptation and stress tolerance of aquatic ectotherms: linking physiology and ecology in a multi-stressor landscape. Journal of Experimental Biology. 224(1).

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LENGTH-WEIGHT RELATIONSHIPS AND NEW FISHERY DATA OF THE THREATENED EUROPEAN SPINY LOBSTER *Palinurus elephas* (J.C. FABRICIUS, 1787) FROM THE REMOTE PSARA-ANTIPSARA ISLANDS, NORTH-EAST AEGEAN SEA, GREECE.

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Abstract
The European spiny lobster *Palinurus elephas* is a threatened fishery resource, classified by IUCN as "Vulnerable", yet it is considered as data-poor species and studies from the eastern Mediterranean sub-basin are almost absent. The current study presents new data regarding the species' allometric growth and its fishery from Psara and Antipsara islands, northeast Aegean Sea, Greece. The sampling period was conducted from March to August 2018. Sampling frequency was 15 days approximately, and trammel nets of 30-36 mm mesh size were used. In total, 63 individuals were collected, and morphometric parameters (Carapace Length, Carapace Width, Weight) were measured. Further data regarding the local spiny lobster fishery were obtained by questionnaires and interviews. The species' allometric profile was negative. Also, all fishermen are operating within a depth range, close to the maximum known depth that the species occurs.
Keywords *Decapoda, Threatened species, Animal growth, east Mediterranean Sea.*
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1. Introduction

The European spiny lobster *Palinurus elephas* (Fabricius, 1787), is a threatened species that occurs and harvested in Mediterranean and adjacent Atlantic waters from North Africa to Scotland, UK (Groeneveld *et al.* 2013). The European spiny lobster is classified from IUCN as "Vulnerable" mainly due to its continuous overfishing (Goñi, 2014). The relative fisheries data are scarce and usually local or regional. In the Mediterranean region it is considered a delicacy and it can be sold as much as 120 €/kg (Kampouris *et al.* 2020). However, spiny lobster supports only a peripheral fishery, and it is considered as a bycatch species for more than 100 finfish fisheries (Goñi & Latrouite 2005; Groeneveld *et al.* 2013). During 1960-1970 the main fishing gear used was traps, pots, and diving, later there was a gradual, yet significant, gear change from pots and traps to netting, resulting at the increase of fishing pressure. Nowadays, netting is the main fishing gear used (Goñi & Latrouite, 2005; Groeneveld *et al.* 2013) but see Kampouris *et al.* (2020) for further details.

The species occurs in waters from one to 200 m of depth, preferring hard substrates (Groeneveld *et al.* 2013). It is a species with limited information available, especially at populations level, with extensive published data reported from the west and central Mediterranean Sea. The available published data, regarding the Greek spiny lobster fishery, are scarce and regional. The current study presents new data relevant at the species' allometric growth and its fishery characteristics from Psara and Antipsara islands, northeast Aegean Sea, Greece.

2. Material and Methods

Study Site and Sampling Methodology

This study took place at the islands of Psara and Antipsara (Figure 1). The sampling period was conducted from March to August 2018, covering the main fishing season. The sampling locations are marked at Figure 1. The sampling frequency was 15 days, approximately. The soak time varied from 5h to 8h. Trammel nets of 36 mm mesh size (knot to knot) were used and, the net's length ranged from 1.5 km to 2.5 km. The fishing depth ranged from 140-180 meters.

In total, 63 specimens were collected, and morphometric measurements were obtained. The measured characters were (1) the Carapace Length (CL), (2) the Carapace Width (CW), (3) the wet weight (W). Length and width were measured using a digital calliper with an accuracy of 0.5 mm and wet weight was measured with an electronic scale with an accuracy of 1g, for further details see Kampouris *et al.* (2020).

Figure 1. The study area of Psara-Antipsara complex, marked (1-7) are the sampling locations of the present study.

Questionnaires and Interviews
Personal interviews were conducted with five coastal fishermen, representing the 100%, that are actively involved with the local spiny lobster fishery. The questionnaires follow the methodology of Kampouris *et al.* (2020).

3. Results
Fishermen questionnaires
The average age of the fishermen involved with the local spiny lobster fishery at Psara-Antipsara was 53.8 years and their average fishing experience was 27.4 years. Their fishing vessels had an average length of 9.16 m and their crew numbered from one (1/5) to two (4/5) people, including the skipper. Wooden (3/5) or plastic (2/5) vessels are used at the local fishery. The horsepower in average was 20.4 HP. The exclusive fishing gear used was trammel nets. The length of nets ranged from 1 km to 2.5 km, while the mesh size measured knot to knot, ranged from 30-36 mm. The fishermen at the study area do not perform long fishing excursions, since they travel 0.5-4 nm to reach their fishing grounds. The average soaking time is 6 h. The fishing depth ranged from 140 to 180 m (Table 1).

Table 1. Spiny lobster fisheries characteristics at Psara-Antipsara islands based on questionnaires and interviews.

Fishermen Demographics					
	F1	F2	F3	F4	F5
Age (in 2018)	35	67	45	62	60
Fishing experience	17	40	20	25	35
Fishing days per month	15	17	15	20	16
Fishing vessel and gear					
Vessel length	8.95	9.95	9.5	9.9	7.5
Crew number	2	2	2	2	1
Vessel material	wooden	plastic	wooden	plastic	wooden
Vessel horsepower	30HP	30HP	20HP	11.03HP	11.03 HP
Gear type	trammel nets	trammel nets	trammel nets	trammel nets	trammel nets
Net length	1 km	1.5 km	2.5 km	2.5 km	1 km
Mesh size (knot to knot)	30-36 mm	30-36 mm	30-36 mm	30-36 mm	30-36 mm
Fishery Characteristics					
Distance from ports nm	1-4 nm	2-4 nm	0.5 nm	1-4 nm	0.5 nm
Soak time	7 h	5 h	6 h	7 h	5 h
Effective fishing hours	morning	morning	whole day	whole day	morning
Fishing depth (m)	150	140	180	150	160

Morphometrics

Overall, 63 specimens were caught, the average weight was 542.62 g (SD=172.43 g), the average Carapace width (CW) was 75.89 mm (SD=15.63 mm), and the average Carapace Length (CL) was 144.38 mm (SD=30.13 mm) (Table 2). The species' allometric profile was significantly negative allometric (Table 3).

Table 2. **Minimum, Average (Standard Deviation) and Maximum values of CL, CW and W, regarding the measurements of spiny lobsters from Psara-Antipsara islands.**

Parameter	**Carapace Length (CL) (mm)**	**Carapace Width (CW) (mm)**	**Weight (W) (g)**
Minimum	91.41	47.65	341.56
average (SD)	144.38 (30.13)	75.89 (15.63)	542.62 (172.43)
Maximum	215.63	109.63	1255.12

Table 3. **Morphometric relationships' parameters and the allometric profile of Psara-Antipsara islands spiny lobsters.**

CL-W parameters			**CW-W parameters**		
a	*b*	r^2	*a*	*b*	r^2
1.15	0.25	0.86	1.11	0.64	0.78
Allometric profile			**Allometric profile**		
Negative allometric			Negative allometric		

4. Discussion

The spiny lobster is a poorly known species, especially in eastern Mediterranean sub-basin, since relevant studies are almost absent (Goñi & Latrouite 2005; Groeneveld *et al.* 2013). The current study is the first that presents quantitative data regarding the allometric growth of the species from the remote island complex of Psara-Antipsara. Our data were in accordance with all the known earlier studies either form Mediterranean (Tidu *et al.* 2004; Goñi & Latrouite 2005 and references within, Kampouris *et al.* 2020) or from east Atlantic waters (Goñi & Latrouite 2005 and references within).

The local spiny lobster fishery is conducted by a low number of fishermen, all of which were interviewed during the present study. The experience and in-depth knowledge of fishermen is a valuable source of information and wider, long-standing collaborations should be established (e.g., Azzurro *et al.* 2019). A striking finding of the present study was that the spiny lobster fishing is taking place within a narrow depth range (140-180 m), close to the maximum known depth that the species occurs (200 m).

Spiny lobsters have been characterised as keystone species (Eddy *et al.* 2014), as they act as good bioindicators, and their natural history help to quantify wide changes in their habitats, if continuous monitoring occurs (Rowland *et al.,* 2018).

The available published fisheries data off Greek waters are quite limited and sporadic and most of the existing studies regard finfish fisheries (Sini *et al.* 2017). Perhaps, the lobster species that is most studied, in Greek waters, is the Norway lobster (e.g., Tsikliras *et al.* 2013), yet still it could be assumed that the species and the fishery are data limited. Studies like the current are valuable, especially when dealing with species such as the spiny lobster, even though that some data based on fishermen interviews might not be so accurate.

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5. References

Azzurro E., Bolognini L., Dragičević B., Drakulović D., Dulčić J., Fanelli E., Grati F., Kolitari J., Lipej L., Magaletti E., Marković O., Matić-Skoko S., Mavrič B., Milone N., Joksimović A., Tomanić J., Scarpato A., Tutman P., Vrdoljak D., Zappacosta F. (2019). Detecting the occurrence of indigenous and non-indigenous megafauna through fishermen knowledge: A complementary tool to coastal and port surveys. Marine Pollution Bulletin 147, 229-236.

Eddy T.D., Pitcher T.J., MacDiarmid A.B., Byfield T.T., Tam J.C., Jones T.T., Bell J.J., Gardner J.P.A. (2014). Lobsters as keystone: Only in unfished ecosystems? Ecological Modelling 275, 48-72.

Goñi R. (2014). *Palinurus elephas*. The IUCN Red List of Threatened Species. Version 2015.2. http://www.iucnredlist.org (accessed: 3-6-2021).

Goñi, R., Latrouite D. (2005). Review of the biology and fisheries of *Palinurus spp*. Species of European waters: *Palinurus elephas* (Fabricius, 1787) and *Palinurus mauritanicus* (Gruvel, 1911). Cahiers de Biologie Marine 46(2), 127-142.

Groeneveld J.C., Goñi R., Díaz D. (2013). *Palinurus* species. In: "Lobsters: Biology, management, aquaculture and fisheries second edition", Phillips B.F. (ed). Wiley-Blackwell, p. 326-356.

Kampouris T.E., Koutsoubas D., Milenkova D., Economidis G., Tamvakidis S., Batjakas I.E. (2020). New Data on the Biology and Fisheries of the Threatened *Palinurus elephas* (Fabricius, 1787) (Decapoda, Achelata, Palinuridae) from the North-West Aegean Sea, Greece. Water 12(9), 2390.

Rowland J.A., Nicholson E., Murray N.J., Keith D.A., Lester R.E., Bland L.M. (2018). Selecting and applying indicators of ecosystem collapse for risk assessments. Conservation Biology 32(6), 1233–1245.

Sini M., Katsanevakis S., Koukourouvli N., Gerovasileiou V., Dailianis T., Buhl-Mortensen L., Daalas D., Dendrinos P., Dimas X., Frantzis A., Gerakakis V., Giakoumi S., Gonzalez-Mirelis G., Hasiotis T., Issaris Y., Kavadas S.G., Koutsogiannopoulos D.D., Koutsoubas D., Manoutsoglou E., Markantonatou V., Mazaris A.D., Poursanidis D., Papatheodorou G., Salomidi M., Topouzelis K., Trygonis V., Vassilopoulou V., Zotou M. (2017). Assembling Ecological Pieces to Reconstruct the Conservation Puzzle Aegean Sea. Frontiers in Marine Sciences 4**,** Article 347.

Tidu C., Sardá R., Pinna M., Cannas A., Meloni M.F., Lecca E., Savarino R. (2004). Morphometric relationships of the European spiny lobster *Palinurus elephas* from northwestern Sardinia. Fisheries Research 69(3), 371-379.

Tsikliras A.C., Tsiros V.Z., Stergiou K.I. (2013). Assessing the state of Greek marine fisheries resources. Fisheries Management and Ecology 20, 34-41.

AGE, GROWTH AND FEEDING HABITS OF THE BLACK GOBY, *Gobius niger*, IN CANDARLI BAY, AEGEAN SEA

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Abstract
Age, growth and diet of black goby, *Gobius niger* L., 1758, were determined from 681 specimens collected in Candarli Bay. Total length ranged from 6.2 and 15.9 cm. Age was determined by otolith readings, where a maximum age of 5 years was observed. The von Bertalanffy growth parameters were estimated as L_∞= 17.11 cm, k= 0.323 yr^{-1}, t_0= -1.676. Stomach contents were mainly Mollusca (%IRI=54.21), Crustacea (%IRI= 36.22), Polychaeta (%IRI= 7.72), Foreminifera (%IRI= 1.40) and Teleostei (%IRI= 0.45).
Keywords: *Age and growth; feeding; black goby; Gobius niger; Aegean Sea*
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Introduction

In most parts of the Mediterrenean Sea, black goby (*Gobius niger* L., 1758) is a common species of the bottom trawl catch composition. The black goby has no commercial value primarily due to its small size, and generally becomes part in the discarded catch. As a consequence, knowledge of its biology and ecology are limited (Filiz & Togulga 2009 and reference therein). The species is not consumed as food, yet due to its high proportion in the bottom trawl discard, which contributed to its habitat loss and pollution, the black goby population has evaluated as Least Concern (LC) in Turkey (Fricke *et al*. 2007), thus making any biological data that we could possess of great importance. In this study, information on the age, growth and feeding habits of *G. niger* are presented, based on material collected in Candarli Bay, Aegean Sea.

Material and methods

Candarli Bay was sampled monthly between March 2003 and February 2004. A total of 681 specimens obtained via trawl (n= 68), and fishing line (n= 613). Trawl specimens were sampled by R/V EGESUF (27 m length and 463 HP). A conventional bottom trawl net of 22 mm cod-end mesh size was used and three hauls in same day were carried out and haul durations was stabilized 30 minutes. The vessel speed was maintained at 2.5 knots. Depth of the fishing ground was ranged between 36 and 94 m. Fishing line sampling carried out in first, middle and last weeks of every month. A fishing line named "sinek oltasi" was used (no. 18 needle, 0.20 mm main body and 25 g lead weight). Fish were captured in the Candarli Bay (where they were abundant) in five locations between 09:00 and 12:00 a.m. All specimens were injected in the abdomen region with 4% alcohol and were frozen. Total lengths (TL, cm) were measured to the nearest 0.01 cm.

Sagittal otoliths were removed, cleaned, dried and stored in labeled plastic tubes. For age determination, otoliths were examined under a reflected light stereoscope (x14 magnification). The otoliths were read by three readers and when there was no agreement among the three readings, the otolith was excluded. Age was expressed in years, the birthday of the fish being considered to be 1st January. For the estimation of the individual growth rate, the von Bertalanffy growth equation (VBGE) was calculated. The growth performance index (Φ′, phi-prime) was employed to compare growth rates.

Stomachs were removed from 269 specimens, prey items were identified to group level, measured, counted and weighed on an electronic balance (precision 0.0001 g). Diet composition was evaluated using three measures described by Hyslop (1980): the numerical index (%N); the gravimetric index (%W), and frequency of occurrence (%F). Based on Cortes' (1997) suggestion, the index of relative importance (IRI) was calculated and expressed as a percentage (%IRI). Subsequently, food items were grouped into categories of preference using the method proposed by Morato et al. (1998) and described by Sever et al. (2008).

Results & Discussion

Total length ranged from 6.2 and 15.9 (10.05, SD=1.90) cm. Among the 681 black goby which were otolith's taken, 48 otoliths could not be aged since both they were unreadable (n= 44) and there was no agreement between readers (n= 4). Thus, 633 were used for direct reading on otoliths.

Age determination from direct observations on the otoliths resulted in the establishment of six age groups (0, I, II, III, IV and V) for the population sampled. Age group I and II included 48.0% and 22.9% of the population, respectively. Age group V was consisted of only males (Table 1). The maximum age reached by specimens of black goby (i.e. 5 years) from the Candarli Bay is within the longevity limits observed over the biogeographical distribution area (Table 1). Indeed, if black goby's longevity (5 year) is similar in the Adriatic (Fabi & Giannetti 1985) and the Norwegian coasts (Nash 1984), while the life span seems to be shorter (2-3 years) in the Atlantic lagoons and estuaries. From these results, it can be concluded that the populations living in the Aegean and Adria tic Sea are the ones that reach the maximum age (5) and close the maximum length ever recorded (16.5 cm).

In this study, growth rate of the population has been found relatively low. However, the growth coefficient is highly variable among different studies (k= 0.19-0.91) (Table 2). L∞ estimated in this study was within the observed total length of males and females. Although there were observed differences, no significant differences were found among my Φ value and ones obtained for Mediterranean and Atlantic from previous studies ($p > 0.05$). In the Candarli Bay, the diet of black goby is based on small benthic invertebrates, generally similar to that of other populations (Table 3). However, foraminifera comprised 1.40% of the diet, possibly ingested accidentally, together with the animal constituents of the diet.

Table 1. Comparison of maximum age and age at lengths records.

Area	Study	Locality	Sex	0+	I	II	III	IV	V
Mediterranean	Fabi & Giannetti (1985)	Adriatic Sea	♂	7.7	9.4	11.9	13.5	14.5	15.5
			♀	6.2	7.8	9.5	10.4	11.8	
	Joyeux *et al*. (1991)	Mauguio Lagoon	♂	-	8.8-9.6	9.6-12.0	12.0-13.2	13.6	-
			♀	-	8.4-9.2	9.2-11.6	11.6-12.4	-	-
	Rasotto & Mazzoldi (2002)	Venetian Lagoon	♂	-	7.4	10.1	12.2	12.8	-
	Filiz & Togulga (2009)	Izmir Bay	♂	8.18	10.34	11.93	13.29	14.14	14.78
			♀	6.67	8.10	9.90	11.33	12.26	
			♂+♀	7.76	9.76	11.52	12.96	13.81	14.78
	This study	Candarli Bay.	♂	7.81	10.07	11.82	13.41	14.29	15.13
			♀	7.36	8.13	10.05	11.39	12.25	
			♂+♀	7.65	9.90	11.90	13.30	14.40	15.13
Atlantic	Vaas *et al*. (1975)	Verse Meer Lake	♂	5.5	8.2	9.5	12.0		
			♀	5.5	8.1	9.6	10.5	11.1	
	Nash (1984)	Norwegian coasts	♂+♀	-	4.4	7.1	8.6	9.6	9.3
	Vesey & Langford (1985)	Stanswood Bay	♂+♀	~3	5.6	9.0	10.9	-	-
	Doornbos & Twisk (1987)	Grevelingen Lake	♂+♀	4.7	8.0-8.5	12.2-12.5	-	-	-
	Arruda *et al*. (1993)	Ria de Aveiro Lagoon	♂	7.6	10.8	11.8	-	-	-
			♀	7.2	10.5	11.5	-	-	-
	Silva & Gordo (1997)	Obidos Lagoon	♂	7.8	10.5	12.2	13.5	-	-
			♀	8.0	10.3	11.9	12.0	-	-

Table 2. Comparisons of growth parameters

Area	Study	Sex	L_∞ (cm)	*k* (year^{-1})	t_0 (year)	t_{max}^{A}.	Φ' ^{B}	Locality
Mediterranean	Fabi & Giannetti (1985)	♂	18.52	0.30	-1.689	10.1	2.01	Adriatic Sea
		♀	16.58	0.19	-2.571	15.7	1.72	
	Froese & Pauly (2021)	♂	18.5	0.30		10.0	2.01	Adriatic Sea
		♀	16.9	0.19		15.8	1.73	
	Filiz & Togulga (2009)	♂	16.69	0.30	-2.205	10.0	1.92	Izmir Bay
		♀	14.84	0.32	-1.459	9.3	1.85	
		♂+♀	17.59	0.26	-2.174	11.7	1.90	
	This study	♂	17.62	0.28	-2.053	10.7	1.94	Candarli Bay

		♀	14.10	0.39	-1.198	7.7	1.89	
		♂+♀	17.11	0.32	-1.676	9.4	1.98	
Atlantic	Vesey & Langford (1985)	♂	11.7	0.91	0.32	3.3	2.10	Stanswood Bay
		♀	15.1	0.91	0.32	3.3	2.32	
	Silva & Gordo (1997)	♂+♀	16.66	0.34	-1.910	8.9	1.97	Obidos Lagoon

[A] t_{max}(life-span)= based on 3/k assumption (according to Froese & Binohlan, 2000)
[B] Φ' = log$k$+2 log L_∞

Table 3. Food items of different populations of *G. niger*. (M): Mediterranean; (A): Atlantic

Study	Locality	Method used	Food Items
Casabianca & Kiener (1969)	Corsica coasts (M)	%N	Mollusca, Crustacea
McGrath (1974)	Baltic Sea (A)	%N	Crustacea
Vaas *et al*. (1975)	Verse Meer Lake, Holland	%N	Polychaeta, Crustacea, Mollusca, Teleost
Fabi & Froglia (1983)	Adriatic (M)	%O	Crustacea, Polychaeta
Fjosne & Gjosaeter (1996)	Norwegian coasts (A)	%IRI	Crustacea (80.8%), Teleost eggs/larvae (13.0%), Polychaeta (6.2%)
Labropoulou & Markakis (1998)	Heraklion Bay (M)	%IRI	Crustacea (65.1%), Polychaeta (39.9%), Cumacea
Labropoulou & Papadopoulou-Smith (1999)	Heraklion Bay (M)	%IRI	Polychaeta
Stergiou & Karpouzi (2002)	Mediterranean	%IRI	Polychaeta (46.0%), Crustacea (49.0%), others
Froese & Pauly (2021)	----	%IRI	Crustacea, Mollusca, Teleost
Filiz & Togulga (2009)	Izmir Bay (M)	%IRI	Mollusca (47.53), Crustacea (42.94), Polychaeta (8.26), Foreminifera (1.14), Teleost (0.13)
This study	Candarli Bay (M)	%IRI	Mollusca (54.21), Crustacea (36.22), Polychaeta (7.72), Foreminifera (1.40), Teleost (0.45)

References

Arruda L., Azevedo M., Neto A.I. (1993). Abundance, age-structure and growth and reproduction of gobies (Pisces; Gobiidae) in the Ria de Averio Lagoon (Portugal). Estuarine, Coastal and Shelf Science, 37, 509-523.

Casabianca M.L., Kiener A. (1969). Gobides des etangs corses: systematique, ecologie, regime alimentaire et position dans les chaines trophiques. Vie et Milieu, 20, 611-634.

Cortes E. (1997): A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Canadian Journal of Fisheries and Aquatic Sciences, 54, 726-738.

Doornbos G., Twisk F. (1987). Density, growth, and annual food consumption of gobiid fish in the saline lake Grevelingen, Netherlands. Netherlands Journal of Sea Research, 21, 45-74.

Fabi G., Froglia C. (1983). Food and feeding of *Gobius niger* L. in the central Adriatic Sea (Osteichthyes: Gobiidae). Rapports Commission Internationale pour l'Exploration Scientifique de la Mer Méditerree, 28, 99-102.

Fabi G., Giannetti G. (1985). Groth parameters of the balck goby (*Gobius niger* L.) in the Adriatic Sea, based on otolith readings. Rapports Commission Internationale pour l'Exploration Scientifique de la Mer Méditerree, 29, 87-90.

Filiz H., Togulga M. (2009). Age and growth, reproduction and diet of the black goby, (*Gobius niger*) from Aegean Sea, Turkey. Journal of FisheriesSciences.com 3(3): 243-265.

Fjosne K., Gjosaeter J. (1996). Dietary composition and the potential of food competition between-group cod (*Gadus morhua* L.) and some other fish species in the littoral zone. ICES Journal of Marine Science, 53, 757-770.

Fricke R., Bilecenoglu M., Sari H.M. (2007). Annotated checklist of fish and lamprey species (Gnathostoma and Petromyzontomorphi) of Turkey, including a Red List of threatened and declining species. Stuttg. Beitr. Nat. Kd. A Biol., 706, 1-169.
Froese R., Binohlan C. (2000). Empirical relationships to estimate asymptotic length, length at first maturity, and length at maximum yield per recruit in fishes, with a simple method to evaluate length frequency data. Journal of Fish Biology, 56, 758-773.
Froese R., Pauly D. (2021). FishBase [World Wide Web electronic publication]. Retrieved from http://www.fishbase.org
Joyeux J.C., Bouchereau J.L., Tomasini J.A. (1991). Croissance et structure demographie de la population de *Gobius niger* Linne, 1758. (poission teleosteen) dans uno lagune nord mediterraneene. Cahiers de Biologie Marine, 32, 415-437.
Labropoulou M., Markakis G. (1998). Morphological-dietary relationships within two assemblages of marine demersal fishes. Environmental Biology of Fishes, 51, 309-319.
Labropoulou M., Papadopoulou-Smith K.N. (1999). Foraging behaviour patterns of four sympatric demersal fishes. Estuarine Coastal and Shelf Science, 49, 99-108.
McGrath D. (1974). Preliminary studies on the feeding of *Gobius niger* L. and *Gobius flavescens* (Fabricius) (Pisces, Gobiidae) in the Northern Baltic proper. Contr. Asko Lab., 4, 25 pp.
Morato T.M., Sola E., Gros M.P., Menezes G., Pinho M.R. (1998): Trophic relationships and feeding habits of demersal fishes from the Azores: importance to multispecies assessment. In. International Council for the Exploration of the Sea ICES CM 1998/O: 7, 34 pp.
Nash R.D.M. (1984). Aspects of biology of the black goby, *Gobius niger* L., in: Oslofjorden, Norway. Sarsia, 69, 55-61.
Rasotto M.B., Mazzoldi C. (2002). Male traits associated with alternative reproductive tactics in *Gobius niger*. Journal of Fish Biology, 61, 173–184.
Sever T.M., Filiz H., Bayhan B., Taskavak E., Bilge G. (2008). Food habits of the hollowsnout grenadier, *Caelorinchus caelorhincus* (Risso, 1810), in the Aegean Sea, Turkey. Belgian Journal of Zoology, 138, 81-84.
Silva M.N., Gordo L.S. (1997). Age, growth and reproduction of the black goby, *Gobius niger*, from Obidos Lagoon, Portugal. Cahiers De Biologie Marine, 38, 175-180.
Stergiou K.I., Karpouzi V.S. (2002). Feeding habits and trophic levels of Mediterranean fish. Reviews in Fish Biology and Fisheries, 11, 217-254.
Vaas K.F., Vlasbom A.G., de Koeijer P. (1975). Studies on the black goby (*Gobies niger*, Gobiidae, Pisces) in the Verse Meer, SW Netherlands. Netherlands Journal of Sea Research, 9, 56-68.
Vesey G., Langford T.E. (1985). The biology of the black goby, *Gobius niger* L. in an English south-coast bay. Journal of Fish Biology, 27, 417-429.

ASSESSMENT OF DEMERSAL FISHERY RESOURCES IN PAGASITIKOS GULF (CENTRAL AEGEAN SEA)

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Abstract
Experimental sampling with a commercial bottom otter trawl was conducted in Pagasitikos gulf a semi-enclosed landlocked gulf located in the northwestern part of the Aegean Sea. Six hauls of 30 min duration each, were conducted in the eastern, western, and central part of Pagasitikos gulf, in depths ranging from 60 to 97 meters. In total 32 species were sampled with the highest species number recorded in the western and eastern sampling sites (22 species) followed by the central sampling site (18 species). Abundance, biomass, and diversity indices exhibited minor differences among sampling areas however not statistically significant. SIMPER analysis indicated that species mainly responsible for the similarities in abundance withing sample areas were *Pagellus bogaraveo, Pagellus erythrinus, Trigla lucerna* and *Parapenaeus longirostris*. Hierarchical cluster analysis indicated the presence of two major groups at the east with 80.4% similarity and at the west with 87.4% similarity.

Keywords: Demersal resources, Pagasitikos gulf, SIMPER analysis, cluster analysis, Aegean Sea
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1. Introduction

Pagasitikos gulf is a semi-enclosed landlocked gulf located in the northwestern part of the Aegean Sea (Figure 1a) highly influenced both by anthropogenic activities (inflow of nutrients at the northern and western parts) as well as by water exchange between the gulf and the Aegean Sea at its south part (Trikeri channel) resulting in the development of functional sub-areas within the gulf. Thus, the inner part is characterized by eutrophic conditions with sporadic formation of harmful algal blooms while the central part acts as a buffer with mesotrophic characteristics influenced by the oligotrophic outer area (Petihakis et al. 2003).

According to Caragitsou et al. (2001) about 90 fish species are present in Pagasitikos gulf, many of which are of high commercial importance. Pagasitikos consists of a unique case for Greek fisheries, since it is one of the few areas where fishing with commercial bottom otter trawls is prohibited throughout the year. The closed nature of the gulf further exacerbates the pressure to the commercial fish resources due to the sensitivity of the ecosystem to agricultural, industrial, and anthropogenic pollutants. The aim of this study was to assess the abundance, biomass, and biodiversity of demersal fish stocks in Pagasitikos gulf using a commercial bottom otter trawl.

2. Materials and Methods

Experimental sampling was carried out on 2 consecutive days in May 2021 at depths ranging between 61 and 87 meters, during May 2021 with a commercial bottom otter trawl. In total six hauls of 30 min duration each, were conducted in the eastern, western, and central part of Pagasitikos gulf, in depths ranging from 60 to 97 meters (Figure 1b). During each haul the geographic position and depth at the start and finish were recorded. Numerical abundance for each species was expressed in number of individuals and biomass in kilograms of individuals in one hour trawl. Assessment of the community structure was based on univariate analysis with the use of diversity indices. Diversity was calculated by means of the (log_2) Shannon–Wiener index (Shannon and Weaver, 1949), species richness (R) was calculated after Margalef (1958) and species evenness (E) after Pielou (1977). Multivariate analysis assessed similarities among sample areas using hierarchical cluster analysis based on normalized abundance of sampled species, using a Eucledian distance matrix with complete linkage method performed with Orange software (3.29.3) (Demsar et al. 2013). Data for statistical analysis were evaluated for normal distribution by employing the Shapiro-Wilk test for normality and homogeneity of variance by employing Levene's test. Welch’s ANOVA was employed for comparisons since data were normally distributed but violated the assumption of homogeneity of variance (Krishnamoorthy & Mathew 2007). Statistical analysis was performed with Jamovi Software (2.0.0) (The Jamovi project 2021).The similarity percentage program SIMPER (Clarke 1993) was used to investigate the contributions of individual species to the observed similarities amongst sampling areas.

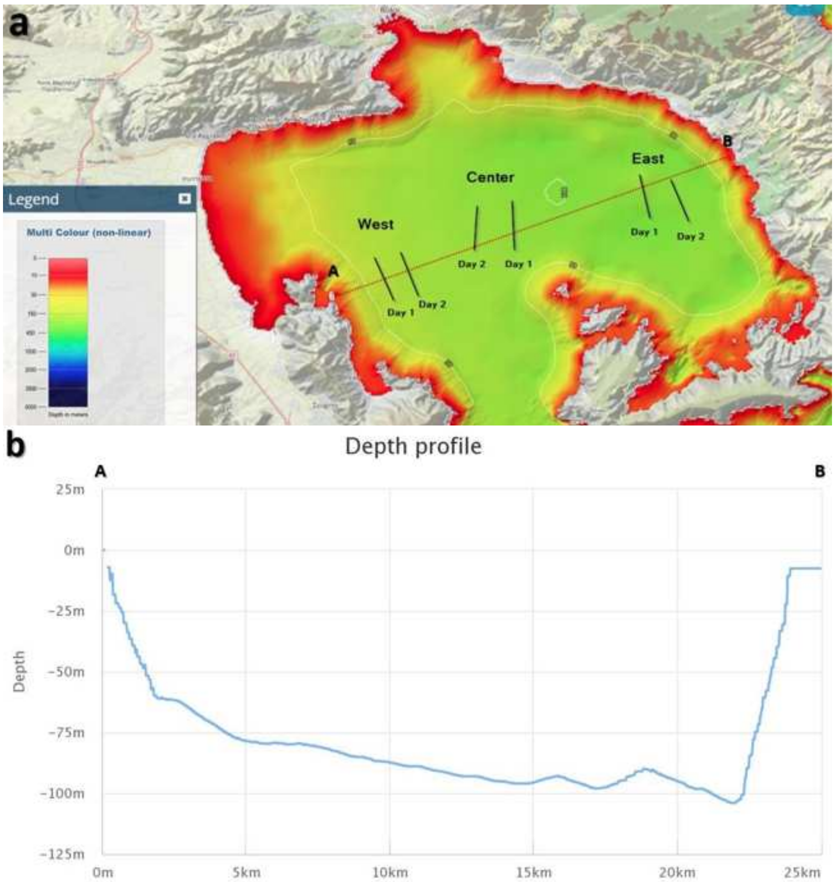

Figure 1. Bathymetric map of Pagasitikos gulf, haul location and timing (a); Depth profile along the transect A-B (b).

3. Results

In total 32 species were sampled including 28 fish species (27 teleostei, 1 chondrichthyes), 3 cephalopods and one crustacean. Highest species number was recorded in the western and eastern sampling sites (22 species) followed by the central sampling site (18 species). Abundance data was visualized in a two-way matrix with values represented by color (the higher a certain value, the darker the represented color) to facilitate the identification of similarities and differences between sites (Figure 2).

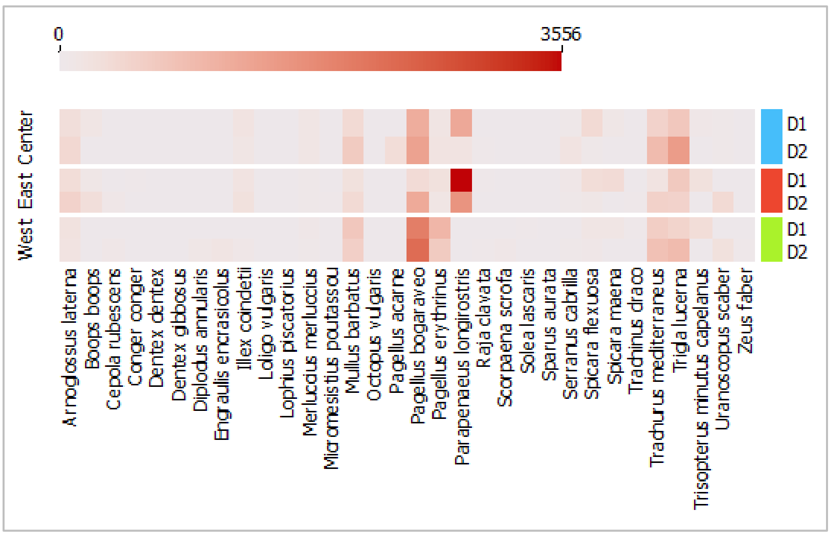

Figure 2. Heat map of the species abundance at each area and sampling day (D).

The major species contributing to the dissimilarity between the eastern and western sampling areas (Figure 2) were: *Parapenaeus longirostris, Pagellus bogaraveo* and *Pagellus erythrinus*. The major species contributing to the dissimilarity between the western and central sampling areas were: *Pagellus bogaraveo, Parapenaeus longirostris, Pagellus erythrinus* and *Trigla lucerna*. The major species contributing to the dissimilarity between the eastern and central sampling areas were: *Parapenaeus longirostris, Pagellus bogaraveo, Trigla lucerna* and *Trachurus mediterraneus*. Numerical abundance (number of individuals per hour trawl) and biomass (kg per hour trawl) were highest at the eastern site followed by western and central sites (Figure 3).

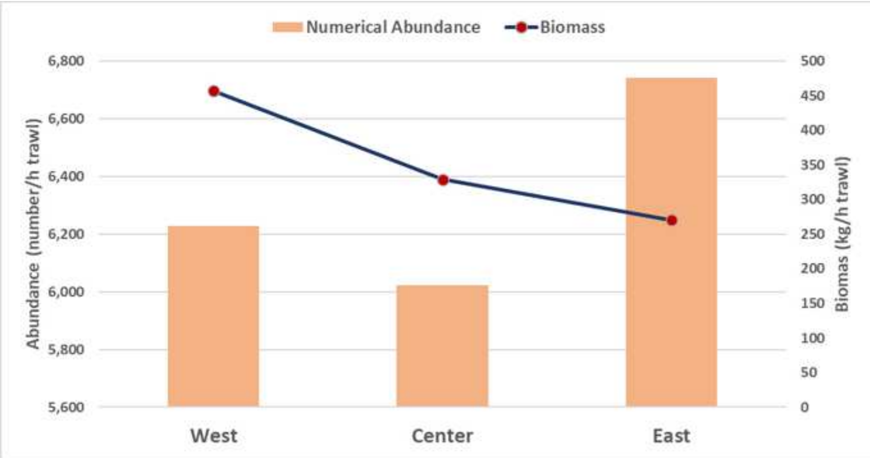

Figure 3. Numerical abundance and biomass at each sampling area.

Table 1. Abundance (%) of major taxa at each sampling site comprising more than 85% of the total abundance (O: Osteichthyes, Ce: Cephalopods, Cr: Crustacea).

Species	Phyllum	Sampling area West	Sampling area Center	Sampling area East
Arnoglossus laterna	O	3.2	5.6	6.2
Illex coindetii	Ce	1.1	2.5	3.3
Mullus barbatus	O	9.9	8.5	4.6
Pagellus acarne	O		5.2	
Pagellus bogaraveo	O	32.9	20.5	11.5
Pagellus erythrinus	O	12.9	2.8	2.8
Parapenaeus longirostris	Cr		11.8	37.1
Spicara flexuosa	O	1.6	3.6	
Spicara maena	O	2.1	1.8	5.1
Trachurus mediterraneus	O	10.9	11.46	5.5
Trigla lucerna	O	10.9	17.6	8.7
Trisopterus minutus capelanus	O	4.3	1.0	4.6
Cumulative abundance (%)		**89.8**	**92.36**	**89.4**

Species richness was higher at the eastern area followed by western and central areas (Figure 4). Species richness is a measure of the number of species present at a site. Sites with more taxa are considered richer, likely to be more ecologically complex and potentially may even be more important from environmental and ecosystem functionality perspectives. Shannon diversity and Pielou's evenness indices exhibited higher values at the central area followed by the eastern and western areas (Figure 4). Species diversity is a measure of how many different types of taxa are present in communities and is widely viewed as a proxy for ecosystem health, resilience, and function. Pielou's evenness measures how abundances are distributed among the species and compares the actual diversity to the maximum possible diversity value. Simpson index exhibited higher values at the central area followed by the western and eastern areas (Figure 4). Simpson index is a measure of diversity with higher values representing higher diversity.

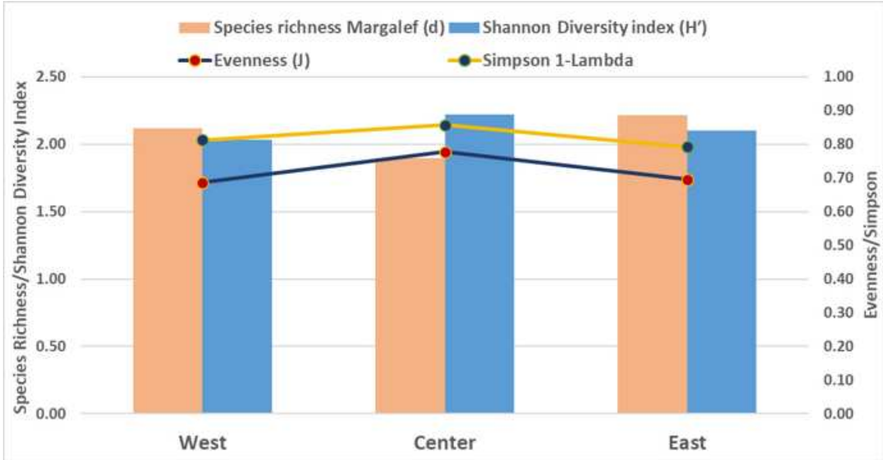

Figure 4. Diversity indices at each sampling area.

The top ranked species responsible for the observed similarities within each sample group, their abundance and contribution are shown in Table 2.

Table 2. Top ranked species responsible for the observed similarities within sample groups, their average abundance (numbers/h trawling), individual contribution (%) and cumulative contribution to the average similarity value.

Species	Average Abundance	Contribution %	Cumulative contribution%
Group Western - Average similarity: 76.58			
Pagellus bogaraveo	2,180	42.56	42.56
Pagellus erythrinus	857	14.05	56.60
Trachurus mediterraneus	724	12.62	69.22
Mullus barbatus	660	12.45	81.68
Group Central - Average similarity: 65.35			
Pagellus bogaraveo	1,302	30.44	30.44
Trigla lucerna	1,117	18.39	48.83
Trachurus mediterraneus	726	13.11	61.94
Mullus barbatus	536	9.96	71.90
Group Eastern - Average similarity: 59.47			
Parapenaeus longirostris	2,600	41.00	41.00
Trigla lucerna	609	12.87	53.87
Pagellus bogaraveo	805	8.88	62.74
Arnoglossus laterna	434	8.38	71.12

Hierarchical clustering of the abundance of sampled species is shown in figure 5. Based on hierarchical clustering, two major groups were identified. A group at the east part of the gulf with 80.4% similarity and a group at the west part with 87.4% similarity.

Figure 5. Hierarchical clustering with complete linkage method of the normalized abundance of sampled species, using a Eucledian distance matrix.

Discussion

Numerical abundance was higher at the western area and lowest at the eastern, whereas biomass exhibited the opposite trend. The most dominant species at the western area were *Pagellus bogaraveo* and *Pagellus erythrinus* with 45.8% of the total numerical abundance. The most dominant species at the eastern area of the gulf were *Parapenaeus longirostris* and *Pagellus bogaraveo* with 48.5% of the total numerical abundance. The most dominant species at the central area of the gulf were *Pagellus bogaraveo* and *Trigla lucerna* with 38.1% of the total numerical abundance. SIMPER analysis indicated that the species mainly responsible for the similarities in abundance withing sample areas were *Pagellus bogaraveo, Pagellus erythrinus, Trigla lucerna* and *Parapenaeus longirostris*. Diversity indices exhibited minor differences between sites, however not statistically significant. Cluster analysis indicated the presence of two major groups, one at the east with 80.4% similarity and one at the west with 87.4% similarity.

An important and worrisome preliminary finding of the present study was the total absence of the Norway lobster (*Nephrops norvegicus*), an over-exploited important commercial resource in the study area (Lolas & Vafidis 2021). According to Smith & Papadopoulou (2003), *Nephrops* density was estimated at 0.64 individuals m^{-2} during May in Pagasitikos gulf. Species environmental preferences depend on a plethora of biotic and abiotic parameters, namely prey abundance, predator avoidance, temperature, salinity, depth, and bottom substrate (Rose & Leggett 1989; Smith et al. 1991; Swain 1993). The geographical distribution of a species can further depend on the population density and may exhibit considerable annual variation (MacCall 1990; Swain & Wade 1993), in addition fish density may vary within a specified area due to the occurrence of gradients in oceanographic parameters (Gunderson 1993).

References

Caragitsou E., Siapatis A., Anastasopoulou A. (2001). Seasonal structure of fish larvae assemblages in the Pagasitikos Gulf (Greece). Rapp Comm Int Mer Médit 36, 250.

Clarke K.R. (1993). Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.

Demsar J., Curk T., Erjavec A., Gorup C., Hocevar T., Milutinovic M., Mozina M., Polajnar M., Toplak M., Staric A., Stajdohar M., Umek L., Zagar L., Zbontar J., Zitnik M., Zupan B. (2013).

Orange: Data Mining Toolbox in Python, Journal of Machine Learning Research 14(Aug), 2349−2353.

Gunderson D.R. (1993). Surveys of fisheries resources. John Wiley and Sons, New York, 248 pp.

Krishnamoorthy K., Lu F., Mathew, T. (2007). A parametric bootstrap approach for ANOVA with unequal variances: Fixed and random models. Computational Statistics & Data Analysis, 51(12), 5731-5742.

Lolas A., Vafidis D. (2021). Population Dynamics, Fishery, and Exploitation Status of *Norway Lobster* (Nephrops norvegicus) in Eastern Mediterranean. Water, 13(3), 289.

MacCall A.D. (1990). Dynamic geography of marine fish populations. University of Washington Press, Seattle, WA. 153 pp.

Margalef D.R. (1958). Information theory in ecology. Gen. Syst. 3, 36–71.

Petihakis G., Triantafyllou G. Theodorou, A. (2003). A time dependent ecosystem operational tool for Pagasitikos gulf. Simulation of biogeochemical variables in space and time. In EGS-AGU-EUG Joint Assembly (p. 3454).

Pielou, E.C. (1977). Mathematical Ecology. John Wiley and Sons, New York.

Rose G.A., Leggett W.C. (1989). Interactive effects of geophysically-forced sea temperatures and prey abundance on mesoscale coastal distributions of a marine predator, Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 46, 1904-1913.

Shannon, C.E., Weaver N. (1949). The Mathematical Theory of Communication. Univ. of Illinois Press, Urbana, Illinois, USA.

Smith, C.J., Papadopoulou, K.N. (2003). Burrow density and stock size fluctuations of *Nephrops norvegicus* in a semi-enclosed bay. ICES Journal of Marine Science, 60(4), 798-805.

Smith, S.J., Perry, R.I., Fanning, L.P. (1991). Relationships between water mass characteristics and estimates of fish population abundances from trawl surveys. Environ Monit Assess 17, 227-245.

Swain, D.P. (1993). Age- and density-dependent bathymetric pattern of Atlantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence. Can J Fish Aquat Sci 50, 1255-1264.

Swain, D.P., Wade E.J. (1993). Density-dependent geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence. Can J Fish Aquat Sci 50, 725-733.

The jamovi project (2021). Jamovi (Version 1.6) [Computer Software]. Retrieved from https://www.jamovi.org

EFFECTS OF ALIEN SPECIES ON THE SUSTAINABILITY OF SMALL-SCALE FISHERIES (A CASE STUDY IN THE ISLAND OF CRETE)

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Abstract
The Island of Crete due to its geographical location receives an influx of Lessepsian migrants across the Suez Canal. Through structured questionnaires using personal interviews, social and demographic data of small-scale coastal fishermen were investigated and the presence of alien species in landings was assessed. Significant lower percentage than expected of young family members exhibiting the intention to follow the fisherman occupation in the future. The majority of fishermen in this case study (60%) are highly dependent on fishing and are thus vulnerable to potential impacts of invasive species. A large number of Lessepsian migrant species in the bycatch indicated a significant effect of their establishment. Multivariate analysis identified three major groups of target species and bycatch. With the use of a logistic regression classification algorithm the principal contributing feature to the presence of target species was identified as the fishing area followed by fishing gear, whereas the principal contributing feature to the presence of bycatch was identified as the fishing gear used followed by fishing area.

Keywords: Alien species, small-scale fishery, sustainability, Lessepsian migration, Island of Crete.
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1. Introduction

The opening of the Suez Canal in 1869 has joined two biogeographic regions resulting in Red Sea species to migrate through the canal to the Mediterranean. These alien species after crossing the Suez Canal and reaching the Mediterranean Sea, experience lower biodiversity and biomass, compared to their endemic environment the Red Sea, facilitating their adaptation and establishment in the Eastern Mediterranean (Galil & Zenetos, 2002).A significant number of alien (Lessepsian) species that have migrated through the Suez Canal have successfully established themselves in the eastern Mediterranean (Saygu et al., 2020). Warming waters in the eastern Mediterranean have facilitated their establishment increasing their abundance and threatening the native fauna (Saygu et al., 2020). The ongoing climate change is expected to exacerbate the rates of invasion (Corrales et al., 2018). This ongoing migration poses a threat to the endemic biodiversity and impacts fisheries.

The main economic and social impacts of invasive alien marine species are negative impacts on human health and decreases in economic production of activities based on marine environments and resources such as fisheries. These effects have related social impacts through decreases in employment, in economic activities directly affected by invasive alien species, but also through decreases in people's welfare from the reduced quality of their environments and natural surroundings (Bax et al., 2003). Though less frequent, alien marine species may also have positive impacts, such as the creation of new economic activities through fisheries and aquaculture and increased employment in invasive alien marine species management projects and programs. However, the net social benefits caused by the introduced marine species are usually negative. The aim of the study was to investigate social and demographic data of a local population of small-scale coastal fishermen, in relation to the Lessepsian migration (Erythrean invasion) and assess potential impacts on the fishery.

2. Materials and Methods

Using personal interviews through structured questionnaires, social and demographic data of small-scale coastal fishermen were investigated and the presence of alien species in their landings was assessed. The study area included the Island of Crete which due to its geographical location, receives an influx of Lessepsian migrants across the Suez Canal. In total 15 sampling locations scattered throughout the island of Crete (9 north and 6 south) were visited and 40 structured questionnaires were completed through personal interviews. Chi-Square Goodness-of-Fit Test and Chi-Square Test for Association were employed to assess the intention of young family members to follow the fisherman occupation in the future (Larson & Farber, 2012). Statistical analysis was performed with Jamovi Software (2.0.0) (The jamovi project, 2021). Multivariate analysis assessed similarities among target species and bycatch through hierarchical cluster analysis based on normalized abundance of sampled species, using a Eucledian distance matrix with complete linkage method performed with Orange software (3.29.3) (Demsar et al., 2013). A logistic regression classification algorithm with ridge regularization was used to compute the contribution of each feature both to the presence of target species and bycatch. Prediction was inferred, by measuring the increase in the prediction error of the model after permuting the feature's values.

3. Results

Chi-Square Goodness-of-Fit Test (Table 1) indicated significant difference between observed and expected intention of young family members to follow the fisherman occupation in the future, with significantly lower percentage (31%) than expected (50%, Tzanatos, 2006).

Table 1. Chi-Square Goodness-of-Fit Test for the intention of children to follow the fisherman occupation.

Fisherman		Number of individuals	Proportion	χ^2 Goodness of Fit		
Yes	Observed	11.00	0.31	χ^2	**df**	**p**
	Expected	18.00	0.50	5.44	1	0.02
No	Observed	25.00	0.69			
	Expected	18.00	0.50			

Chi-Square Test for Association (Table 2) indicated significant difference in the intention of young family members to work as small-scale fishermen in the future, with fishermen families in north Crete exhibiting significantly higher percentage of family members willing to follow the fisherman occupation (42%) compared to the South (8%).

Table 2. Chi-Square Test for Association for the intention of children to follow the fisherman occupation between north and south Crete.

Fisherman occupation		North Crete	South Crete	χ^2 Test for Association		
Secondary	Observed	10.00	0.925	χ^2	**df**	**p**
	Expected	7.33	0.606	4.19	1	0.041
Tertiary	Observed	14.00	0.075			
	Expected	16.67	0.394			

Chi-Square Goodness-of-Fit Test indicated no significant difference between observed and expected (60%, Tzanatos, 2006) number of fishermen, highly dependent on fishing. Furthermore, Chi-Square Test for Association indicated no significant difference in the number of fishermen highly dependent on fishing between north (68%) and south (47%) Crete. The recorded sighting of discarded species in the study group is shown in Figure 1.

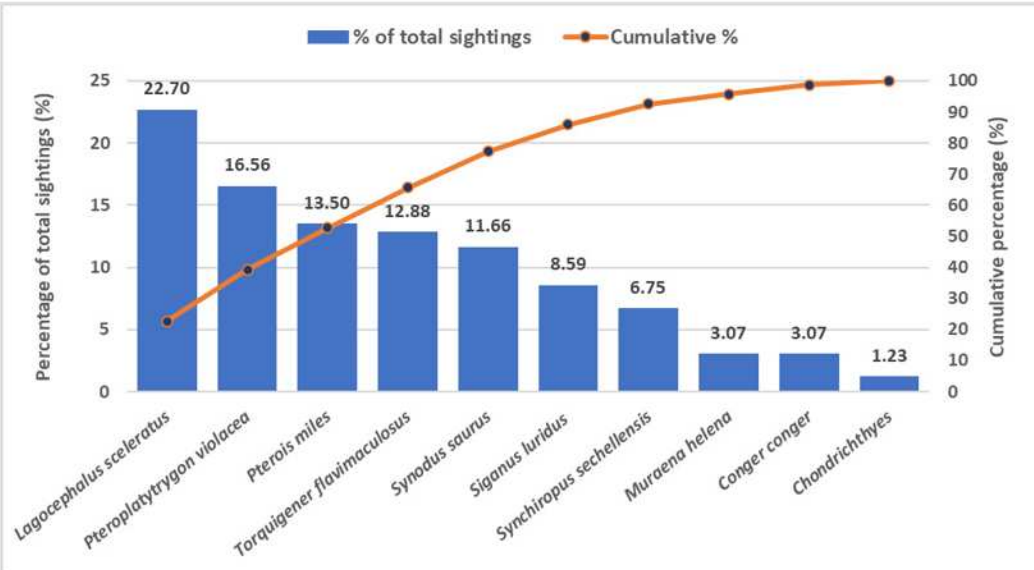

Figure 1. Pareto chart of the recorded sightings of discarded species.

Multivariate analysis, using hierarchical clustering on the sightings of target species (Figure 2) and bycatch (Figure 3), identified three major groups accordingly.

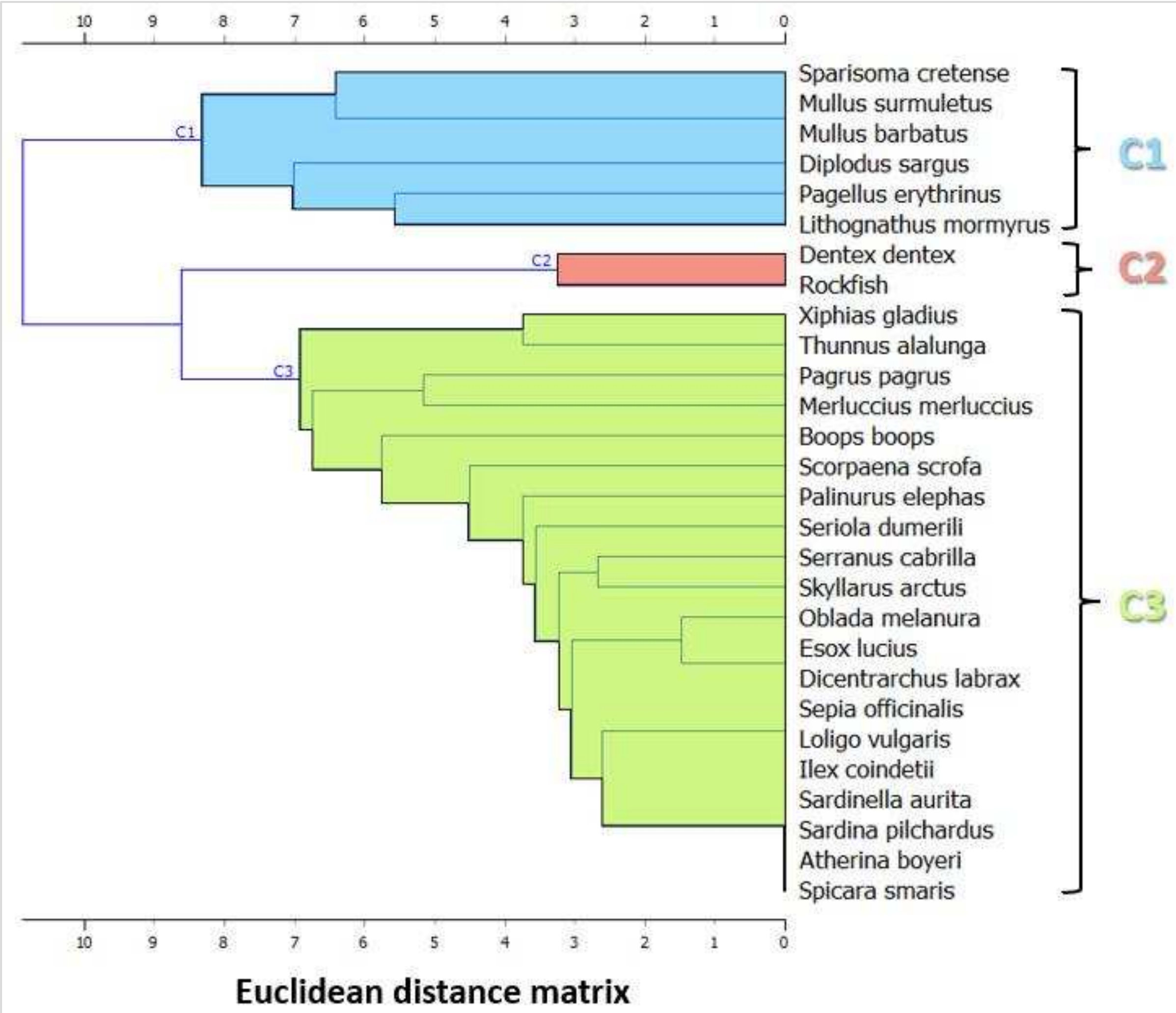

Figure 2. Hierarchical clustering with complete linkage method of the normalized presence of target species, using a Euclidean distance matrix.

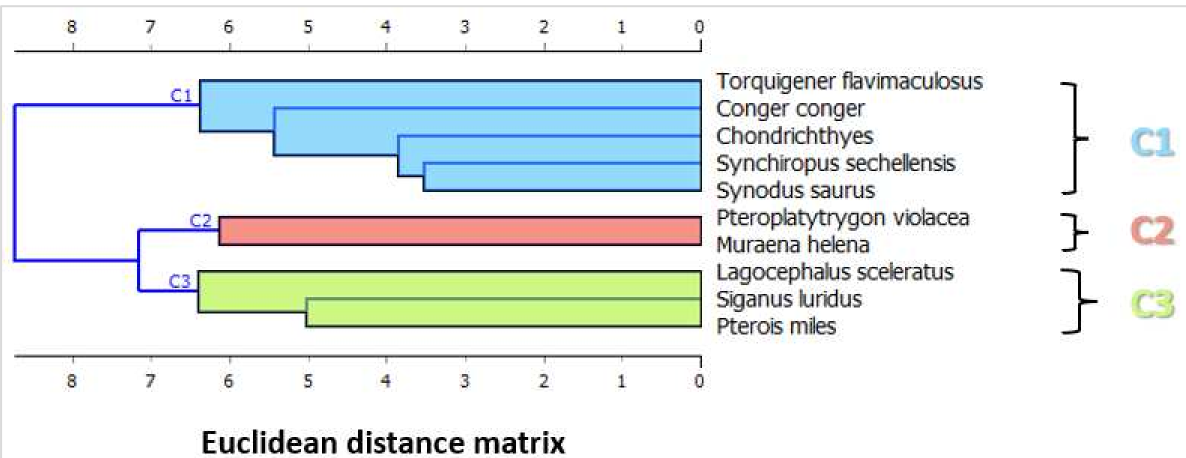

Figure 3. Hierarchical clustering with complete linkage method of the normalized presence of bycatch, using a Euclidean distance matrix.

The logistic regression classification algorithm used to compute the contribution of each feature to the presence of target species, identified that the principal contributing feature was fishing area (Figure 4) followed by fishing gear. The logistic regression classification algorithm used to compute the contribution of each feature to the presence of bycatch, identified that the principal contributing feature was the fishing gear used (Figure 5) followed by fishing area.

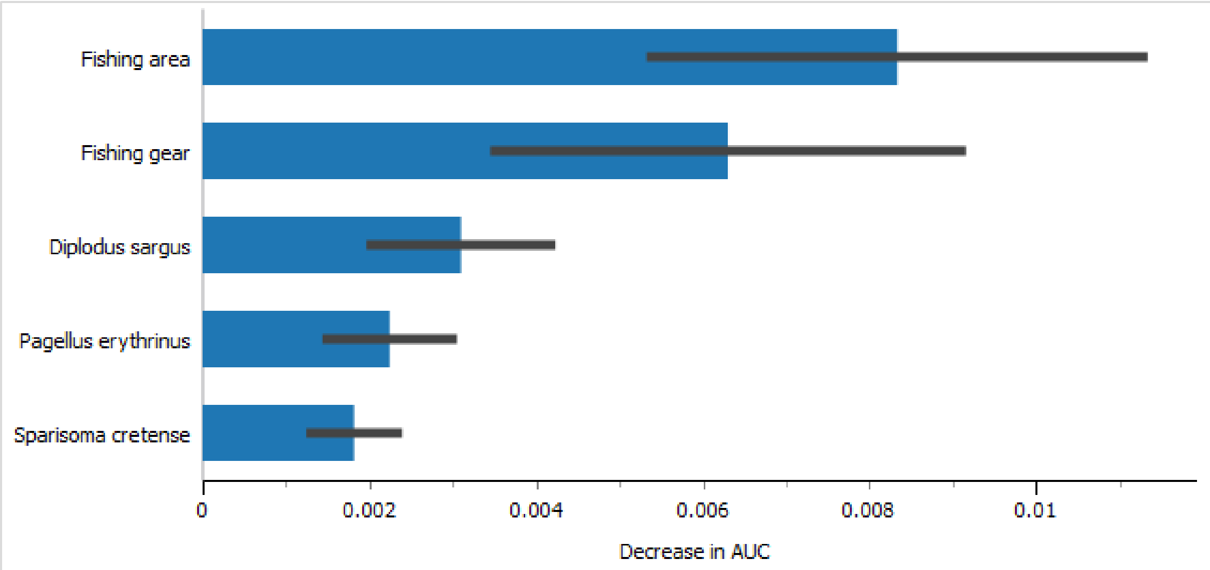

Figure 4. Top 5 contributing features to the presence of target species according to their importance.

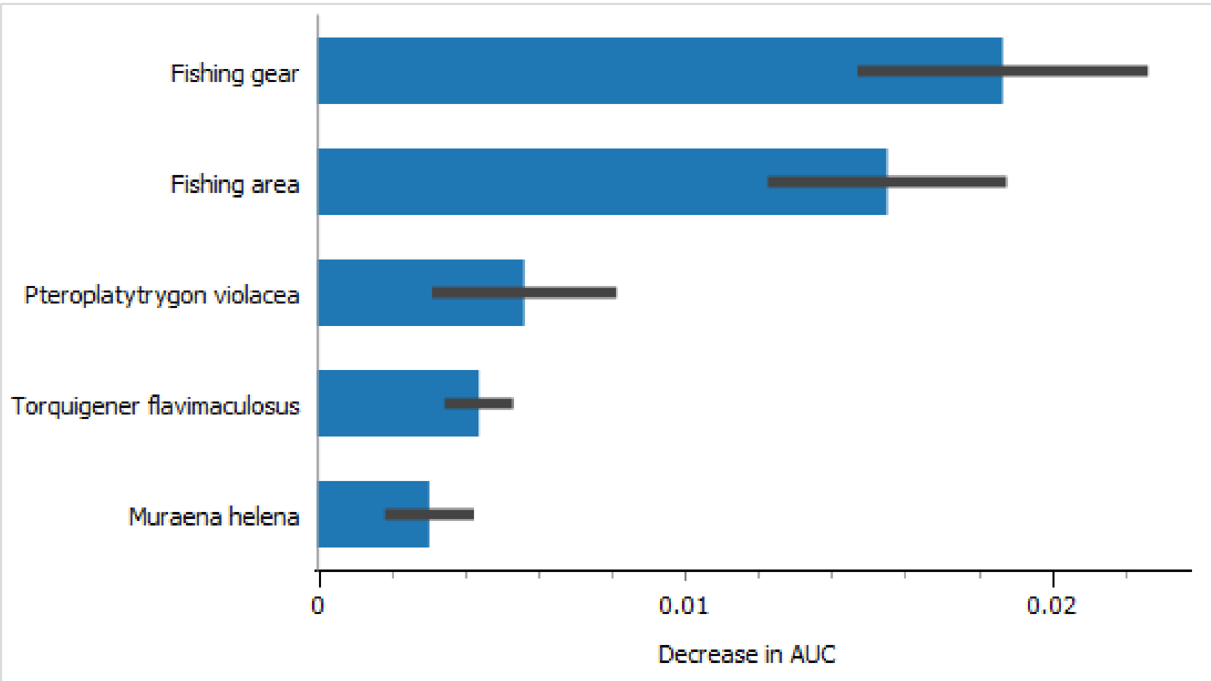

Figure 5. Top 5 contributing features to the presence of bycatch according to their importance.

Discussion

Invasive alien species are a well-recognized driver of social-ecological change globally, as they affect biodiversity, ecosystem services and human well-being. Understanding the effects (benefits and costs) of invasive species on livelihoods and human well-being is important for guiding policy formulation and management (Shackleton et al., 2019)The significantly lower than expected percentage of young family members eager to follow the fisherman occupation in the future, indicated a direct effect of the established Lessepsian migrants in the livelihoods of the small-scale fishermen in Crete. The majority of the fishermen that participated in the study, were highly dependent on small scale fishing (60% of the total) highlighting their vulnerability to potential impacts of invasive species. The immediate effect of established alien species is evident in the bycatch which is comprised by a large proportion of known Lessepsian migrantsMarine invasive species present a regional and global problem that requires regional and global solutions. Before introduced marine alien species can be managed, it is necessary to determine the risks of their introduction, establishment and spread, their potential impacts on the ecosystem, and economic activities. Effective management responses need to determine the risks and potential net benefits of prevention and management. Possible measures will need to address environmental impacts and net socioeconomic benefits, while accounting for regional differences in perspectives, technical capacity, and financial resources.

References

Bax N., Williamson A., Aguero M., Gonzalez E., Geeves W. (2003). Marine invasive alien species: a threat to global biodiversity. Marine policy, 27(4), 313-323.

Corales X., Coll M., Ofir E., Heymans J.J., Steenbeek J., Goren M., Edelist D., Gal G. 2018. Future scenarios of marine resources and ecosystem conditions of the Eastern Mediterranean under impacts of fishing, alien species and sea warming. Sci. Rep. 8, 14284. https://doi.org/10.1038/s41598-018-32666-x.

Demsar J., Curk T., Erjavec A., Gorup C., Hocevar T., Milutinovic M., Mozina M., Polajnar M., Toplak M., Staric A., Stajdohar M., Umek L., Zagar L., Zbontar J., Zitnik M., Zupan B. (2013). Orange: data mining toolbox in Python. the Journal of machine Learning research, 14(1), 2349-2353.

Galil B. S., Zenetos A. (2002). A sea change—exotics in the Eastern Mediterranean Sea. In Invasive aquatic species of Europe. Distribution, impacts and management (pp. 325-336). Springer, Dordrecht.

Larson R., Farber B. (2019). Elementary statistics. Pearson Education Canada.

The jamovi project (2021). Jamovi (Version 1.6) [Computer Software]. Retrieved from https://www.jamovi.org

Tzanatos E. (2006). Structure, characteristics and management of small-scale coastal fishing in Greece (Doctoral dissertation). 160 p

Saygu I., Heymans J. J., Fox C. J., Özbilgin H., Eryaşar A. R., Gökçe G. (2020). The importance of alien species to the food web and bottom trawl fisheries of the Northeastern Mediterranean, a modelling approach. Journal of Marine Systems, 202, 103253.

Shackleton R.T., Shackleton C.M., Kull C.A. (2019). The role of invasive alien species in shaping local livelihoods and human well-being: A review. Journal of environmental management, 229, 145-157.

BATHYMETRIC DISTRIBUTION OF THE FAMILY OCTOPODIDAE IN THE NORTH AEGEAN SEA, EASTERN MEDITERRANEAN

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Abstract

9 species belonging to 4 genera of the Octopodidae Family have been recorded in the Greek seas. With the exception of *Octopus vulgaris*, *Eledone moschata* and *Eledone cirrhosa*, most species of the Family are not fished or landed. This study, utilizing material and data collected from the MedITS Programme survey in the North Aegean, has determined the depth distribution of the Family's species in this part of the Mediterranean Sea. Eight out of the nine species of the Family were recorded during the MedITS Programme survey in the North Aegean. *E. cirrhosa* and *Octopus salutii* were present in all 5 depth zones from 0 to 800m depth, while *Macrotritopus defilippi* was caught only in one depth zone (200 to 500m depth). *E. moschata* was the most abundant in the shallower waters (depth zone from10 to 50 m), *E. cirrhosa* was the most abundant in the intermediate depth zones (51-100, 101-200 and 201-500 m), and *O. salutii* in the deepest (501-800 m) zone. *B. sponsalis* was caught almost exclusively in the 201-500 m deep zone. Considering abundance values, *O. vulgaris* showed maximum abundance in the shallowest zone. The same exists also for *E. moschata* which had maximum abundance in the shallowest depth zone, while the congeneric *E. cirrhosa* had its greatest abundance shortly after 200 m. *O. salutii* was most abundant in the 201-500 m zone. *Pteroctopus tetracirrhus* was mostly recorded in the depth zone of 201-500 m. Finally, the maximum abundance of *Scaeurgus unicirrhus* was observed in the depth zone of 101-200 m.
Keywords: *Octopodidae, Depth-zone, North Aegean Sea, Eastern Mediterranean Sea*
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1. Introduction

Octopodidae (d'Orbigny, 1840) is one of 13 families in the order Octopoda and includes the vast majority of octopuses, with over 200 species worldwide. They have eight peristomal arms, each of which has one or two rows of suckers. They lack mantle lateral fins and their endoskeleton is limited to a cartilaginous inner shell. Octopods are the only Cephalopod class that is not adapted to swimming but to benthic life. They are found in all oceans within a range of a few meters to very large depths (> 3000 m). The juveniles of many species spend some time on the plankton and the adults of some species sometimes swim in open waters to move between reefs. Octopuses are found in a wide range of habitats. Many species live on rocky or coral reefs where there is easier to find shelter and hide. Some pygmy species spend their lives safely between corals or clusters of algae. Others inhabit sandy or muddy bottoms where they can be buried to avoid predators. Most species are nocturnal and only a few hunt during the day. They possess an exceptional camouflaging ability that allows them to match the color and the texture of their environment. Some species of the Octopodidae Family play an important role in fisheries worldwide as they are a popular food in most parts of the world. This also applies in the Mediterranean and particularly in Greece, where 9 species belonging to 4 genera have been recorded (Roper *et. al.*, 1984).

Most species of the Octopodidae Family are not landed in the areas of their geographic distribution, including the Greek Seas and the Mediterranean, for two main reasons. The first is that they have no commercial value and even when caught as a by-catch, they are discarded. The second is that some of the species live in deep waters where there are no fishing grounds at least when considering the Greek Seas. Commercial fishing is usually active at depths down to 400 m where deeper dwelling Octopodidae are unlikely to be caught. Research programs with deep sea sampling are the only way to surface species that would be otherwise impossible to. MedITS Programme is a field survey which is carried out in Greek waters within the National Data Collection Framework, and enables the fisheries scientific community to collect data for many species that are either discarded from commercial fishing, or live deeper than where commercial fishing operates. The purpose of this study is to interpret the experimental fishery data and to describe the depth distribution of the Octopodidae Family species in the North Aegean. Gaining more knowledge on non-commercial species today may prove useful in the future, as known commercial fish stocks are declining and the need for food is likely to lead us fishing less conventional species. Furthermore, these species often play in important role within the communities they are found and consequently the available information about them is an important input for ecosystem models, and for ecosystem-based fisheries management programs (Dimarchopoulou, 2019).

2. Materials and Methods

The sampling area for collection of biological samples was the North Aegean (Figure 1), which extends north of 38° 40'. It is characterized by a series of deep ditches and basins from Northeast to Southwest, at depths approaching 1500 m. All data are from MedITS - Mediterranean International Trawl Survey - carried out from 1998 to 2016. It is part of a European funded sampling Programme aiming to monitor the benthic and demersal fish stocks of the Mediterranean. This action is implemented within the National Data Collection Framework, as defined by the European Union (EU Regulations 199/2008 and Decision 2019/93 / EU) and in accordance with the MEDITS instruction manual, version 9, 2017. Unfortunately it was not carried out every year throughout this period, therefore data were available for the years 1998-2001, 2003-2006, 2008, 2013, 2014 and 2016, 12 in total. The specifications of the fishing gear (trawls, doors, wires, etc.) and the sampling methodology are standard and common to all the coastal EU member states that carry out the program, according to the guidelines of the MedITS manual (MedITS-Handbook, 2017).

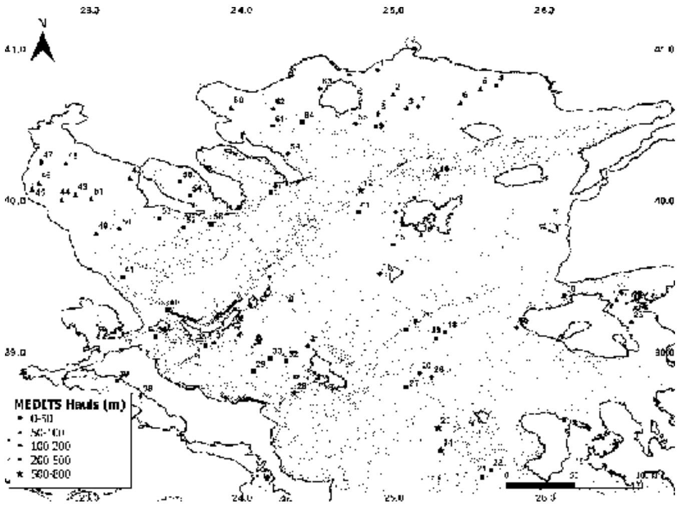

Figure 1. Study area and sampling stations with depth zone.

3. Results

During the 12 years of sampling included in the study, all species were present in more than one depth zone, except *Macrotritopus defilippi* which was recorded only in the 201-500 m depth zone. *Eledone cirrhosa* and *Octopus salutii* were the two species present in all 5 depth zones. Three species (*Bathypolypus sponsalis*, *Eledone moschata* and *Octopus vulgaris*) were observed at 4 depth zones, while *Pteroctopus tetracirrhus* and *Scaeurgus unicirrhus* were recorded at 3 depth zones. Six species, all except *M. defilippi* and *P. tetracirrhus*, were recorded during all sampling years. *Octopus macropus*, is absent from the present study, due to a complete lack of data during sampling. The species with highest total biomass (absolute number of kg) in all samples was *E. cirrhosa*, followed by *O. vulgaris*, while *M. defilippi* had the least. *O. vulgaris* was the species with the highest biomass in the shallower depth zone (10-50 m), *E. cirrhosa* had the highest biomass in the next three depth zones (51-100, 101-200 and 201-500 m), while the highest biomass in the deepest zone (501-800 m) was that of *O. salutii* (Table 1).

Table 1. Total biomass (kg) of the Octopodidae Family species per depth zone in the N. Aegean.

	Depth zone (m)					
SPECIES	10-50	51-100	101-200	201-500	501-800	Total
B. sponsalis	0.09	0	2.66	69.68	0.45	72.88
E. cirrhosa	19.84	136.96	88.78	231.17	0.51	477.26
E. moschata	165.91	87.25	29.56	34.15	0	316.87
M. defilippi	0	0	0	6.88	0	6.88
O. salutii	0.96	0.45	0.2	58.92	5.23	65.76
P. tetracirrhus	0	0	0.4	50.46	0.44	51.31
O. vulgaris	208.41	110.48	42.42	8.53	0	369.84
S. unicirrhus	0	0.10	8.61	1.98	0	10.70

O. vulgaris had the highest average biomass per haul in all sampling stations. The average biomass per haul in each depth zone followed the pattern of absolute biomass (Figure 2). *E. moschata* was the species with the highest average abundance (number of individuals per haul) in the 10-50 m zone, *E. cirrhosa* in the next 3 zones (51-100, 101-200 and 201-500 m), while the most abundant species in the deepest zone was *O. salutii* (Figure 2).

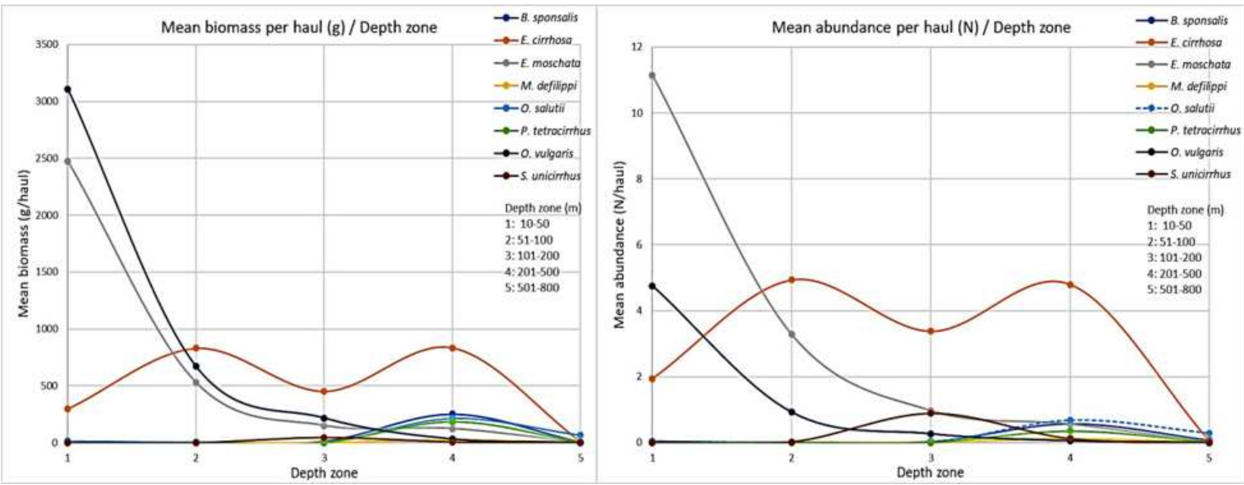

Figure 2. Mean biomass and abundance of the Octopodidae Family species per depth zone in the North Aegean.

In terms of observations per sampling station, *E. cirrhosa* was the most recorded, since it was recorded from 387 stations in total, with most of them (178) being in the 201-500 m depth zone. This zone was also the only one where the least recorded species (19 stations), *M. defilippi*, was observed (Figure 3).

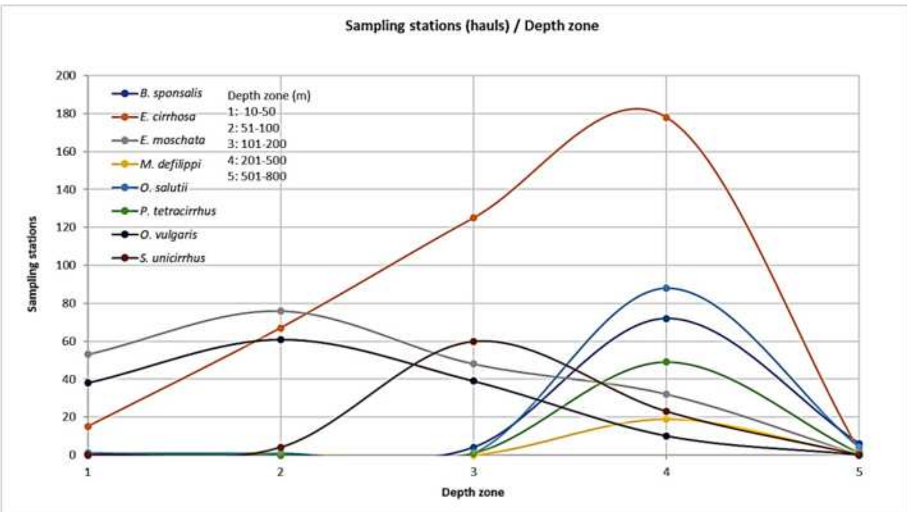

Figure 3. Number of observations of the Octopodidae Family species per depth zone in the North Aegean.

The percentage of each species individuals caught per depth zone varied: some species had their presence shared in several depth zones, some occasional, and others inhabit one specific zone exclusively (Table 2).

Table 2. Percentage (%) distribution of the Octopodidae Family species per depth zone in the N. Aegean.

	Depth zone (m)				
SPECIES	10-50	51-100	101-200	201-500	501-800
B. sponsalis	1.71		3.43	91.43	3.43
E. cirrhosa	4.42	27.68	22.61	45.19	0.10
E. moschata	45.74	33.13	11.64	9.49	
M. defilippi				100.00	

O. salutii	0.46	0.92	0.46	87.61	10.55
P. tetracirrhus			1.01	97.98	1.01
O. vulgaris	59.40	28.31	9.68	2.61	
S. unicirrhus		1.87	82.24	15.89	

Another interesting fact regarding the depth distribution of each species is the frequency of its occurrence at the sampling stations of a particular depth zone. *E. moschata* for example, recorded maximum values of both abundance and biomass in the 10-50 m zone and was observed in 79% of the stations of that zone. As total biomass and abundance decreased in deeper zones, so did the percentage of stations that it was observed in (Table 3).

Table 3. Percentage (%) of presence of the Octopodidae Family species in total sampling stations of each depth zone in the N. Aegean.

	Depth zone (m)				
SPECIES	10-50	51-100	101-200	201-500	501-800
B. sponsalis	1.49	0.00	2.03	25.99	7.59
E. cirrhosa	22.39	40.61	63.45	64.26	2.53
E. moschata	79.10	46.06	24.37	11.55	0.00
M. defilippi	0.00	0.00	0.00	6.86	0.00
O. salutii	1.49	0.61	0.51	31.77	5.06
P. tetracirrhus	0.00	0.00	0.51	17.69	1.27
O. vulgaris	56.72	36.97	19.80	3.61	0.00
S. unicirrhus	0.00	2.42	30.46	8.30	0.00

Regarding the depth zones and the distribution of species in each one of them, it was observed that all but the 51-100 m zone, had one single species making up for more than 50% of the total biomass of the zone. This was most pronounced also in the deepest depth zone i.e. the 501-800 m. Almost 80% of that zone's total biomass belonged to *O. salutii*. The most diverse zone was the 201-500 m, where each of four species made up for more than 10% of total biomass and all species were observed. Three species were responsible for almost 100% of total biomass of the 51-100 m zone, with a share of over 25% each. Nearly 95% of the total biomass of the shallowest zone belonged to *O. vulgaris* and *E. moschata* (Figure 4).

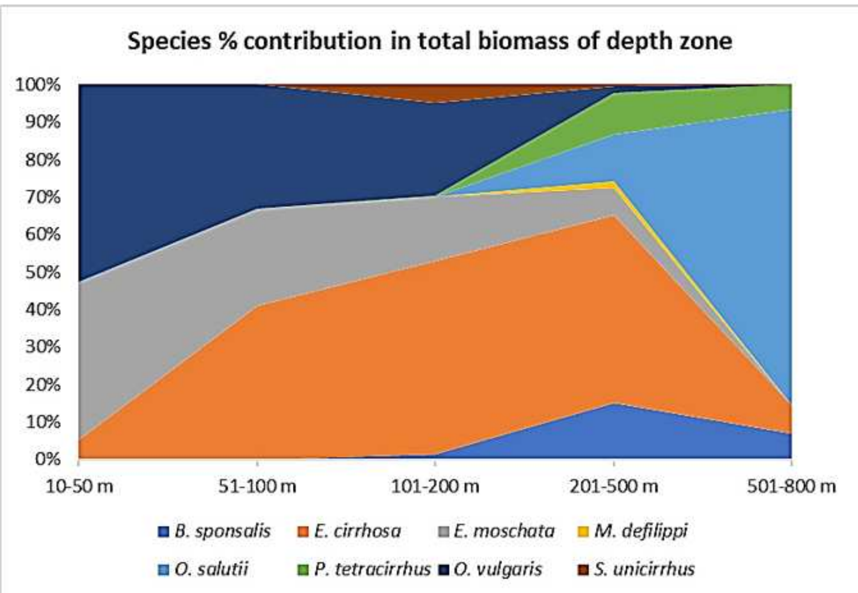

Figure 4. Contribution (%) of each Octopodidae Family species in total biomass of each depth zone in the North Aegean.

4. Discussion

During the sampling *B. sponsalis* was observed almost exclusively in the 201-500 m depth zone, where 91.4% of the total catch was recorded, with a maximum abundance at about 300 m. According to the existing bibliographic data from the scientific literature, the maximum abundance should be observed somewhat deeper, at depths between 400 and 700 m. But the fact is that it is a species that has not been studied sufficiently and mostly in the Western Mediterranean (e.g. Quetglas *et al.*, 2001).The pattern of the *E. cirrhosa* depth distribution in the North Aegean according to our sampling data, showed occurrence of the species from the shallowest depth zones down to 500 m, with higher abundance down to 300 m. That is in accordance to the depth distribution of the species that has been described so far by previous studies in the Mediterranean and the Atlantic (Tursi *et. al.*, 1995; Orsi Relini *et al.*, 2006). According to the literature, *E. moschata* is recorded at depths down to 450 m (Silva *et al.*, 2004), which is almost identical to the results of our sampling. Similar was the case of *O. salutii*, with the results of our sampling faithfully following the literature references (Norman *et. al.*, 2014), suggesting that it is observed at depths of a wide bathymetric range from 50 to 700 m with the maximum abundance being observed at depths from 250 to 500 m. Almost 88% of individuals were actually recorded in the 201-500 m depth zone. *O. vulgaris* was another species for which our sampling results were in full accordance to the existing scientific literature. It is known to be abundant at depths down to 200 m (Roper *et. al.*, 1984; Belcari *et. al.*, 2002) and our study confirmed that over 97% of individuals were observed down to that exact depth. Scientific knowledge about *P. tetracirrhus* is limited. It is known to inhabit wide range of depths, from 25 to 720 m (Norman *et. al.*, 2014). During our study, almost all individuals of the species were observed in the 201-500 m zone exclusively, though the distribution inside the zone was wide with a small peak near 400 m. *S. unicirrhus* is another species that has not been studied widely. According to the literature it has been observed from 50 to 500 m (Norman *et. al.*, 2014). The same is true in the case of our study. All individuals were caught in the three zones that include these depths: 51-100, 101-200 and 201-500 m, with the majority of individuals (~ 82%) observed in the 101-200 m zone.

In conclusion, the distribution of the Octopodidae Family species per depth zone in the North Aegean was the following: The shallowest depth zone was dominated by *O. vulgaris* and *E. moschata*. The zone 51-100 m was dominated by *E. cirrhosa*, with *O. vulgaris* and *E. moschata* still having a fairly strong presence. Similar was the case of the 101-200 m zone in which, however, *S. unicirrhus* appeared as well. The 201-500 m zone was characterized by the greatest diversity since all eight species were caught (the only zone where *M. defilippi* was recorded), with *E. cirrhosa* predominating. The deepest zone was characterized by the almost exclusive presence of *O. salutii*.

References

Belcari P., Cuccu D., González M., Srairi A., Vidoris P. (2002). Distribution and abundance of *Octopus vulgaris* Cuvier 1797, (Cephalopoda: Octopoda) in the Mediterranean Sea. Scientia Marina, 66 (Suppl. 2), 157–166.

Dimarchopoulou D., Tsagarakis K., Keramidas I., Tsikliras A. C. (2019). Ecosystem models and effort simulations of an untrawled gulf in the central Aegean Sea. Front. Mar. Sci. 6, 648.

MEDITS-Handbook. Version n. 9 (2017). MEDITS Working Group, pp 106.

Norman M. D., Finn J. K., Hochberg F. G. (2014). Family Octopodidae. In: "Cephalopods of the World. An Annotated and Illustrated Catalogue of Cephalopod Species known to Date. 3. Octopods and Vampire Squids", Jereb P., Roper C. F. E., Norman M. D., Finn J. K. (eds). FAO Species Catalogue for Fishery Purposes, 4/3, p. 36–215.

Orsi Relini L., Mannini A., Fiorentino F., Palandri G., Relini G. (2006). Biology and fishery of *Eledone cirrhosa* in the Ligurian Sea. Fisheries Research 78, 72–88.

Roper G. F. E., Sweeney M. J., Nauen E. (1984). FAO species Catalogue. Vol. 3 - Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Syn., 125 (Vol.3), Rome, pp. 277.

Quetglas A., Gonzalez M., Carbonell A., Sanchez, P. (2001). Biology of the deep-sea octopus *Bathypolypus sponsalis* (Cephalopoda: Octopodidae) from the western Mediterranean Sea. Marine Biology 138, 785-792.

Silva L., Ramos F., Sobrino I. (2004). Reproductive biology of *Eledone moschata* (Cephalopoda: Octopodidae) in the Gulf of Cádiz (south-western Spain, ICES Division IXa). Journal of the Marine Biological Association of the UK 84, 1221–1226.

Tursi A., D'Onghia G., Lefkatidou E., Maiorano P., Panetta P. (1995). Population biology of *E. cirrhosa* (Mollusca, Cephalopoda) in the north Aegean Sea (eastern Mediterranean Sea). Vie et Milieu 45, 139–145.

MORPHOMETRIC MEASUREMENTS OF DATA-DEFICIENT ELASMOBRANCH SPECIES FROM THE MEDITERRANEAN SEA

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Abstract
The study provides morphometric measurements of seven data-deficient elasmobranch species caught in the Aegean Sea, with four of them issued as protected by National and International Conventions. The studied species are too rare to occur frequently enough to have morphometric measurements from the Mediterranean Sea and all are listed as threatened or data deficient by the IUCN. Information on the date, gear-type used, and depth was provided, and for one specimen DNA barcoding analysis was also performed.
Keywords: *meristic measurements, Aegean Sea, Greece*
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1. Introduction

Severe gaps of knowledge exist for the Mediterranean Elasmobranchii despite the massive efforts that are taking place throughout the basin (Serena *et al.* 2020). Lack of information on basic biological parameters (e.g., morphometrics, diet, trophic levels, etc.) do persist throughout the Mediterranean, with most of the information derived from the western basin (Bradai *et al.* 2012), whereas these information are still scarce from the Eastern part.

In this study, species-specific morphometric data of seven elasmobranch species were presented, namely *Dasyatis marmorata* (Steindachner 1892); *Gymnura altavela* (Linnaeus 1758); *Aetomylaeus bovinus* (Geoffroy Saint-Hilaire 1817); *Hexanchus griseus* (Bonnaterre 1788); m common stingray, *Dasyatis pastinaca* (Linnaeus 1758) and marbled stingray, *Dasyatis marmorata* (Steindachner 1892); *Heptranchias perlo* (Bonnaterre 1788); *Isurus oxyrinchus* (Rafinesque 1810); and *Centrophorus cf. uyato*. Morphometric relationships and growth parameters were also estimated for the latter five species. Four of the above-mentioned species are protected species issued by National and International Conventions. In the case of *Dasyatis marmorata*, a DNA barcoding analysis was also conducted, because the identification of *Dasyatis* spp. in the Mediterranean is very problematic (Serena *et al.* 2020).

2. Material and Methods

Samples were obtained during monthly in-situ surveys to auction markets and landing sites in the North Aegean Sea during January-December 2019. All individuals found alive were immediately released back in the sea without taking any measurements.

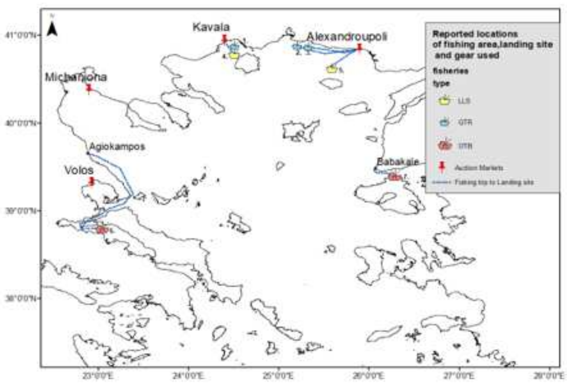

Figure 1. Reported locations of the specimens caught indicated with the number and the ports that were landed as provided by the fishers.

All dead individuals were collected and measured. A taxonomic expert confirmed the identification of each specimen in situ and pictures were also collected for facilitating further confirmation and validation. All measurements were obtained based on Serena (2005). In addition, fishers were asked to provide information regarding the fishing gear used, and the location of captures (Figure 1).

3. Results and Discussion

Overall, the morphometric measurements of 10 individuals from 7 elasmobranch species (3 batoid and 4 shark species, Tables 1 and 2, respectively) were presented.

Table 1: Morphometric measurements of the ray species examined from the North Aegean Sea, 2019.

Species	*G. altavela*	*D. marmorata*	*A. bovinus*	
Measurements (cm)	Specimen 1	Specimen 1	Specimen 1	Specimen 2
Total length	41.4	47	78.5	110
Disc length	30.6	22.27	26.9	39.4
Disc Width	60.8	24.86	44.6	64.3
Post-dorsal length	NA	16.9	28.4	41
Post-anal length	13.3	17.4	41	51
Pre-orbital length	5.1	4.2	4	6
Pre-oral length	5.8	3.05	5	7.1
Pre-nasal length	4.3	2.61	3.6	5.4
Inter orbital width	5.4	3.7	5	9.1
Eye length	1.3	1.82	1	1.4
Mouth width	5.7	–	3.2	4.2
Tail length	10.9	–	52.3	73
Spiracle length	5.1	1.04	6.4	7
Number of tail spines	0.2	0.1	0.1	0.1
Width between fifth pair of gills	7.7	6.8	4.1	5.7
Width between first pair of gills	10.2	6	6.5	9
Inter gill length	1.2	0.82	1	1.2
Total weight (Kg)	1.5	NA	1	3.4
Stomach weight (Kg)	0.021	NA	0.057	0.135
Sex	Male	Male	Male	Male
Maturity stage	I	I	I	I

Morphometric measurements are associated to a variety of fisheries parameters such as the life-history traits, population structure and identification of marine organisms (Francis 2006) providing crucial information for species conservation. Given that overexploitation from fisheries is the main driver for the vast decline in the Mediterranean (Dulvy *et al.* 2016) and the fact that severe gaps of knowledge still persist, especially in the Eastern Mediterranean basin, the information presented is of paramount importance especially for the critically endangered species. Although the number of individuals per species did not provide robust information (e.g., growth curves, etc.), the absence of similar studies makes this effort significant. The second record of *D. marmorata* from the Greek waters and its northernmost distribution (Bradai *et al.* 2015) is presented. The increasing number of records from Turkey (Yeldan & Gundogdu 2018) and the two new records from Greece indicate either an increase in the scientific effort towards these species or a natural expansion of *D. marmorata* towards the northern parts of the Mediterranean, because of the increasing water temperature. Similar patterns have been also observed in other native thermophilic species like e.g., *Alectis alexandrina* (Geoffroy Saint-Hilaire 1817; Naasan Aga Spyridopoulou *et al.* 2020). Similar studies are crucial for advancing our understanding about elasmobranchs in the Eastern Mediterranean. Albeit, the mortality of such threatened species is not encouraged, because they are not commercial, they are bycaught (Serena *et al.* 2020).

Table 2: Morphometric measurements of the shark species examined from the North Aegean Sea, 2019.

Species	*H. griseus*			*C. uyato*	*I. oxyrinchus*	*H. perlo*
Measurements (cm)	Specimen 1	Specimen 2	Specimen 3	Specimen 1	Specimen 1	Specimen 1
Total Length	150	117.6	166.6	86.3	154	85
Pre-anal Length	80.5	69.9	96.2	50.3	117.8	44
Pre-caudal length	101	81.3	112.5	71	96.6	58.3
Pre-first dorsal length	80.2	63.6	88.7	27.5	57.5	41.9
Pre-Second Dorsal Length	NA	NA	NA	59.5	108	NA
Pre-Orbital Length	7.2	6	7.3	4.4	7.9	4.1
Pre-Pectoral Length	30	23	26.8	19.3	38.5	17
Pre-Pelvic Length	68.5	96	73	8.6	91.4	32.4
Pectoral fin anterior margin Length	20	15.3	19	11	27.7	9.4
Pectoral fin posterior margin	NA	NA	NA	6.5	20.5	8.6
Pectoral-fin base	8.9	9.5	10.5	10.2	13.9	4
Pelvic-fin base	15.5	10.5	13.8	7.8	6.6	6
Pelvic-fin length	18.7	14.3	16	8.6	11.2	8.2
Upper caudal anterior margin Length	NA	NA	NA	15.2	33.5	26.2
Lower caudal anterior margin Length	NA	NA	NA	8.8	22.5	8
First dorsal anterior margin	12	6	10.3	7	18.6	6.3
First dorsal base	11.7	8.4	10.4	9.9	13.6	4.9
First dorsal fin height	NA	5.2	6.5	3.4	14.6	4.1
First dorsal fin length	15.5	11	NA	11.6	17.4	6.9
Anal-fin anterior margin	5.9	7	8	NA	2.7	3.5
Anal-fin base	9.7	6.6	8.6	NA	2.2	5.3
Anal-fin length	13.1	8.7	11.5	NA	2.8	6.4
Intergill Length	1.7	1.5	1.6	NA	3	0.8
Second Dorsal Spike	-	-	-	4	-	-
Fork Length	119	93	126.8	76.3	147.8	64.9
Eye Length	3.8	3	3.5	2.7	2.5	2.6
Head Length	29.6	23.6	28.2	18.3	38.2	16.2
Weight without intestines (kg)	8.5	5.4	14.5	2.3	24.4	1.7
Sex	Female	Male	Female	Male	Male	Female

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References

Bradai M.N., Notarbartolo di Sciara G., Serena F., Mancusi C. (2015). *Dasyatis marmorata*. The IUCN Red List of Threatened Species 2015: e.T161748A48919544.

Bradai M.N., Saidi B., Enajjar S. (2012). Elasmobranchs of the Mediterranean and Black Sea: status, ecology, and biology bibliographic analysis. Studies and Reviews-General Fisheries Commission for the Mediterranean (91).

Dulvy N.K., Allen D.J., Ralph G.M., Walls R.H.L. (2016). The conservation status of sharks, rays, and Chimaeras in the Mediterranean Sea [Brochure]. IUCN, Malaga, Spain.

Francis M.P. (2006). Morphometric minefields—towards a measurement standard for chondrichthyan fishes. Environmental Biology of Fishes 77(3-4), 407–421. doi:10.1007/s10641-006-9109-1.

Kruschel C., Zubak I., Schultz S.T., Dahlke S. (2012). New records of the parrot fish, *Sparisoma cretense*, and the cleaver wrasse, *Xyrichtys novacula*, by visual census in the Southern Adriatic. ANNALES Serhistnat 22(1), 47.

Naasan Aga Spyridopoulou R., Langeneck J., Bouziotis D., Giovos I., Kleitou P., Kalogirou S. (2020). Filling the Gap of Data Limited Fish Species in the Eastern Mediterranean Sea: A Contribution by Citizen Science. Journal of Marine Science and Engineering 8(2), 107. https://doi.org/10.3390/jmse8020107.

Serena F. (2005). Field identification guide to the sharks and rays of the Mediterranean and Black Sea. Food & Agriculture Org.

Serena F., Abella A.J., Bergnesi F., Barone M., Colloca F., Ferretti F., Fiorentino F., Jenrette J., Moro S. (2020). Species diversity, taxonomy, and distribution of Chondrichthyes in the Mediterranean and Black Sea. The European Zoological Journal 87(1), 497-536.

Yeldan H., Gundogdu S. (2018). Morphometric relationships and growth of common stingray, *Dasyatis pastinaca* (Linnaeus, 1758) and marbled stingray, *Dasyatis marmorata* (Steindachner, 1892) in the northeastern Levantine Basin. Journal of the Black Sea/Mediterranean Environment 24(1), 10-27.

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RESTRICTED LAGOONS SUBJECTED TO DIFFERENTIAL TIDAL FORCING

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Abstract
Differential tidal forcing in a lagoon with at least two inlets can be effected either by differences in tidal amplitude or by a phase lag in the tide arrival in different inlets. Such differential tidal forcing may considerably improve the renewal time of the lagoon. The mechanism of water-renewal enhancement is explored through numerical experiments in lagoons of idealized geometry and is found to be due to transient flow-through conditions that develop during each tidal cycle as a result of the differential forcing. Further numerical experiments are needed in order to parameterize the mechanism.
Keywords: *idealized lagoon, differential tidal forcing, water renewal mechanism*
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1. Introduction

Lagoons are valuable water bodies known to be vulnerable to pollution loading, in recent times. This is especially so for restricted lagoons, with limited hydraulic exchange with the open sea with which they communicate. In such cases the remedies for improving lagoon water quality focus on the enhancement of water exchange, which can be achieved either by changing the dimensions of existing passes by dredging, filling and jettying, or by creating additional passes (Miller *et al*. 1990).

Fourniotis *et al*. (2018) showed that it is possible for lagoons to have widely varying renewal times, even though they are classified as restricted and be of similar dimensions and tidal forcing, provided they have at least two tidal inlets. Their analysis showed that, in the cases they analyzed, this was due to the fact that tidal forcing in the inlets was either of different amplitude or of different phase. Fourniotis *et al*. (2021) examined the renewal time of the Papas lagoon, a restricted lagoon having three tidal inlets, and using numerical simulations they showed that the residence time could be significantly reduced if in one of the inlets the amplitude of the tide would be reduced to half, or if a phase lag with the tide at the other inlets could be created.

Given the importance of the water renewal time of a lagoon, it is important to examine in detail the mechanism that causes the renewal time to be reduced under the conditions just described. To this end, rather than focusing on a specific lagoon, which is bound to have peculiarities in its bathymetry and coastline, a series of numerical experiments were carried out, simulating tidal flow in a generic lagoon of idealized geometry, having two tidal inlets differentially forced. Some of the more interesting results of these experiments are discussed below.

2. Material and Methods

2.1 The problem posed

The lagoon used for the simulations was set to be 5 km long, 1 km wide and 2 m deep, having two tidal inlets connecting it with the adjacent seas. The length of the inlets was 260 m, their width 50 m and their depth 1 m. These are typical dimensions of a lagoon such as, for example, the Papas Lagoon, in Western Greece. Two scenarios were examined. In Scenario No 0, both open seas, with which the lagoon was connected through the inlets, had a semi-diurnal tide of amplitude 0.2 m. In the Scenario No 1, the semi-diurnal tide was of 0.2 m in amplitude in the northern (with respect to the lagoon) sea, whereas in the western sea the amplitude of the tide was 0.1 m, i.e., half the size of the northern tide. Both seas were 40 m in their outer boundary depicted in Figure 1.

2.2 The hydrodynamic code

All numerical simulations presented herein have been performed using the commercially available CFD (Computational Fluid Dynamics) numerical model MIKE21 Flow Model FM. The MIKE21 Flow Model FM is a modelling system for two dimensional (2D) numerical modelling of free-surface flows developed by the Danish Hydraulic Institute (DHI). It is applicable to the numerical simulation of hydraulic and environmental phenomena. The details of the code can be found in DHI (DHI, 2018). The simulations were based on the numerical solution of the 2D Reynolds averaged Navier-Stokes (RANS) equations for unsteady, incompressible flow, with the assumption of hydrostatic pressure. The turbulence closure is achieved by means of an eddy viscosity concept, which is specified for the horizontal transport. The model proposed by Smagorinsky (1963) is used for the horizontal eddy viscosity.

2.3 Computational Domain and Grid

The numerical domain for the two-dimensional simulations covers the entire lagoon and parts of coastal waters in front of the tidal inlets of the lagoon. Specifically, a truncated computational domain was formed covering the entire orthogonal lagoon and the adjacent open waters outside of the lagoon, i.e. the northern and western sea (Figure 1). Further, in the vicinity of the tidal inlets, i.e. inside, upstream (inflow from the open sea) and downstream (outflow to the lagoon) a much finer grid is required for adequate simulation of the tidal propagation from the open sea to the lagoon. Thus, a non-uniform computational mesh was generated consisting of three zones in the horizontal. The first zone covers the area inside and in the vicinity of tidal inlets and consisted of cells, the characteristic dimension of which is 5 m, increasing outwards. Adjacent to first zone lies the second zone covers the lagoon's body where the grid size increases to 45 m inside the lagoon. The third zone covers the coastal and offshore area of the open sea waters where the grid size increases to 175 m. The overall number of horizontal cells in the whole domain is approximately 7000. The specific discretization keeps the computational cost at a reasonable level, while trying to enhance, to the degree possible, the accuracy of the simulations. The details of the implementation of numerical method of the commercial code are outside the scope of the present paper. These details have been presented in DHI (2021).

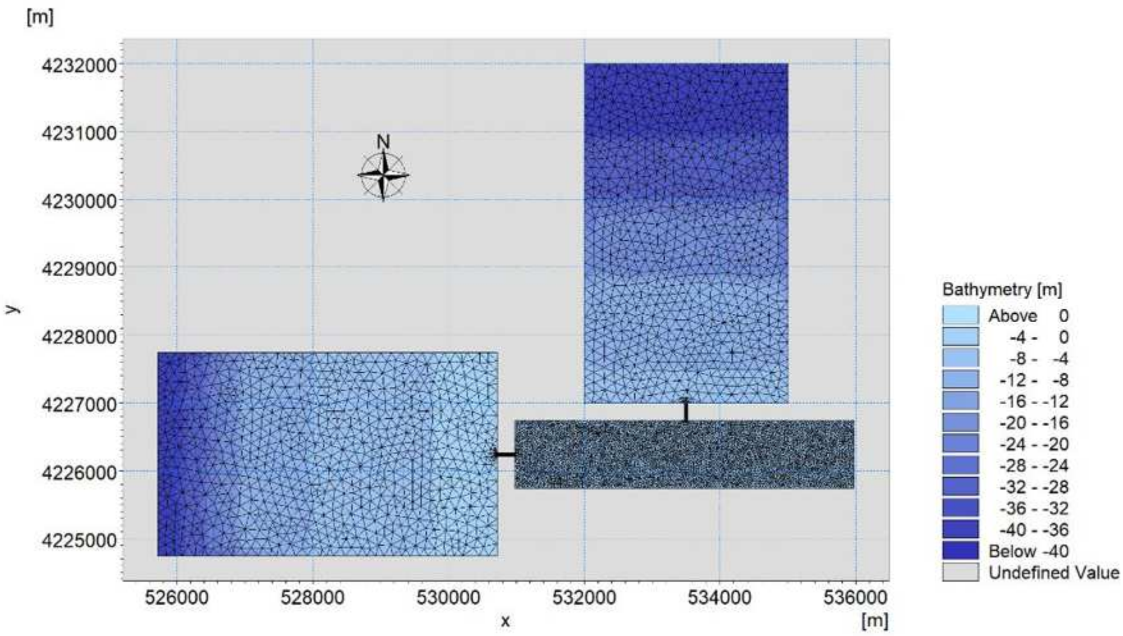
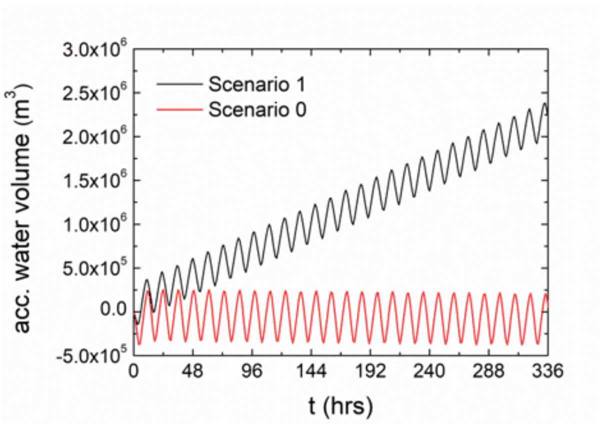


Figure 1. Numerical domain for the wider area of the lagoon, covering part of the open sea waters, bathymetry and computational mesh.

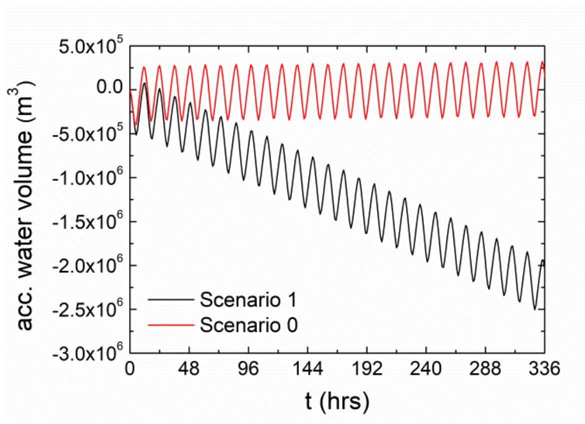
3. Results

The numerical experiments described below simulate the tide-induced flow in the idealized lagoon. We focus on tidal flow, excluding wind-induced flow or density driven flows, because it can be argued (e.g., Fourniotis *et al*. 2018) that the most stringent conditions for water renewal in a lagoon is pure tidal flow. The essential difference in the hydraulic exchange flow function in each of the two scenarios described above, can be seen by observing Figure 2. From that figure it follows that, in Scenario No 0 (red line), the tidal prism, i.e., the volume of water that enters through the inlet channel during flood flow, is equal to the flow that then exits during ebb flow. Thus, the cumulative volume of water exchanged (red line, Figure 2) oscillates around zero (for both inlets, in the Figure 2 (a) and (b), respectively). In contrast, in Scenario No 1, more water enters in each tidal cycle in the northern inlet channel than the water that exits (black line, in Figure 2b), and, as a result, the cumulative discharge is negative, and the black curve decreases monotonically (in an oscillatory way, of course). The opposite happens in the western (black line, Figure 2a) inlet channel, so that more water exits than enters in each cycle, and the cumulative discharge curve increases monotonically.

The explanation for this behavior can be given by observing the flow field in corresponding time instances for each of the two scenarios. An instant for the Scenario No 0 is shown in Figure 3. The flow, depicted by the depth-averaged velocity vector field, can be seen to be essentially confined in an area in the vicinity of each inlet, with no large-scale flow of any consequence developing within the lagoon. In contrast, the same instant for the Scenario No 1, depicted in Figure 4, shows that a large scale, flow-through pattern has developed, with flow entering the northern inlet channel, where the amplitude of the tide is larger, and exiting the western channel, where the tidal amplitude is smaller. It is important to note that this is a transient state of the flow, which occurs for a limited time interval during each tidal cycle. As expected, the simulations show that the free-surface gradient which accompanies this feature of the flow, is very short-lived (a few minutes in the simulated cases), so that no free-surface gradient of long duration is present. This happens because the time scale of the tidal celerity entering the lagoon, which is $L/\sqrt{gh}$, L being the length of the lagoon and h its depth is much smaller than the period of the tidal forcing (e.g., US Army Coastal Engineering Manual, 2008). This flow-through condition develops always at the same time interval of the tidal cycle. This time interval, as well as the flow strength, depend on the important features of this phenomenon, including the distance between the two inlet channels. Further experiments, which are under way, aim at clarifying the exact nature of this dependence. These details are important in order to be able to design with precision the optimum location of any additional inlets that might be deemed necessary in lagoons, in order to improve the renewal time of their waters. In the past, lack of this knowledge has led to the addition of tidal inlets that have had minimal improvement in the lagoon's water renewal time (e.g., Cladas *et al.* 2016).



(a)



(b)

Figure 2. Cumulative water volume exchanged at (a) the western inlet and (b) the northern inlet.

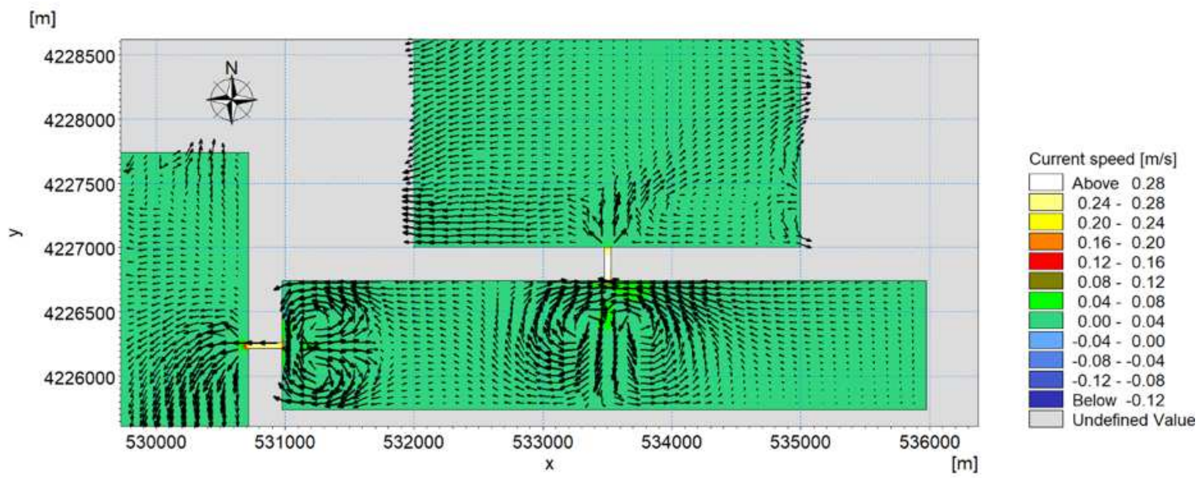


Figure 3. Depth-averaged velocity field for a time instant of Scenario No 0. Note that for the thicker vectors their length is not proportional to the corresponding speed, because the vector length has been truncated in order to make the figure legible.

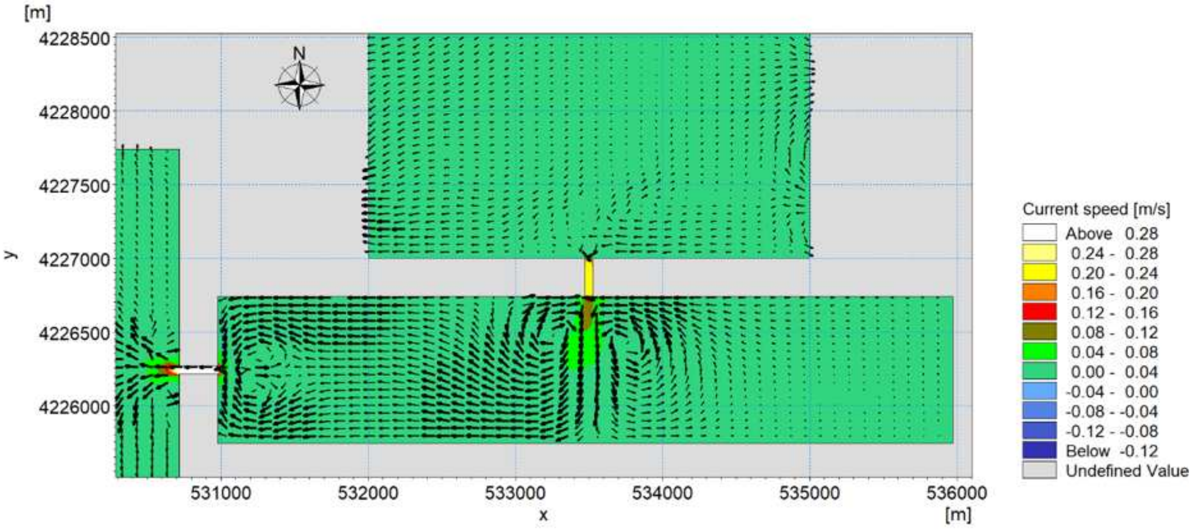


Figure 4. Depth-averaged velocity field for the time instant of Scenario No 1 corresponding to the instant of Scenario No 0 depicted in Figure 3. This instant documents the transient occurrence of flow-through conditions. The same holds for the thicker arrows as for Figure 3.

4. Conclusions

The mechanism that leads lagoons, with two or more tidal inlets subjected to differential tidal forcing, to an improved water renewal time, is examined by simulating the flow which develops in an idealized lagoon. The mechanism has been found to be highly transient and the enhancement of the renewal time is due to the formation of flow-through conditions, which last for a short time-interval during each tidal cycle. Further numerical experiments are needed before the mechanism is parameterized. This parameterization will be valuable in the selection of the optimum site of additional inlet channels that might be considered in case a lagoon is deemed to benefit from enhanced renewal of its waters.

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References

Cladas Y., Papantoniou G., Bekiari V., Fragkopoulu N. (2016). Dystrophic crisis event in Papas Lagoon, Araxos Cape, Western Greece in the summer 2012. Mediterranean Marine Science 17, 32-38. doi:10.12681/mms.1409.

DHI (2021). MIKE 21 Flow Model FM, Hydrodynamic and Transport Module, Scientific Documentation. https://manuals.mikepoweredbydhi.help/latest/MIKE_21.htm#MIKE_21_Documentation

DHI (2018). MIKE 21 Flow Model FM, Hydrodynamic Module-User Guide. DHI Software. 132p.

Fourniotis N.Th., Horsch G.M., Leftheriotis G.A. (2018). On the Hydrodynamic Geometry of Flow-Through versus Restricted Lagoons. Water 10(3), 237. https://doi.org/10.3390/w10030237.

Fourniotis N.Th., Leftheriotis G.A., Horsch G.M. (2021). Towards enhancing tidally-induced water renewal in coastal lagoons. Environmental Fluid Mechanics 21, 343-360. https://doi.org/10.1007/s10652-020-09776-0

Miller J.M., Pietrafesa L.J., Smith N.P. (1990). Principles of hydraulic management of coastal lagoons for aquaculture and fisheries. FAO Fisheries Technical Paper. No. 314. Rome, FAO, 88p.

Smagorinsky J. (1963). General circulation experiments with the primitive equations I. The basic experiment. Mon. Weather Rev. 91(3), 99-165. https://doi.org/10.1175/1520-0493(1963)091<0099:GCEWTP>2.3.CO;2

US Army Coastal Engineering Manual (2008). Part II, Chapter 6.

METHODOLOGICAL CONSIDERATIONS IN MAPPING A HOSTILE SEACLIFF WITH TERRESTRIAL LASER SCANNER

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Abstract
A considerable effort was made to monitor a hostile sea cliff, which is under erosion and bounds an ecologically important sea-turtle nesting beach in Zakynthos Island (Greece), and to predict rates of recession. Various issues (logistics, the unstable nature of the seacliff and methodological approach) that complicated the implementation of the project are addressed and discussed. The final outcome fulfilled the project requirements but also helped the involved personnel to gain experience and manage issues under challenging conditions.
Keywords: *terrestrial laser scanner, sea cliff monitoring, coastal processes, Zakynthos Island*
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1. Introduction

Erosion of sea cliffs (or coastal cliffs) stem from the combination of marine and subaerial processes that result in weakening of the internal consistency, leading in gradual or sporadic erosion/recession and mass/rock failure (Sunamura 2015). It is well established that seacliff instability/erosion can be responsible for significant economic and even human losses (Marques *et al*. 2013). The conditions and the mechanisms of sea cliff failure have been the subject of numerous studies all over the world with a big variety of topographic and remote sensing surveying techniques, both terrestrial and aerial (Esposito *et al*. 2018). For small terrains, low-cost terrestrial methods are apparently preferable. In recent decades, a common method used for observing short-term sea cliff dynamics is terrestrial laser scanning (TLS) because it produces high resolution measurements and 3-D geometries of the cliffs and they offer easy and fast deployment in the field (Kuhn & Prufer 2014). However, measurements of cliff volumes (along with beach morphological) changes are subject to errors stemming usually from the TLS set-up, affecting consequently the accuracy of the resulting estimations. Since sea cliff short-term erosion rates may vary, when they are low, they are apparently more easily affected by erroneous measurements.

Gerakas embayment is one of the six sea-turtle (*Caretta-caretta)* nesting beaches located in Laganas Bay, in Zakynthos Island (Greece) (Figure 1a). The embayment has a great ecological interest, and it is managed by the National Marine Park of Zakynthos (NMPZ), but it is also attractive to tourists due to the beach beauty. The southeastern-most part of Gerakas beach is protected from NE to SE winds and waves by a small sea cliff promontory. The intensified erosion of a small part of the sea cliff (Figure 1b,c) has been documented by visual observations, information from local people and from the Management Agency of the NMPZ that have noticed considerable rates of retreat the last years.

The unique morphological characteristic of the study area is that the seacliff has a “double aspect”, meaning that it is exposed to wave action both from the east and west (Figure 1). The potential loss of this formation could result in the (i) creation of an isolated islet in the southeastern tip of the Gulf of Laganas, (ii) alteration in the coastal hydro- and sediment-dynamics and (iii) possible unforeseen changes of Gerakas rookery. In this context, the NMPZ appointed a baseline study in order to map the seacliff and to evaluate its erosional rate, examining also local hydrodynamics as a factor that controls erosion from the sea. Other physical agents that profoundly contribute to cliff erosion, such as rain, the intense seismicity of the region (Chousianitis 2009), the textural characteristics of the cliff etc, are not considered in this research since they demand a multi-disciplinary approach that was proposed for a future investigation program (Panagou *et al*. 2020). The present study focuses solely on the problems, considerations and methodological approaches that were followed in order to map and monitor this hostile landform with topographic surveying techniques.

2. Material and Methods

The implementation of the project had to face problems concerning the (i) logistics, (ii) nature of the sea cliff and (iii) methodology to be used. Concerning logistics, the duration of the project should not exceed 24 months, the budget was limited and there were serious difficulties with cliff accessibility that also had an impact on the use of modern mapping techniques.

Figure 1. (a) Location of the study area (red circles pointing to the studied cliff; green arrow: nesting beach), (b) east aspect of the sea-cliff, (c) west aspect of the sea-cliff (dashed yellow line: unconformity), (d and e) instability features (arrows), (f) scattered boulders in shallow waters prohibiting easy approach to the beach.

Regarding the nature of the cliff, two geological units are discernible, the Gerakas and Kalogeras Formations (Upper Pliocene - Lower Pleistocene) (Triantaphyllou *et al*. 1997 - Figure 1c). Gerakas Formation consists mainly of massive silty mudstones with a variety of marine fossils and bioturbated sandy beds, and it is unconformably overlain at a low-angle discordant contact by Kalogeras Formation consisting of marine calcareous sandstones and massive mudstones. The overall appearance and the absence of vegetation indicates active erosion but there was no historical information on the sea cliff retreat rates and on geotechnics. Instability features of both formations can be easily distinguished, also from available photos (Figure 1d,e). At the middle section, Gerakas Formation is fully exposed forming a characteristic concave shape (Figure 1b,c). The sea cliff cannot be approached from the shore or from the top, whereas small boats can access the two small fronting beaches only if they manage to perfectly maneuver in-between scattered boulders of various sizes in shallow waters (Figure 1f).

Concerning methodology, the long-term rate of sea cliff retreat was evaluated using historical (1945 to 2014) aerial photos, topographic maps, temporal orthophotographs and satellite images. In relation to the topographic data needed to evaluate the short-term erosional rate of the sea cliff and to compare with older/temporal data, the following issues were addressed: (a) the base of the seacliff was not accessible from none of the two aspects, and also the acute cliff geometry did not permit access from the top; (b) UAV topographic mapping was not available at the time of the survey. Thus, for the accurate mapping of the seacliff and taking also into account its confined and small size, the convex shape of the embayment and the need for surveying from a safe distance (to avoid accidents due to potential small-scale failures), the Terrestrial Laser Scanner (TLS) was selected as the most appropriate method. The TLS is a remote sensing technique that determines the position of a point measuring the horizontal angle, the vertical angle and the slope distance. The accuracy of the measurement is a main function of the range, reflectivity of the material (Voegtle *et al*. 2008), complexity of the scanned surface (Abellan *et al*. 2009) and angle of incidence (Lichti & Jamtsho 2006). For most applications more than one scan is needed to represent the 3D geometry of the study area.

Sea cliff topographic surveying was conducted in three periods (May 2014, March 2015 and April 2016) using a Global Navigation Satellite System (GNSS), a Total Station (TS) and a TLS. A 6 m inflatable was used to transport personnel and instruments. In-situ observations verified that cliff erosion is continuous and still active. Overall, the seacliff attains steep slopes, locally with an irregular micro-relief. Many small-scale features and deposits (rills and gullies, rockfalls, debris cones, mass flows etc), the intense micro-relief and the absence of vegetation, all manifest the intense erosional processes that take place and the unstable nature of the sea cliff, but also of the wider area. Both sea cliff aspects are fronted by small beaches, the western being larger (60 m long by 10 m wide) than the eastern one (30 m long and 7 m wide), consisting of sand to pebbles/cobbles.

2.1. Geomatics

For the topographic measurements (Figure 2), initially, a small geodetic network was established by stainless survey point nails at both sides of the seacliff. Two dual signal GNSS geodetic receivers were used for triangulation. The base receiver was installed in a fixed station of the Greek Geodetic Reference System (GGRS87) and the rover at the stations of the established network. The accuracy of the measurements is 5 mm ± 0.5 ppm multiplied by the baseline length. The operational space for GNSS observations was limited as the steep cliff face affected observation of satellite signals, and increased multipath effects.

Figure 2. TLS scanning of the east (a, b) and west (c, d) seacliff aspects from different positions (red arrows: instability features; red dashed line: recent mud flow).

The network was expanded by a geodetic TS. In order to register the point clouds, which would be later acquired by the TLS, checkboard targets and distinguishable natural features around the beach and the sea cliff face were measured by TS during each survey. Ideally, an extensive network of reflective targets should have been established covering the sea cliff spatially and at various elevations. Nevertheless, the soft and unstable cliff proved to be so hazardous that did not permit safe climbing for establishing fixed targets at higher positions. Therefore, targets settled only at low elevations and near the cliff toe. Additionally, characteristic geomorphologic features were measured at higher elevation (replacing targets role) and leaving a "hard heritage" to post-processing (registration) operations.

A compact sized TLS was installed in front of and at the side of both aspects of the cliff (Figures 2, 3). Repeated scans from adjacent stations around the cliff toe ensured full seacliff coverage. Even though several methods are proposed for choosing the optimal distance for scanning a cliff (Gulyaev & Buckeridge 2004), these were unsuitable due to the local geomorphology that prohibited scanning efforts from inaccessible and highly unstable elevated positions. Shadows (data voids) appeared mainly at the top of the sea cliff and at the large talus of the east side due to difficulties in approaching the cliff and its unfavorable exposure. The average point spacing of the merged scans was between 1 and 3 cm. The effort to avoid shadow areas produced abundant data that raised computational problems. Post-processing included geo-registration, cloud-to-cloud comparison (using the Multiscale Model to Model Cloud Comparison [M3C2] algorithm (Lague *et al*. 2013), terrain analysis, profiles comparison and volume analysis.

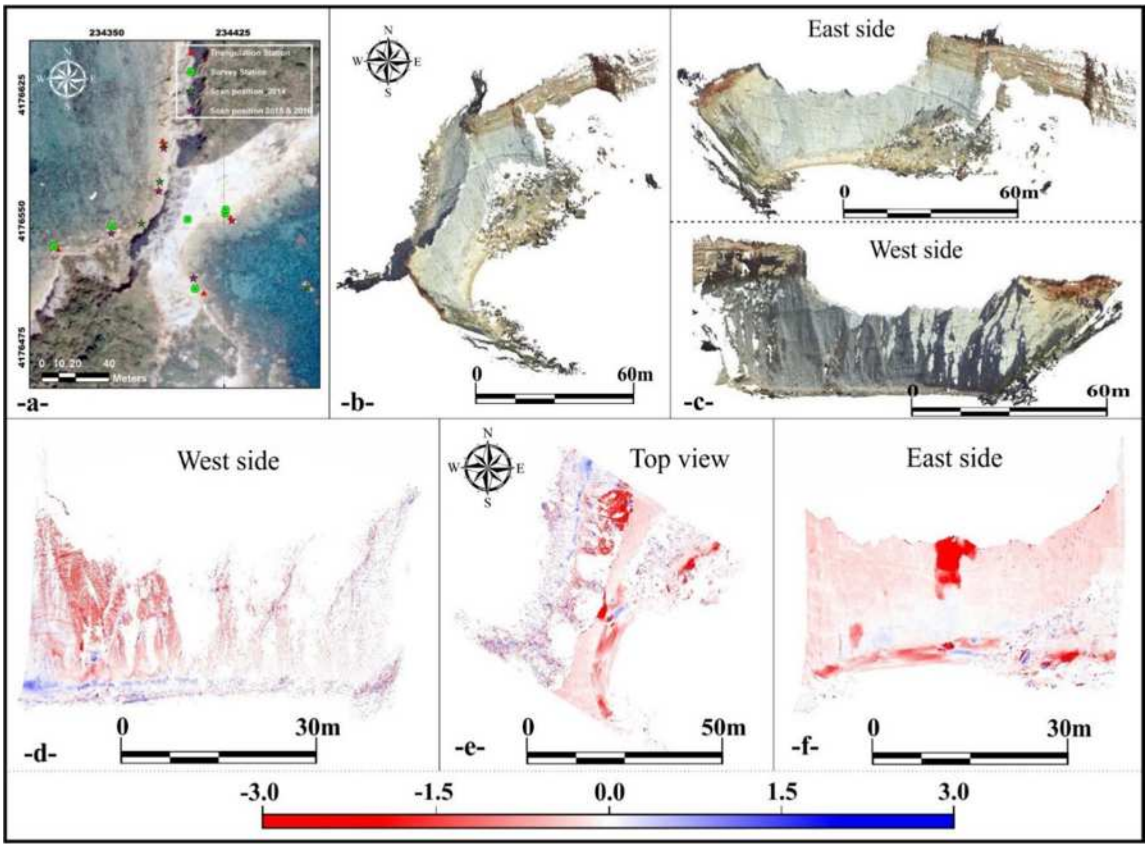

Figure 3. (a) Setup of the geodetic network and distribution of the scan positions, (b) a 3D view of a point-cloud, (c) point-clouds of the east and west aspects, (d - f) temporal "Cloud to Cloud" comparisons between 2014-2016 (M3C2 algorithm results), restricted to the eroding section of Gerakas Formation (scale bar: M3C2 distances).

3. Results and Discussion

Successive TLS surveys combined with older spatio-temporal data, along a small but vastly eroded and hostile part of the double aspect sea cliff in Gerakas embayment, provided evidence of its retreat and crude estimations of its recession rates (0.16 m/yr for the last 20 years – Panagou *et al*. 2020). The literature review revealed that there is no similar research related to the study of double-aspect coastal cliffs, signifying the uniqueness of the research, whose pilot results assign questions but can also provide the initiatives for methodologically integrated studies (i.e. combined with UAVs, geotechnical studies etc) and cliff erosion modelling of similar coastal environments.

During the 2-years monitoring period, the TLS point-clouds comparisons reveal the spatial distribution of morphological changes giving an instant perception of cliff/beach mass fluctuations (erosion and deposition) that locally reach 3.0 m of momentary erosion (Figure 3d-f).

3.1. Cliff monitoring issues

TLS monitoring has detected micro-morphological changes, also contributing to the study of the evolution of the erosional features/processes. However, data voids (shadows) could not be avoided, especially at the top of the cliff. This is a common problem when scanning seacliffs from a lateral point of view, a fact that can reduce the efficiency of geometric reconstruction of the cliff (Martino & Mazzanti 2014). This problem could be overcome in the future utilizing a small copter (UAV), flying in a low height, offering images of a high quality and measurement accuracy almost equivalent to the TLS.

Also, there are errors deriving from the measurement procedure (laser characteristics, target characteristics, the scanner setup) and errors stemming from data processing (alignment of scans) (Olsen *et al*. 2009). The largest contribution in inaccuracies arises from geo-referencing of the point-clouds in a national coordinate system. The inaccessible and unstable Gerakas seacliff made geo-referencing of the clouds a mandatory procedure. From a geodetic point of view, the errors could be avoided by establishing an arbitrary "closed and fixed" network of stations, commonly applied to deformation monitoring of infrastructure constructions, from which fixed marked points would be distributed spatially (planimetrically and vertically) and would be measured periodically. In our case it was impossible to have fixed points spatially distributed all over both seacliff aspects, because of the specific hostile coastal environment and the restricted financial resources.

Although scanning in a hostile and dangerous environment, the TLS measurements managed to produce results of high accuracy and of unprecedented detail, which permitted volume analysis and comparisons and contributed with the rest of the available and collected data in modelling and in the crude estimation of the Gerakas sea cliff retreat (Panagou *et al*. 2020). The results of this expedition were beneficial not only for the Management Agency of the NMPZ but also for the involved personnel that gained experience working in a hostile environment and managing to handle diverse and puzzling situations met during field operations.

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References

Abellán A., Jaboyedoff M., Oppikofer T., Vilaplana, J. (2009). Detection of millimetric deformation using a terrestrial laser scanner: experiment and application to a rockfall event. Natural Hazards and Earth System Science 9(2), 365-372.

Chousianitis K. (2009). Seismic source properties and ground deformation study of the broader area of Cephalonia and Zakynthos Islands using Geophysical and Space techniques. Doctorate Thesis, Department of Geophysics-Geothermics, University of Athens.

Esposito G., Salvini R., Matano F., Sacchi M., Troise C. (2018). Evaluation of geomorphic changes and retreat rates of a coastal pyroclastic cliff in the Campi Flegrei volcanic district, southern Italy. Journal of Coastal Conservation 22, 957-972. https://doi.org/10.1007/s11852-018-0621-1.

Gulyaev S.A., Buckeridge, J.S. (2004). Terrestrial methods for monitoring cliff erosion in an urban environment. Journal of Coastal Research 20(3), 871-878.

Kuhn D., Prufer S. (2014). Coastal cliff monitoring and analysis of mass wasting processes with the application of terrestrial laser scanning. A case study of Rügen, Germany. Geomorphology 213, 153–165.

Lichti D., Jamtsho S. (2006). Angular resolution of terrestrial laser scanners. The Photogrammetric Record 21(114), 141-160.

Lague D., Brodu N., Leroux J. (2013). Accurate 3D comparison of complex topography with terrestrial laser scanner: Application to the Rangitikei canyon (N-Z). Journal Photogrammetric Remote Sensing 82, 10-26.

Marques F., Matildes R., Redweik P. (2013). Sea cliff instability susceptibility at regional scale: a statistically based assessment in the southern Algarve, Portugal. Natural Hazards and Earth System Science 13(12), 3185-3203.

Martino S., Mazzanti, P. (2014). Integrating geomechanical surveys and remote sensing for sea cliff slope stability analysis: The Mt. Pucci case study (Italy). Natural Hazards and Earth System Science 14(4), 831-848.

Olsen M.J., Johnstone E., Driscoll N., Ashford S., Kuester F. (2009). Terrestrial Laser Scanning of Extended Cliff Sections in Dynamic Environments: Parameter Analysis. Journal of Surveying Engineering 135(4), 161-169.

Panagou Th., Hasiotis T., Velegrakis A., Karambas Th., Oikonomou E., Dimitriadis C. (2020). Erosion status of a sea cliff promontory bounding an ecologically important beach. Journal of Coastal Conservation 24(39) https://doi.org/10.1007/s11852-020-00756-6.

Sunamura T. (2015). Rocky coast processes: with special reference to the recession of soft rock cliffs. Proceedings of the Japanese Academy Series B91, 481–500.

Triantaphyllou M., Drinia H., Dermitzakis M. (1997). The Plio-Pleistocene boundary in the Gerakas section, Zakynthos (Ionian Islands) – Biostratigraphical and paleoecological observations. N. Jb. Geol. Palaont. Mh., 1, 12-30.

Voegtle T., Schwab I., Landes T. (2008). Influences of different materials on the measurements of a Terrestrial Laser Scanner (TLS). The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, Vol. XXXVII. Part B5, 1061-1066.

THE ACOUSTIC REPERTOIRE AND THE CHARACTERISTICS OF DOLPHIN WHISTLES IN GREECE

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Abstract
Four species of dolphins can be found permanently in the Greek Seas. However, there is no extensive description of the sounds they use to communicate, such as whistles and clicks they use to echolocate. A list of whistles could be used to indicate possible variations in acoustic characteristics between different populations in the Mediterranean or Atlantic, and could also contribute to the recognition of dialects. They could also be a first step in creating algorithms for the automatic detection of whistles. The present study incorporated an analysis of simultaneous visual and acoustic data provided by Marine Conservation Research (MCR) for the years 2003, 2007 and by MCR and the Pelagos Cetacean Research Institute for 2013. In total 2540 minutes of recordings of good quality with low environmental noise were used for the analysis. A total of 4834 whistles were detected in the recordings, of which the 3325 were of good quality, with a signal to noise ratio (SNR) deemed high enough for subsequent analysis. The results showed a significant difference between the four species in specific descriptive indices. It was also found that the whistles from the populations of Greece seem to differ from other populations of the Eastern Mediterranean. For future research, it is important to investigate further the repertoire of whistles produced in Greek waters, how they may be sub-divided by species, and to examine the chronological sequence of their production.
Keywords: *Bioacoustics, marine mammals, Oceanography.*
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1. Introduction

In the marine environment animals often use sound for communication, food search and navigation. Among the Cetaceans, baleen whales (mysticetes) typically produce low frequency sounds, that undergo little absorption and therefore propagate over long distances (Tyack & Miller 2002), while the toothed whales (odontocetes) tend to produce higher frequency vocalisations, that are more directional and undergo greater absorption. The main types of vocalization produced by dolphins include clicks (which can incorporate a wide range of frequencies and are used for echolocation), burst pulses (composed of rapid series of pulsed sounds that may be produced in both foraging and social contexts), and whistles (narrowband tonal signals used for communication between conspecifics) (Au 1993).

Out of the thirteen species of cetaceans identified in the Greek Seas (Frantzis 2009; Notarbartolo di Sciara & Bearzi 2010; Frantzis 2018), eight may be considered most common: the bottlenose dolphin *(Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*), the common dolphin (*Delphinus delphis*), the Risso's dolphin (*Grampus griseus*), the Cuvier's beaked whale (*Ziphius cavirostris*), the harbor porpoise (*Phocoena phocoena relicta*), the fin whale (*Balaenoptera physalus*), and the sperm whale (*Physeter macrocephalus*). The species minke whale (*Balaenoptera acutorostrata*), humpack whale (*Megaptera novaeangliae*), rough-toothed dolphin (*Steno bredanensis*), false killer whale (*Pseudorca crassidens*) and the species Indian Ocean humpback dolphin (*Sousa plumbea*) are occasionally/rarely present in Greek waters (Frantzis 2018).

Bottlenose dolphins (*Tursiops truncatus*) are found mainly in coastal areas, bays, straits and between islands and in semi-enclosed bays (Frantzis *et al.* 2009). The frequency range of their acoustic repertoire is from 0.1 to 165 kHz (Southall *et al*. 2019). The bottlenose dolphin emits a wide variety of signals whistles, clicks and click arrays, burst pulses and bi-phonations (whistles and clicks produced at the same time) (Boisseau 2005; Jones *et al.* 2020). Whistles may act as signatures (Sayigh *et al.* 2010) indicative of each individual. In Greece, a study, which was carried out in Mytilene, found whistles of central frequency 9.5 kHz and average duration 0.6 s (Kyriakou *et al.* 2019). The striped dolphin in Greek waters is found in deep, pelagic waters, rarely near the coast or in shallow waters. It is widely distributed in all Greek Seas and is systematically found in the Gulf of Corinth (Frantzis *et al.* 2009). This species' acoustic behaviour has not been well studied; the repertoire of signals is from

1 kHz to 34 kHz (whisltes) (Azzolin *et al.* 2013; Southall *et al.* 2019). The species also produces clicks, it can also produce whistles and click simultaneously and it has been found to produce "Nacchere", clicks at night with most energy at lower frequencies and a distinctive tone (Manghi *et al.* 2003). In Greece, some analysis has been conducted with data from the Ionian Sea (Azzolin *et al.* 2013). Risso's dolphin (*Grampus griseus*) is found in deep water areas of Greece such as southwestern Crete, often in Myrtoon Pelagos and the Gulf of Corinth, at a distance of 0.5-32 km from the coast (Frantzis *et al.* 2009). As for its acoustics, the dolphin has been little studied; it produces clicks from 3 to 72 kHz (Neves 2013). The species also produces broadband clicks, barks, buzzes, grunts and whistles; it also appears to emit whistles and pulses at the same time (Neves 2013). The common dolphin (*Delphinus delphis*) is found in shallow and coastal waters, often in the Inner Ionian and in areas of the Aegean (Frantzis *et al.* 2009). The species produces social sounds like whistles from 0.3- 44 kHz (Southall *et al.* 2019). This species also produces clicks but in general the common dolphin's acoustic behavior is not very well known.

In Greece, the dolphins' acoustic repertoire has not been extensively described, so the present study aims at the first comprehensive analysis of the whistles of dolphin species from Greek waters, using data from simultaneous acoustic and visual surveys, where the recorded species were observed and visually confirmed. Such a list could be used to show variations in the acoustic characteristics of local populations from other populations in the Mediterranean or Atlantic (Papale *et al.* 2014; Lopez 2009; Azzolin *et al.* 2021; Gregorietti *et al*. 2021). In addition, it could be the basis for further research on the management of large volumes of data from static hydrophones and the automatic recognition of dolphin sounds.

2. Material and Methods

In 2003 a survey was conducted by the International Fund for Animal Welfare's (IFAW) with a 14 m sailing vessel the RV "Song of the Whale" (SOTW). The main study areas were the Ionian Sea, the Channel of Sicily and Malta, but for the present study only the data collected in the Ionian Sea was used. A second survey was conducted in 2007 in the eastern part of the Mediterranean with the new specially designed 21 m sailing vessel renamed "Song of the Whale" (Boisseau *et al.* 2010). In 2013, Marine Conservation Research (MCR) in collaboration with the Pelagos Cetacean Research Institute (PCRI) conducted visual and acoustic research in the Thracian Sea and the rest of the Aegean. Two observers during the day were on watch Boisseau *et al.* 2010) and the acoustic monitoring was performed with two hydrophones with an approximately flat response between 10 Hz and 40 kHz, towed 200 m behind the boat. (Boisseau *et al.* 2010). Two-minute listening stations were performed at 15-minute intervals during which cetacean clicks or whistles were recorded by an operator (Ryan *et al.* 2014). For the data analysis a combination of visual and audio data was used for the four species of dolphins regularly encountered in Greek waters. Audio files, recorded with a sample rate of 48 kHz, were selected for analysis based on their spectral content according to quality (high Signal to Noise Ratio); this generated a dataset of 47 audio files. Any whistles in these files were characterised using several descriptive indices for spectrograms generated with Raven Pro (Charif *et al*. 2010). The descriptive indices for each whistle were obtained through manual measurement of the fundamental contour (first harmonic) and included the average entropy and the aggregate entropy (entropy describes the disorder in a sound), the maximum frequency, the minimum frequency (the upper and the lower limit of the selection of the sound with respect to the spectrogram's frequency axis), the peak frequency (the frequency at which the selection has the maximum power), the 90% bandwidth (the difference between the 5% and 95% of total frequency range of the sound), the delta frequency (the difference between the maximum and minimum frequency), the 90% duration (the difference between the 95% and 5% time) and the delta time (duration).

The statistical analysis has been made with the use of the R programming language. The Shapiro-Wilk test was used to check the normality of the data. The non-parametric Kruskal-Wallis test (one-way ANOVA on ranks) was selected to test the differentiation of species in relation to selected descriptive indices, and finally a non-parametric Mann Whitney Wilcoxon U test was used for pairwise comparisons between species.

3. Results and discussion

Most of the recordings were made in the North Agean Sea, as most of field research was focused on this area. There were also observations of dolphins in the Ionian, Central and South Aegean Seas and a few in the Levantine Sea. Those areas with few observations are not indicative of species distribution, but the dispersion of that data rather reflects heterogenous survey effort. The acoustic data were confirmed by simultaneous visual observations of dolphins, and this way the whistles were distinguished per species. The total number of recorded whistles of bottlenose dolphins was 738, of which 538 were deemed to have sufficiently high quality (high SNR) for subsequent analysis. The total sample of recorded whistles of striped dolphins was 2479 and of these, 1666 were of good quality. The sample of Risso's dolphins' whistles was quite small, due to the limited number of observations (n = 2), and all 6 recorded whistles used for analysis. The total sample of recorded whistles of common dolphins were 44 and of these, 29 were used for subsequent analysis.

Out of 169 simultaneous visual observations and acoustic recordings of dolphins, 103 recordings did not contain whistles, 19 contained whistles with low SNR (low quality) that could only be used for temporal sequence analysis, and 47 were suitable for analysis. A total of 2540 minutes of recordings were extracted from the sample, as they were of good quality with low ambient noise. A total of 4834 whistles were detected in the recordings that were analysed, of which 1509 were of low quality; 3325 whistles were of good quality and used for subsequent analysis. Descriptive statistics were performed (Table 1) and the non-parametric Kruskal Wallis test (one-way ANOVA on ranks) was used to compare the medians of the samples. The results (Table 2) showed that there was a significant difference between the four species as regards the descriptive indices minimum frequency, maximum frequency, peak frequency, duration and 90% bandwidth. The four species were found to have similar total and average entropy (Charif *et al*. 2010), as well as 90% duration. To further investigate the differences in the descriptive indices between species, a Mann Whitney Wilcoxon U test was performed for each species pair (Table 3).

Table 1. Results of descriptive statistics for the main whistles' parameters of the four species of dolphins (N=sample size, SD= Standard Deviation)

			Description indices				
Species	**N**		**Min frequency (Hz)**	**Max Frequency (Hz)**	**Peak frequency (Hz)**	**Total Entropy (bits)**	**Duration (s)**
Bottlenose dolphin	538	Min	554.9	1942	1453	1.63	0.07
		Max	16369.9	24000	18375	8.22	2.43
		Mean	7984.4	13287	10020	6.18	0.58
		SD	2367.6	3313.2	2759	1.2	0.39
Striped dolphin	1666	Min	989.5	1683	1500	1.81	0.04
		Max	22057.8	24000	22406	8.2	3.93
		Mean	8735	13674	10785	5.72	0.55
		SD	2188.5	3023.6	2519.5	1.1	0.33
Rissos dolphin	6	Min	5827	13526	8203	6.26	0.89
		Max	9780	21105	13453	8.09	1.45
		Mean	7388	16632	10977	7.12	1.11
		SD	1637.3	2790.8	2335	0.6	0.2
Common dolphin	29	Min	3504	4826	3984	3.91	0.18
		Max	11174	19598	16031	7.77	1.82
		Mean	6479	13161	8426	6.62	0.88
		SD	2103.58	3041.73	2818.2	1.1	0.46

The results of the pairwise comparisons reveal that there is a significant difference in the minimum frequency of whistle contours between bottlenose and striped dolphins, common and bottlenose dolphins, and common and striped dolphins. For maximum frequency, there is a difference between bottlenose and striped dolphins, bottlenose and Risso's dolphins, common and Risso's dolphins, and striped and Risso's dolphins. In terms of peak frequency, bottlenose dolphins differ from striped dolphins and common dolphins, and common dolphins differ from striped dolphins. In terms of the duration, there are differences between bottlenose and common dolphins, bottlenose and Risso's dolphins, striped and Risso's dolphins, and common and striped dolphins. Finally, for bandwidth, there is a significant difference between bottlenose and common and Risso's dolphins, striped dolphins and Risso's dolphins, and common and striped dolphins.

Table 2. Results from the Kruskal-Wallis test for all descriptive indices by species. * denotes results significant at the 95 % level.

Kruskal-Wallis test										
		Min.Freq (Hz)	**Max.Freq (Hz)**	**Peak.Freq (Hz)**	**Agg.Entropy (bits)**	**Avg.Entropy (bits)**	**Bandwidth 90% (Hz)**	**Delta.Freq (Hz)**	**Delta.Time (s)**	**Duration 90% (s)**
	Chi-squared	2577	2546	473	2346	2294	497	2968	2241	450
Species	df	1708	1902	307	2275	2240	256	2583	2118	248
	P-value	<0.001*	<0.001*	<0.001*	0.14	0.21	<0.001*	<0.001*	0.03*	0.61

Table 3. Results from the Mann Whitney Wilcoxon U test for all descriptive indices by species. * denotes results significant at the 95 % level.

Mann Whitney Wilcoxon U test						
Species pairs		**Min. Frequency**	**Max. Frequency**	**Peak Frequency**	**Delta Frequency**	**Delta Time**
Bottlenose vs Striped	p	<0.001*	0.035*	<0.001*	0.654	0.131
Bottlenose vs Common	p	<0.001*	0.611	0.002*	0.010*	<0.001*
Bottlenose vs Risso's	p	0.471	0.013*	0.294	0.007*	0.002*
Common vs Striped	p	<0.001*	0.302	<0.001*	0.004*	<0.001*
Common vs Risso's	p	0.168	0.012*	0.061	0.154	0.2141
Striped vs Risso's	p	0.082	0.021*	0.83	0.003*	<0.001*

The results of this study describe the general characteristics of the whistles of four dolphin species in Greek waters. It is the first analysis performed in Greece that compares these species from data collected from free-ranging populations. Comparison of the whistles with previous studies from the Eastern Mediterranean, Ionian, Egypt, Sardinia suggest there are differences in relation to the characteristics of the whistles recorded in the present study (Lopez 2011; Azzolin *et al.* 2013; Neves 2013). These differences found in the present study could probably relate to the genetic differentiation of populations in the various regions of the Eastern Mediterranean and Greece. This geographical diversity is attributed to possible genetic variation of populations (Bourret *et al.* 2007). Differentiation of the whistles can also depend on the behaviours and the group composition (Heiler *et al*. 2016).

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References

Au W. W. L. (1993). The Sonar of Dolphins ~Springer-Verlag, New York.

Azzolin M., Papale E., Lammers M.O., Gannier A., Giacoma C. (2013). Geographic variation of whistles of the striped dolphin (*Stenella coeruleoalba*) within the Mediterranean Sea. J. Acoust. Soc. Am. 134(1), 694–705.

Azzolin M., Gannier A., Papale E., Buscaino G., Mussi B., Ardizzone G., Giacoma C., Silvia Pace D. (2021). Whistle variability of the Mediterranean short beak common dolphin. Aquatic Conservation: Mar Freshw Ecosyst., 31(S1), 36– 50.

Boisseau O., Lacey C., Lewis T., Moscrop A., Danbolt M., McLanaghan R. (2010). Encounter rates of cetaceans in the Mediterranean Sea and contiguous Atlantic Area. Journal of the Marine Biological Association of the United Kingdom 90(8), 1589–1599.

Boisseau O. (2005). Quantifying the acoustic repertoire of a population: The vocalizations of free-ranging bottlenose dolphins in Fiordland, New Zealand. Journal of the Acoustical Society of America 117 (4), 2318-2329.

Bourret V. J. R., Mace M. R. J. M., Crouau-Roy B. (2007). Genetic variation and population structure of western Mediterranean and northern Atlantic *Stenella coeruleoalba* populations inferred from microsatellite data. J. Mar. Biol. Assoc. U.K. 87, 265–269.

Charif R.A., Strickman L.M., Waack A.M. (2010). Raven Pro 1.4 User's Manual. The Cornell Lab of Ornithology, Ithaca, NY.

Fearey J., Elwen S.H., James B.S. et al. (2019). Identification of potential signature whistles from free-ranging common dolphins (Delphinus delphis) in South Africa. Anim Cogn 22, 777–789.

Frantzis A. (2018). A long and deep step in range expansion of an alien marine mammal in the Mediterranean: First record of the Indian Ocean humpback dolphin *Sousa plumbea* (G. Cuvier, 1829) in the Greek Seas. Bioinvasions Records 7, 83–87.

Frantzis A. (2009). Cetaceans in Greece: Present status of knowledge. Initiative for the Conservation of Cetaceans in Greece, Athens, Greece, 94 pp.

Gregorietti M., Papale E., Ceraulo M., de Vita C., Pace D.S., Tranchida G., Mazzola S., Buscaino G. (2021). Acoustic Presence of Dolphins through Whistles Detection in Mediterranean Shallow Waters. Journal of Marine Science and Engineering 9(1), 78. https://doi.org/10.3390/jmse9010078

Heiler J., Elwen S. H., Kriesell H. J., Gridley, T. (2016). Changes in bottlenose dolphin whistle parameters related to vessel presence, surface behaviour and group composition. Anim. Behav. 117, 167–177.

Jones B., Zapetis M., Samuelson M.M., Ridgway, S. (2020). Sounds produced by bottlenose dolphins (Tursiops): a review of the defining characteristics and acoustic criteria of the dolphin vocal repertoire. Bioacoustics 29 (4), 399-440.

Kyriakou K., Katsanevakis S., Trygonis V. (2019). Quantitative analysis of the acoustic repertoire of free-ranging bottlenose dolphins (*Tursiops truncatus*) in N Aegean, Greece, recorded in the vicinity of aquaculture net pens. 14th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions (ICZEGAR), June 27–30.

López B.D. (2009). The bottlenose dolphin *Tursiops truncatus* foraging around a fish farm: effects of prey abundance on dolphins' behaviour. Curr. Zool. 55 (4), 243–248.

Lopez B.D. (2011). Whistle characteristics in free-ranging bottlenose dolphins in the Mediterranean Sea: influence of behavior. Mammalian Biology. 76 (2): 180–189. doi:10.1016/j.mambio.2010.06.006.

Manghi G., Fossati M., Pavan C., Priano M. (2003). Diel occurrence of characteristic acoustic emission in feeding striped dolphin (Stenella coeruleoalba). Proceedings of the seventeenth annual conference of the European cetacean society, 9-13 March 2003

Neves S. (2013). Acoustic behaviour of Risso's dolphins, *Grampus griseus*, in the Canary Islands, Spain. PhD Thesis, University of St Andrews.

Notarbartolo di Sciara G., Bearzi G. (2010). National Strategy and Action Plan for the conservation of cetaceans in Greece, 2010-2015. Initiative for the Conservation of Cetaceans in Greece, Athens. 55 pp.

Papale E., Azzolin M., Cascão I. et al. (2014). Acoustic divergence between bottlenose dolphin whistles from the Central–Eastern North Atlantic and Mediterranean Sea. Acta Ethol 17, 155–165.

Ryan C., Cucknell A.C., Romagosa M., Boisseau O., Moscrop A., Frantzis A., McLanaghan, R. (2014). Final report. A Visual and Acoustic Survey for Marine Mammals in the Eastern Mediterranean Sea during Summer 2014.

Sayigh L.S., Esch H.C., Wells R.W., Janik V.M. (2010). Facts about signature whistles of bottlenose dolphins, Tursiops truncatus. Anim. Behav. 74, 1631–1642.

Southall B.L., Finneran J.J., Reichmuth C., Nachtigall P.E., Ketten D.R., Bowles A.E., Ellison W.T., Nowacek D.P., Tyack P.L. (2019). Marine Mammal Noise Exposure Criteria: Updated Scientific Recommendations for Residual Hearing Effects. Aquatic Mammals, 45(2), 125-232, DOI 10.1578/AM.45.2.2019.125

Tyack P. L., Miller E. H. (2002). Vocal anatomy, Acoustic and Communication and Echolocation. In: Marine Mammal Biology, An Evolutionary Approach. (Hoelzel, A. R., ed). Blackwell, Durhan. pp. 142-184.

OCEAN CIRCULATION EFFECTS ON EUTROPHICATION IN THERMAIKOS GULF

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Abstract
One of the most significant environmental problems of Thermaikos Gulf is the occasional formation of extended eutrophication phenomena (red tides, mucilaginous aggregates). Herein, we investigate the contribution of hydrodynamic processes on the formation of such events, under the effects of different meteorological and river discharge conditions. We conducted field observations (physical-biological measurements), microscopy analysis of phytoplankton samples, satellite ocean color image analysis, and implemented high-resolution numerical hydrodynamic simulations with updated river discharge outflows to detect eutrophication events and relate them with the prevailing physical processes and ocean circulation patterns. The eutrophication events were mainly associated with the dominance of southerly winds. Northerly winds contribute on the renewal of the Gulf imposing a two-layer flow, especially along the eastern coasts.
Keywords: *Ocean Dynamics, Red Tides, Ocean Modeling, Observations*
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1. Introduction

Thermaikos Gulf is a typical, river-fed, microtidal, semi-enclosed, coastal inlet of the east-central Mediterranean Sea. It is an important coastal ecosystem susceptible to several anthropogenic pressures, strong river discharges and variable meteorological and ocean (met-ocean) conditions. One of the most significant environmental problems of the region is the occasional formation of extended eutrophication phenomena (phytoplankton blooms, red tides, mucilaginous aggregates), especially over the Northern Thermaikos Gulf (NTG). The hydrodynamic circulation of the NTG is characterized by two seasonal patterns, one during the winter and the other during summer (Hyder *et al*. 2002; Krestenitis *et al*. 2012). The intrusion of the denser Aegean Sea Waters (ASW) usually takes place through the deeper layers along the eastern coasts of the Gulf while the water masses of the Gulf exit towards the open sea through the surface layers (Balopoulos & Friligos, 1993; Krestenitis *et al*. 2012). The major lateral freshwater input of NTG consists of four rivers (Gallikos, Axios, Loudias, and Aliakmonas) together with a large complex system of canals and trench drains, mainly located at the western coast (Vokou *et al*. 2018). The hydrography of the region, the major circulation patterns, and several physical processes (e.g., coastal upwelling, dense water formation, renewal, river plume dynamics, vertical mixing) play a significant role on the variability of the ecological and biogeochemical characteristics, the pollutants transport, and furthermore the water quality of the Gulf. Eutrophication events are very common, especially pronounced over the northern region, and have recently raised great concern among the public, municipal, and regional authorities, and the scientific community. The main motivation of this study is to investigate the influence of the predominant circulation patterns on the appearance of eutrophication events based on an integration of satellite, in situ and modeling data.

2. Material and Methods

The seasonal in situ measurements refer to the physical (temperature and salinity) and biological (chlorophyll-a: chl-a) parameters, whereas water samples for phytoplankton microscopy analysis were also collected in the water column of three sampling stations, located at the inner- (Station S2), central- (Station S1), and outer- (Station S3) Gulf (Figure 1a). The observational period covered three seasons in 2017. Phytoplankton samples were examined using an inverted epi-fluorescence microscope (Nikon Eclipse TE 2000-S, Melville, MSA) with phase contrast. Phytoplankton identification was based on taxonomic keys and papers and counting was done using the inverted microscope method following the same protocol described in Stefanidou *et al*. (2019). For biomass estimation, the dimensions of 30 individuals of each abundant species were measured using a digital microscope camera (Nikon DS-L1). Mean cell volume estimates were calculated using the appropriate geometric formulae. Satellite ocean-color data were also used to derive the chl-a concentrations over the entire study region. All the collected raw satellite images refer to Sentinel-2 and Sentinel-3 datasets, derived from EU's Earth Observation Programme platform, Copernicus (https://scihub.copernicus.eu/). The numerical simulations were implemented with the FLOW module of the Delft3D (Delft3D-FLOW) modeling system (https://oss.deltares.nl/web/delft3d) in a 3-dimensional (3-D), sigma-layer configuration for the investigation of the NTG hydrodynamic circulation (Delft3D-Thermaikos model; Androulidakis *et al*., 2021). Information about the model set-up (e.g., initial, boundary, forcing conditions, river parameterization and input) and its good performance were discussed by Androulidakis *et al*. (2021).

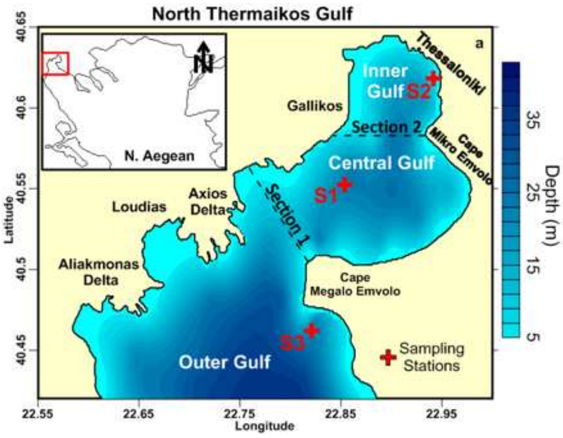

Figure 1. (a) Bathymetry of the Northern Thermaikos Gulf, located in the Northwestern Aegean Sea (insert in the upper left). The major topographic features of the Gulf are shown: inner-, central- and outer-Gulf, Cape Megalo Emvolo, Cape Mikro Emvolo, city of Thessaloniki, Gallikos, Axios, Loudias and Aliakmonas rivers. The locations of three sampling stations are also marked with red crosses. Dashed black lines indicate the borders between the three sub-regions (Sections 1 and 2).

3. Results

The distribution of chl-a in the water column and the phytoplankton biomass concentration at specific depths for each campaign and sampling station are presented in Figure 2. The chl-a water column distribution derived with the CTD broadly agrees with the distribution of the phytoplankton biomass derived from processing of collected water samples in three depth levels (surface, pycnocline, near-bottom). The peak values and the distributions between the two variables qualitatively agree in all cases despite the very low chlorophyll content of phytoplankton biomass reflecting the physiological state of dominant diatoms and the mixotrophy of other phytoplankters on their chl-a content (Genitsaris *et al*. 2019).

The respective horizontal distribution of chl-a, derived from the satellite ocean color images, is presented in Figure 3. The highest near-surface chl-a values among the three stations were detected over the inner-Gulf (Station S2) in early-December 2017. The phytoplankton biomass also revealed the highest values (extreme value of 85 mg/L) during December, while smaller, but very high concentrations were detected in July (20 mg/L) in agreement with the chl-a concentrations (2-4 mg/m^3). In all seasons, Station S3 (outer-Gulf) revealed very low and homogenous vertical distribution of chl-a (~1 mg/m^3) and phytoplankton biomass (<5 mg/L) indicating lower primary production of nano- and micro-phytoplankton over this area, located closer to the open sea boundary (influence of ASWs).

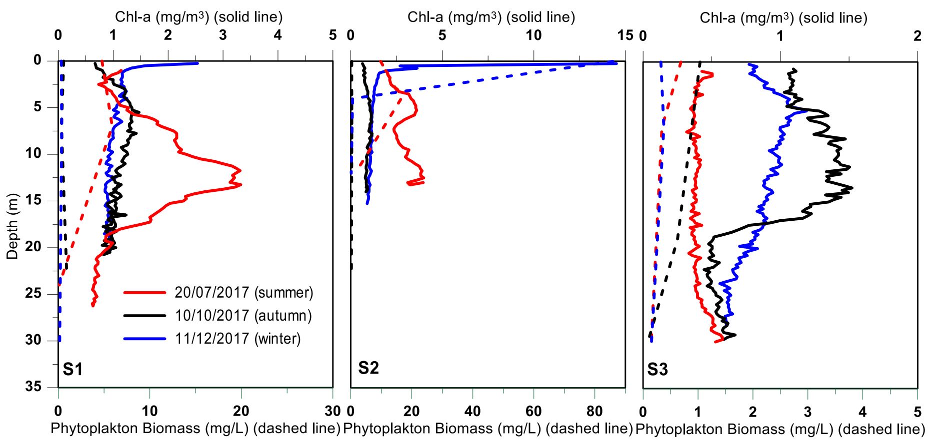

Figure 2. Seasonal vertical profiles of chl-a measured by SBE 19plus CTD (mg/m^3; solid lines) and phytoplankton biomass (mg/L; dashed lines) at S1 (red line), S2 (black line), S3 (blue line) stations.

High chl-a concentrations were detected by the satellite ocean-color sensor in early-July 2017 (Figure 3a). Different surface distribution of satellite-derived chl-a was observed in the ocean color satellite images between the two campaigns of summer 2017 (5 and 20 July). The eutrophication event in late-June and early-July was related to an extensive phytoplankton bloom in spring/early-summer 2017 and *Noctiluca* red tides that contributed to a "dirty sea" phenomenon of mucilaginous aggregates (Genitsaris *et al*. 2019). The December 2017 red tide event was also discussed by Genitsaris *et al*. (2019), which was related to the autotrophic ciliate species *Mesodinium rubrum*. Based on the ocean color images, the chl-a concentrations were significantly high south of the Axios Delta on 5 December (Figure 3d) but were also increased in the inner-Gulf a few days later, on 10 December (Figure 3e), in agreement with the field observations. Generally, both in situ (Station S3) and satellite data support the lower eutrophication state over the southeastern part of the NTG, while the higher chl-a concentration values usually appeared in the inner-Gulf and especially along the western coasts, where the river deltas are located. The year of 2017 was unique, with two very intense eutrophication events (late-June to early-July 2017 and December 2017) and two periods (renewal events) characterized by clear waters (late-July 2017; October 2017) over the entire NTG.

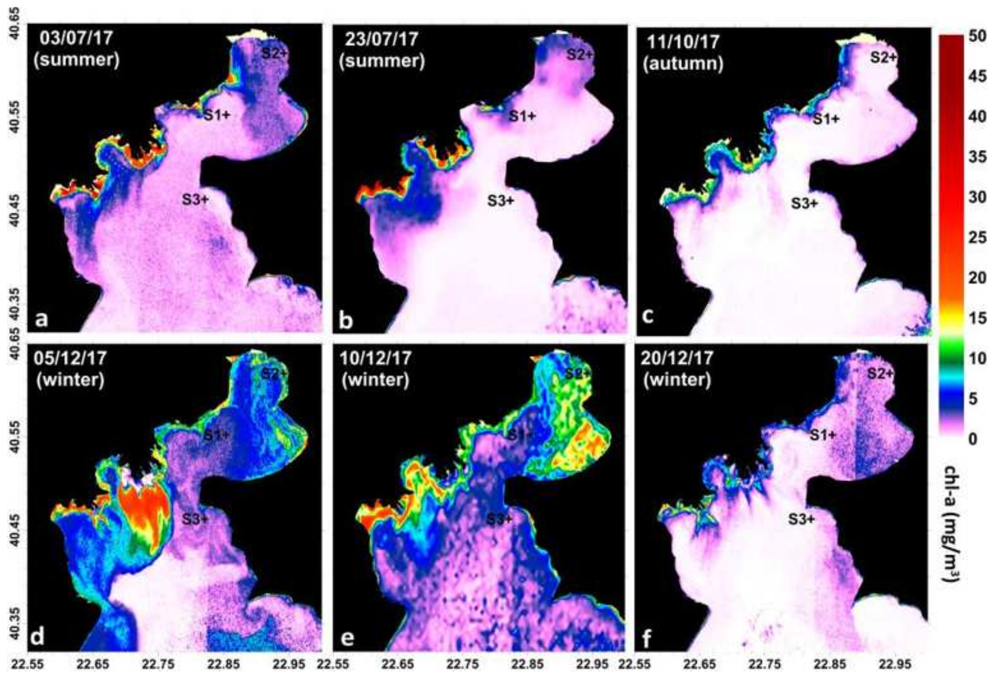

Figure 3. Horizontal distribution maps of chl-a concentrations (mg/m^3), derived from the available Sentinel-2 and Sentinel-3 satellite on (a) 03/07/17, (b) 23/07/17, (c) 11/10/17, (d) 05/12/17, (e) 10/12/17, (f) 20/12/17.

The Delft3D-Thermaikos model simulated the ocean circulation during a full annual cycle in 2017, covering the two major eutrophication events in late-June and early-July, and December and the two renewal events in late-July and October. The southerly winds induced a general northward spreading of surface waters imposing an anticyclonic circulation in early-July (Figure 4b). Two distinct circulation features emerged from the surface current fields; an anticyclonic pattern in the central- and inner-Gulf and a second anticyclonic eddy in the outer-Gulf, keeping the two water masses separated. A similar anticyclonic circulation pattern prevailed in the central-Gulf a few days later (Figure 4d), on 20 July, under weak northerly winds (Figure 4c), with smaller river discharge rates (Figure 1b) and limited spreading of brackish waters, especially over the central-Gulf. Stronger northerly winds that prevailed in mid-July (not shown) played a role on the southward advection of the riverine plume, away from the northern central- and inner-Gulf regions. The small period of strong northerly winds that occurred in mid-July played a role on the different eutrophication levels and physical distribution between the two campaigns. Significantly strong northerly winds (>10 m/s; Figure 4e) prevailed in October over the entire study region imposing a respective southwestward spreading of surface waters with currents higher than 0.5 m/s. During the eutrophication event in early-winter 2017 (11/12/17), the prevailing southerly winds induced a respective northward surface circulation pathway. The simulated Sea Surface Temperature (SST) distribution indicating two separate water masses between the inner- (colder and less saline) and outer-Gulf (warmer and saltier). Although southerly winds may drive surface waters towards the North, they can also confine the inner-Gulf surface waters and enhance eutrophication phenomena especially when riverine brackish waters are trapped in the northern part of the NTG.

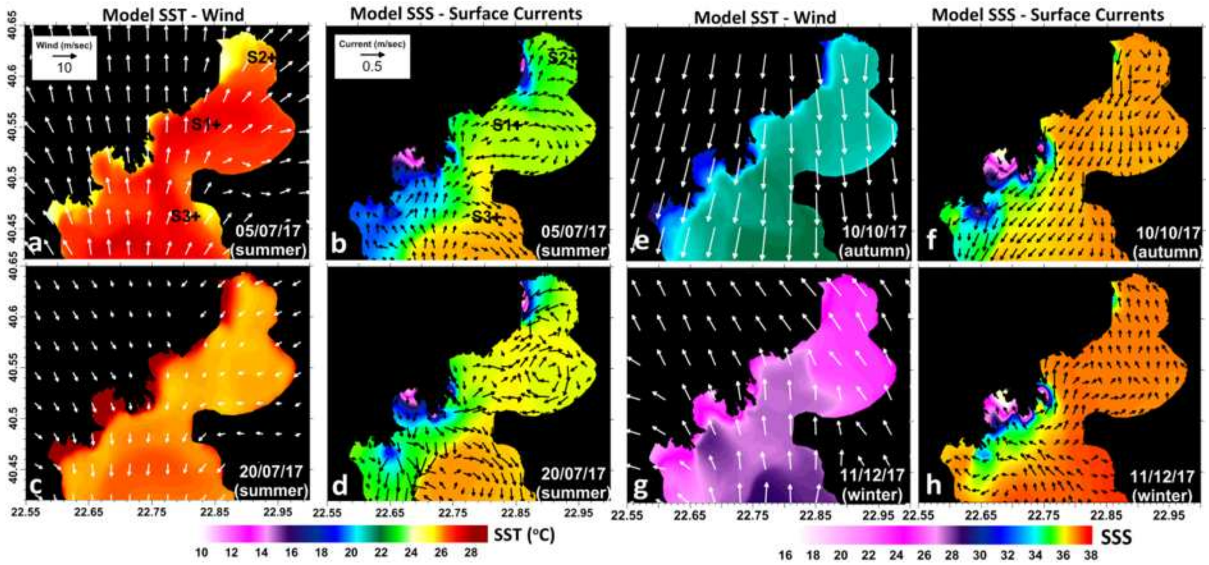

Figure 4. Horizontal distribution of Sea Surface Temperature (SST; °C) and Sea Surface Salinity (SSS), derived from the Delft3D-Thermaikos simulations, overlaid with wind vectors (WRF-METEO-AUTh; m/s) and simulated surface current vectors (m/s), respectively on (a)-(b) 05/07/17, (c)-(d) 20/07/17, (e)-(f) 10/10/17 and (g)-(h) 11/12/17.

4. Conclusions

The eutrophication events (e.g., "Dirty Sea mucilaginous aggregates" in June-July 2017 and "Red Tide" in December 2017), described in the study, were mainly associated with the dominance of southerly winds, which affect the ocean circulation over the NTG in three ways: 1) they confine the surface waters in the northern parts of the NTG (central- and inner-Gulf) separating the waters masses between the northern and southern regions, 2) they contribute to the northward spreading of nutrient-rich brackish waters towards the northern parts of the Gulf, originated from the main rivers of Thermaikos (Axios and Aliakmonas); the phytoplankton species of the different habitat communities of S1, S2 and S3 were highly connected while S1, located closer to the river deltas showed the largest species pool including nutrient opportunists (Vallina *et al*. 2014), and 3) they impose an anticyclonic circulation, especially in the inner- and central-Gulf weakening their renewal process, which is mainly associated with cyclonic circulation that supplies the NTG along the eastern coasts with clearer Aegean Sea Waters (ASW; Hyder *et al*. 2002; Krestenitis *et al*. 2012; Androulidakis *et al*. 2021). The renewal events were mainly related to northerly winds that enhance the spreading of the coastal waters and dispersing abundant phytoplankton towards the southern parts of the NTG (outflow) and eventually towards the open sea of the Aegean. More data and analyses over the 2017-2020 period are also available confirming the prevailing met-ocean conditions during the eutrophication and renewal periods.

References

Androulidakis Y., Kolovoyiannis V., Makris C., Krestenitis Y., Baltikas V., Stefanidou N., Chatziantoniou A., Topouzelis K., Moustaka-Gouni, M. (2021). Effects of ocean circulation on the eutrophication of a Mediterranean gulf with river inlets: The Northern Thermaikos Gulf. Continental Shelf Research 221, 104416.

Balopoulos E.T., Friligos, N.C. (1993). Water circulation and eutrophication in the northwestern Aegean Sea: Thermaikos Gulf. Journal of Environmental Science & Health Part A 28(6), 1311-1329.

Genitsaris S., Stefanidou N., Sommer U., Moustaka-Gouni M. (2019). Phytoplankton blooms, red tides and mucilaginous aggregates in the urban Thessaloniki Bay, Eastern Mediterranean. Diversity 11(8), p.136.

Vallina S.M., Follows M.J., Dutkiewicz S., Montoya J.M., Cermeno P., Loreau, M. (2014). Global relationship between phytoplankton diversity and productivity in the ocean. Nature communications 5(1), 1-10.

Hyder P., Simpson J.H., Christopoulos S., Krestenitis Y. (2002). The seasonal cycles of stratification and circulation in the Thermaikos Gulf Region of Freshwater Influence (ROFI), north-west Aegean. Continental Shelf Research 22(17), 2573-2597.

Krestenitis Y.N., Kombiadou K.D., Androulidakis, Y.S. (2012). Interannual variability of the physical characteristics of North Thermaikos Gulf (NW Aegean Sea). Journal of Marine Systems 96, 132-151.

Stefanidou N., Genitsaris S., Lopez-Bautista J., Sommer U., Moustaka-Gouni, M. (2019). Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experimental heat shock and changing salinity. Oecologia 191, 461–474.

Vokou D., Giannakou U., Kontaxi C., Vareltzidou S. (2018). Axios, Aliakmon, and Gallikos Delta Complex (Northern Greece). In: “The Wetland Book”, Finlayson C., Milton G., Prentice R., Davidson N. (eds). Springer, Dordrecht. p. 1137–1147. doi:10.1007/978-94-007-4001-3_253

SEISMIC EVIDENCE OF FLUIDS IN THE SEDIMENTS OF THE SEMI-ENCLOSED GULF OF GERA (LESVOS, GREECE)

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Abstract

Various types of acoustic anomalies have been observed in the Gulf of Gera (Lesvos Island) during a shallow seismic survey, indicating the presence of fluids in the sediment pores. The observed acoustic signatures were in the form of acoustic turbidity, enhanced reflectors, acoustic masking, acoustic heterogeneity, reverberations and velocity pull downs. These anomalies, which appear in a large area of the gulf, have been grouped in three regions, with each of those groups associated with certain fluid acoustic signatures, depth of occurrence and relation to specific stratigraphic and structural features. The fluids are probably biogenic methane gas; however, some of those may have hydrothermal origins, as fluids appear to leak through a well-developed fault zone in the NE part of the gulf. The fluid distribution might be also related to the extensive fields of biogenic mounds found in both the highstand and transgressive sediments of the gulf. In order to identify the exact nature and origins of these fluids further, targeted sedimentological and geochemical studies should be undertaken.

Keywords: *Gas-charged sediments, hydrothermal fluids, sub-bottom sediment structure, seismic survey, Lesvos Island*

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1. Introduction

The presence of fluids in marine sediments is common and widespread in different oceanographic and geological settings (Judd & Hovland, 2007). Although methane gas has been reported as the most important and abundant fluid in the pores of sub-bottom sediments, submarine groundwater, hydrothermal and petroleum fluids are also found in small quantities. A common characteristic manifesting the presence of fluids in the sediments are acoustic anomalies in sub-bottom profiling records. However, since there are considerable differences in the characteristics (e.g., frequency, energy) of the acoustic (seismic) profiling systems used, the fluids can show a variety of seismo-acoustic signatures (e.g., Mathys *et al*. 2005). Coastal marine environments may host large quantities of fluids, with their origin depending on the environmental setting/conditions and the geology of the surrounding area (Roy *et al.* 2019). In Greece, fluids (usually methane gas) have been observed in the sediments of various shallow marine environments (Papatheodorou *et al*. 1993; Hasiotis *et al*. 1996), although groundwater (Christodoulou *et al*. 2003; Karageorgis *et al*. 2011) and hydrothermal seepages (Dando *et al*. 1995) have been also occasionally observed. This paper presents evidence of various seismo-acoustic signatures of fluids in the semi-enclosed Gulf of Gera (Lesvos, Greece) and discusses their spatial distribution, depth of occurrence, relation with the main stratigraphic and structural features and potential origin.

The Gulf of Gera is a shallow (< 19 m water depth) semi-enclosed embayment located in SSE Lesvos (Figure 1), which is connected to the Aegean Sea through an elongated narrow channel. The inner gulf is separated from the deeper channel by a ramp reaching 10 m near the gulf entrance. The main sediment inputs are from seasonal streams/small rivers, with Evergetoulas River being the most important source. The surrounding land shows various rock formations and fault systems, some of them active (Pavlides *et al*. 2009). A thermal spring is found at the NNE coast (Lambrakis & Stamatis, 2008). Previous studies have focused on the sedimentology and geochemistry of the gulf (Anagnostou & Sioulas, 1989), the sub-bottom structure (Manoutsoglou *et al*. 2018a) and the intriguing seabed mounds found on the seabed (Manoutsoglou *et al*. 2018b).

2. Materials and Methods

The seismic (acoustic) data (Figure 1) were acquired in 2017 using the research vessel of the University of the Aegean (R/V Amfitriti). An Applied Acoustics sub-bottom profiler (SBP) was used, consisting of a CSP-D 700 Joules energy source, a AA251 Boomer plate mounded on a CAT-200 catamaran and a 12-element hydrophone. The research vessel speed was maintained at about 4 knots and positioning was provided by an RTK-DGPS (Topcon HiPer). A total length of about 150 km (covering almost 36 km^2 of the gulf) of seismic profiles were collected using the SonarWiz 6 acquisition and processing software. SBP data processing followed a standard single-channel seismic processing procedure, including bandpass filtering, swell filtering, bottom tracking, gain filters and digitization of specific horizons and features. ArcGIS 10.2 was used for mapping purposes.

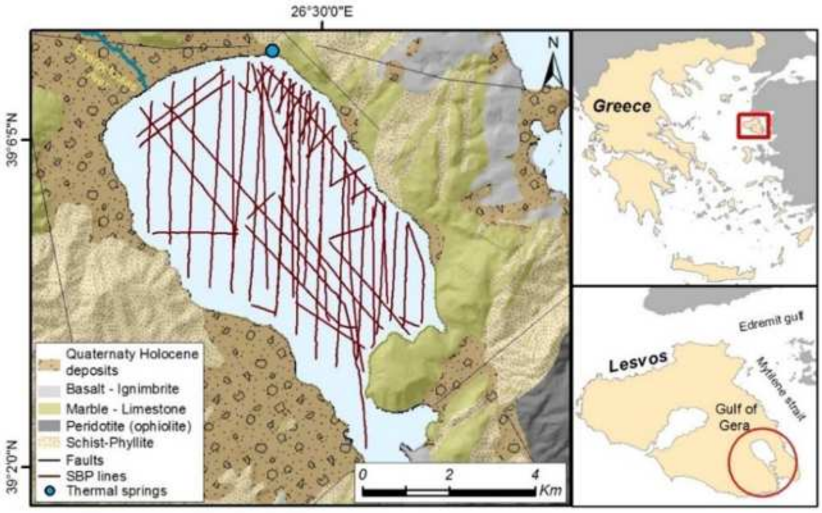

Figure 1. Location of Lesvos island and the Gulf of Gera, also showing the SBP track lines.

3. Results

A maximum penetration of ~65 m under the seabed was achieved during the seismic survey, although this was reduced significantly in areas with water depths shallower than about 10 m. Analysis of the profiles revealed four seismic units (SU) (Figure 2). SU1 is acoustically semi-transparent with few faint internal reflectors indicative of an almost homogeneous surficial layer with few slightly coarser interlayers. The surficial echo is wavy due to numerous small, mounded structures (up to 2.5m in height) that appear on the seafloor in areas with water depths deeper than ~12 m. SU1 has a maximum thickness of ~4 m, becoming more indistinct shallower than ~13-15 m and is interpreted to correspond to the Holocene high stand system tract (HST). It overlies unconformably the SU2 which show wavy reflectors of low to medium intensity. The thickness of SU2 is up to ~8 m and has been interpreted to consist of transgressive sediments (TST) deposited during the last stage of the Holocene transgression. The combined thickness of SU1 and SU2 is usually less than 10 m. Manoutsoglou *et al* (2018b) presented evidence that the wavy surficial echo of SU1 is due to mud mounds that contain a plethora of biogenic fragments and benthic assemblages (mainly bivalves) in a fine-grained matrix, which are confined to the central area of the gulf. Similarly, the wavy reflectors of SU2 resemble those found on the present seafloor and have been interpreted as buried mounds of similar origin developed during the transgression (Manoutsoglou *et al*., 2018a, 2018b).

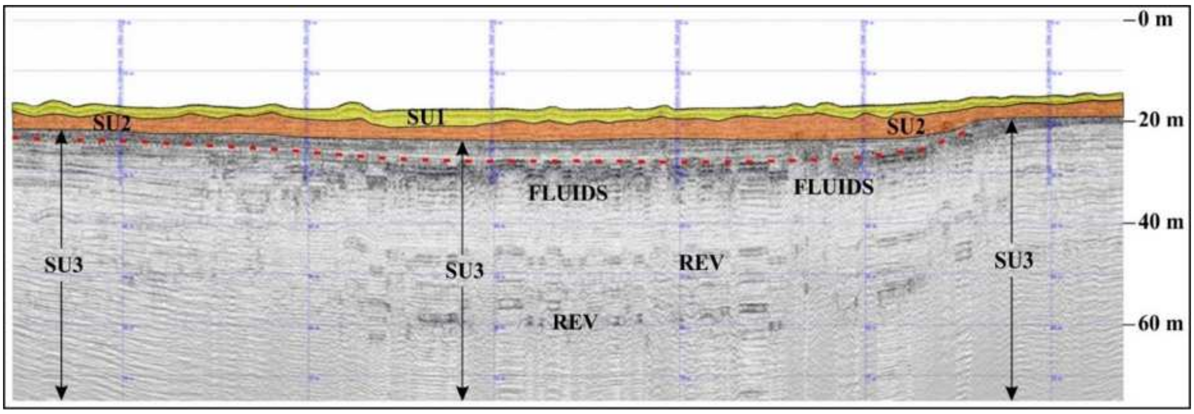

Figure 2. Seismic units SU1, SU2 and SU3 in the Gulf of Gera (dashed red line: inferred base of the lake sedimentary units over acoustic anomalies implying to fluids; REV: reverberation).

The SU3 observed below the SU2 shows parallel reflectors of medium to high intensity suggesting consolidated and/or coarser stratified sedimentary layers. It is locally more than 40 m in thick A number of unconformities are observed within SU3, suggesting low stand sediments as well as older sedimentary units. The top of SU3 appears as a concave seismic reflector; this may indicate the deposition of paleo-lake sediments in the eastern and deeper part of the gulf. An extended fault-zone oriented parallel to the coastline is observed at the NE part of the gulf, affecting SU3 and creating complex patterns. This zone comprises numerous closely spaced faults, which are synthetic and antithetic to the onshore fault systems and locally form small graben or horst structures. Finally, SU4, is interpreted as the acoustic basement; it rarely appears in the profiles (only close to the shoreline) and not found to outcrop. The signal penetration into SU4 is low, so the internal structure can barely be recognized. Nevertheless, its upper erosional surface (where imaged) appears as an uneven high amplitude reflector.

A plethora of acoustic anomalies have been detected in the sediments almost all over the gulf (except for its southwestern part), which are indicative of the presence of fluids within the sediment pores. Since the acoustic manifestation of fluids has been studied mostly for gassy sediments, the general nomenclature of Judd & Hovland (2007), Toth *et al.* (2014), Jasniewicz *et al*. (2019) and references therein are used to describe the observed acoustic characteristics as follows. (i) *Acoustic turbidity*: scattering of acoustic signal producing chaotic reflections of varying spatial extent often smearing or blanking sub-bottom layering (the latter in the case of a very coherent/strong top reflector (curtain)). When acoustic turbidity is of very small (local) extent and the surrounding reflectors are visible, then the terms *acoustic turbidity pockets or plumes* are used. (ii) *Enhanced reflectors*: individual high amplitude reflectors (increased reflectivity due to high concentration trapped fluids in a specific horizon), often observed in association with acoustic turbid zones and masking. (iii) *Acoustic masking*: blanking of underlying reflectors, beneath layers/areas of high fluid concentration (acoustic turbid zones, enhanced reflectors) that cause high signal attenuation and/or high reflection. (iv) *Acoustic heterogeneity*: sudden change of scattering or reflectivity due to changing sound speed resulting from altering fluid concentration and distribution. Penetration is not prohibited by the horizons/areas containing fluids, however acoustic blurring is obvious. (v) *Reverberation (or ringing)*: prolongation of the acoustic signal, which is detected as closely spaced strong multiple reflectors with progressively decreasing amplitude. (vi) *Velocity pull-down*: a downward bending of apparently horizontal reflectors, usually next to the acoustically blanked areas, due to velocity reduction in the overlying sediments coantaining fluids (gas).

Acoustic anomalies have been detected on almost every profile in the dataset and they were grouped in three regions (I-III), each one related with certain fluid acoustic signatures, depth of occurrence and association with specific stratigraphic and structural features (Figure 3a).

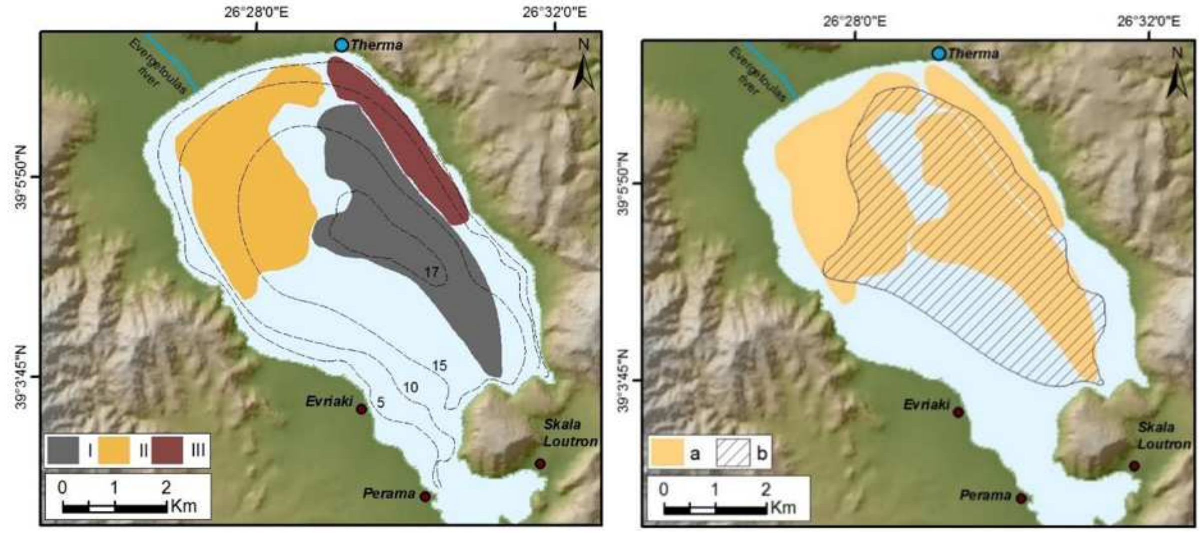

Figure 3. Distribution of the three regions with various acoustic signatures of fluids (left). General fluid distribution (a) and observed aerial extent of the buried micro-relief (b) (right).

In the eastern and deeper part of the gulf an extensive area (region I) of acoustic masking was detected occupying almost 7.3 km^2 (Figure 3a and 4a). Along this region the boundary between SU2 and SU3 appear as a fluid accumulation horizon in the form of enhanced reflectors or as an elongated acoustic turbidity zone that produce complete acoustic blanking to underlying horizons/features. This horizon has been interpreted as the surficial sediments of paleo-lake deposits (Manoutsoglou *et al*. 2018a). Locally, fluids leak and they are observed within SU2 in the form of small turbid pockets/plumes. Reverberations and strong multiples also appear, indicative of the increased concentration of fluids along the fluid accumulation horizon. Occasionally, under the edges of enhanced reflectors, velocity pull downs are observed.

At the north and northwestern parts of the gulf fluids appear a variety of acoustic returns (region II) but are mostly recognized due to the acoustic heterogeneity of the reflected acoustic signals (Figure 3a and 4b, c).

Also, enhanced reflectors and acoustic turbid zones (pockets and plumes) appear in the profiles. The fluids appear to fluctuate in various stratigraphic levels (down to ~40 m under the seabed, along SU3), and they can be recognized even in the base of SU1. The fluid acoustic signatures only partly constrain signal penetration. Region II occupies 7.5 km^2 and develops off Evergetoulas river.

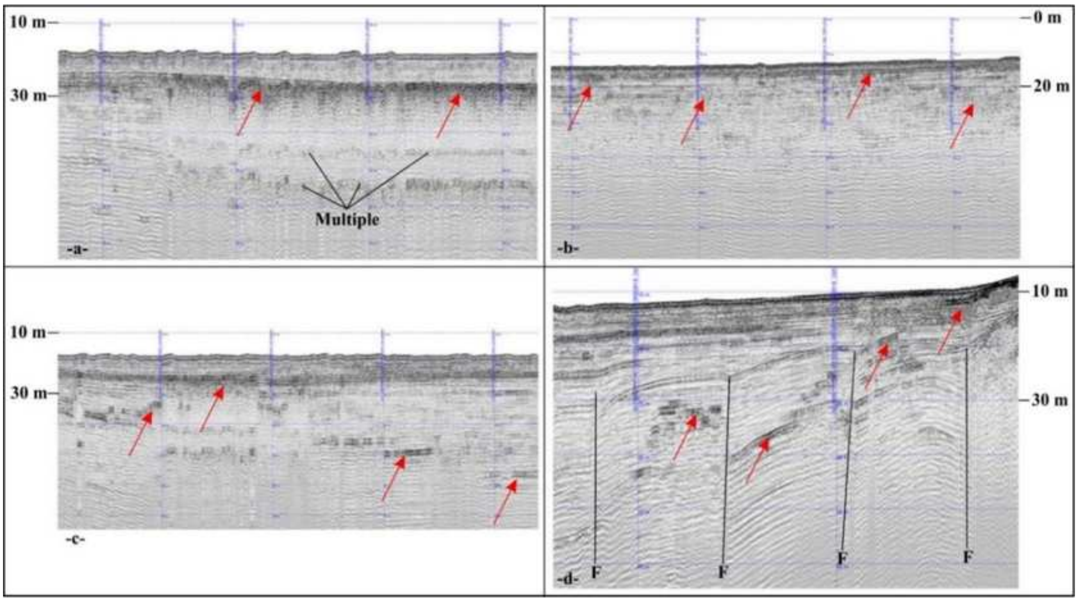

Figure 4. Fluid acoustic signatures (arrows) in the Gulf of Gera: (a) acoustic masking; (b,c) various acoustic anomalies at different stratigraphic levels off Evergetoulas River; (d) fluids associated with fault lines (F).

Acoustic turbidity (pockets), acoustic heterogeneity and small in extent enhanced reflectors were detected along the elongated fault system (region III – 2.3 km^2) that develops parallel to the coastline in the NE part of the gulf (Figure 3a and 4d). Here, manifestation of fluid seepage is a submarine spring observed within the fault zone (Manoutsoglou *et al*. 2018b).

4. Discussion and Conclusions

The analysis of seismic profiles from the Gulf of Gera revealed strong evidence of widespread fluid presence in the sediments over almost half of the surveyed area, mainly in the form of acoustic turbidity, acoustic heterogeneity, acoustic blanking and enhanced reflectors, and locally as reverberations and velocity pull downs. According to the regional geomorphology and tectonic setting the fluids can be of biogenic or hydrothermal origin or related to groundwater flow. The seismic reflectors configuration offshore of Evergetoulas river (Region II) do not show evidence of buried paleo-valleys or typical wedges related to deltaic sedimentation suggesting a small sediment supply from the river. However, the river and the surrounding drainage systems probably had delivered sufficient volumes of fine-grained sediments and organic material, whose bacterial degradation might have produced methane. Methane could have also been generated in anoxic conditions of lake sediments due to microbial decomposition of organic matter, as inferred to be the case in the Region I. The fault zone at the NE gulf is probably associated with the onshore active fault system that provides the conduit for the leakage of hot water towards the surface (thermal spring, Figure 1). Thus, the fluids distributed along the submarine fault zone (Region III), might have a hydrothermal origin, although submarine groundwater flow cannot be excluded.

The spatial distribution of fluids and the occurrence of the fault zone may be also related to sub-bottom distribution of the paleo-mound surfaces (Figure 3b) reported by Manoutsoglou *et al*. (2018a, b). Cukur *et al*. (2015) suggested that groundwater seepage along faults might be responsible for the formation and distribution of carbonate mounds, whilst Spalt *et al.* (2018) proposed that submarine groundwater discharge in coastal bays may provide a favorable environment to shellfish and may also deliver solute inputs to oyster reefs. Furthermore, Judd & Hovland (2007) suggested a causative relationship between gas seepage and the development of various benthic fauna aggregations and buildups. Similar processes might have taken place during the Holocene transgression, enhancing (along with other processes) biogenic mounds build-up.

Finally, it should be noted that in order to identify the exact nature and origins of the extensive fluid occurrences, targeted sedimentological and geochemical studies should be undertaken as well as collection of data by specialized sensors (i.e., Marinaro *et al.* 2006; Etiope *et al.* 2013).

Acknowledgements

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References

Anagnostou C., Sioulas A. (1989). A sedimentological study of the Gulf of Geras (Lesvos) and the possible anthropogenic influence in the geochemical cycle of the region. Bull Geol Soc Greece, XXIII/1:193-207.

Christodoulou D., Papatheodorou G., Ferentinos G., Masson M. (2003). Active seepage in two contrasting pockmark fields in Patras and Corinth Gulfs, Greece. Geo-Marine Letters 23,194-199.

Cukur D., Krastel S., Cagatay N., Damci E., Meydan A., Kim S.P. (2015). Evidence of extensive carbonate mounds and sub-lacustrine channels in shallow waters of Lake Van, eastern Turkey, based on high-resolution chirp sub-bottom profiler and multibeam echosounder data. Geo-Marine Letters 35, 329-340.

Dando P.R., Hughes J.A., Leahy Y., Niven S.J., Taylor L.J., Smith C. (1995). Gas venting from submarine hydrothermal areas around the island of Milos, Hellenic Volcanic Arc. Continental Shelf Research 15 (8), 913-929.

Etiope G., Christodoulou D., Kordella S., Marinaro G., Papatheodorou G. (2013). Offshore and onshore seepage of thermogenic gas at Katakolo Bay (Western Greece). Chemical Geology 339, 115-126.

Jasniewicz D., Klusek Z., Brodecka-Goluch A., Bolalek J. (2019). Acoustic investigations of shallow gas in the southern Baltic Sea (Polish Exclusive Economic Zone): a review. Geo-Marine Letters 39, 1-17.

Judd A., Hovland M. (2007). Seabed Fluid Flow: The impact on Geology, Biology and the Marine Environment, Cambridge University Press.

Hasiotis T., Papatheodorou G., Kastanos N., Ferentinos G. (1996). A pockmark field in the Patras Gulf (Greece) and its activation during the 14/7/93 seismic event. Marine Geology 130, 333-344.

Karageorgis A., Papadopoulos V., Rousakis G., Kanellopoulos Th., Georgopoulos D. (2011). Submarine groundwater discharges in Kalogria Bay, Messinia-Greece: geophysical investigation and one-year high resolution monitoring of hydrological parameters. In: "Advances in the research of aquatic environment-Volume 1", Lambrakis N., Stournaras G., Katsanou K. (eds). Springer, p. 469-476.

Lambrakis N.J., Stamatis G.N. (2008). Contribution to the study of thermal waters in Greece: chemical patterns and origin of thermal water in the thermal springs of Lesvos. Hydrological Processes 22, 171–180.

Manoutsoglou E., Kostopoulos K., Hasiotis T., Velegrakis A. (2018a). The sub-bottom structure of the Gulf of Gera (Lesvos isl.) - Preliminary results. 12th Panhellenic Symposium of Oceanography & Fisheries, Corfu, Greece, 30 - 3 June 2018, p. 88.

Manoutsoglou E., Hasiotis T., Kyriakoudi D., Velegrakis. A., Lowag, J. (2018b). Puzzling micro-relief (mounds) of a soft-bottomed, semi-enclosed shallow marine environment. Geo-Marine Letters 38, 359–370.

Marinaro, G., Etiope, G., Bue, N.L., Favali P., Papatheodorou G., Christodoulou D., Furlan F., Gasparoni F., Ferentinos G., Masson M., Rolin J-F. (2006). Monitoring of a methane-seeping pockmark by cabled benthic observatory (Patras Gulf, Greece). Geo-Marine Letters 26, 297–302.

Mathys M., Thiessen O., Theilen F., Schmidt M. (2005). Seismic characterization of gas-rich near surface sediments in the Arkona Basin, Baltic Sea. Marine Geophysical Research 26, 207–224.

Papatheodorou G., Hasiotis T., Ferentinos G. (1993). Gas-charged sediments in the Aegean and Ionian Seas, Greece. Marine Geology 112, 171-184.

Pavlides S., Tsapanos T., Zouros N., Sboras S., Koravos G., Chatzipetros A. (2009). Using active fault data for assessing seismic hazard: a case study from NE Aegean Sea, Greece. XVIIth International Conference on Soil Mechanics & Geotechnical Engineering (2–3/ 10/2009), Alexandria, Egypt.

Roy S., Senger K., Hovland M., Römer M., Braathen A. (2019). Geological controls on shallow gas distribution and seafloor seepage in an arctic fjord of Spitsbergen, Norway. Marine and Petroleum Geology 107, 237-254.

Spalt N., Murgulet D., Hu X. (2018). Relating estuarine geology to groundwater discharge at an oyster reef in Copano Bay, TX. Journal of Hydrology 564, 785-801.

Toth Z., Spiev V., Jensen J. (2014). Seismo-acoustic signatures of shallow free gas in the Bornholm Basin, Baltic Sea. Continental Shelf Research 88, 228-239.

Interannual variability of Sea Surface Temperature over the NE Mediterranean Sea

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Abstract
Long-term satellite-derived Sea Surface Temperature (SST) observations (2008-2021) were used to investigate the spatial and temporal interannual variability over the Aegean and Ionian Seas. The number of Marine Heat Waves (MHWs) showed an increase of approximately 1.7 event and 21 days of MHWs per decade; the spatially- and annually-averaged number of events was approximately 1 with less than 10 days duration on 2008 and increased to more than 3 events with almost 30 days by 2021. We also focus on the on the variability of the coastal zone that showed clear increasing trends during the 14-year period, with significant slopes especially in the northern Aegean Sea coasts. In particular, the year of 2021 showed significant high SST levels both in winter (January-February) and summer (July-August) during a prolonged period of an atmospheric heat wave.
Keywords: *Marine Heat Waves, coastal zone, SST variability, summer of 2021*
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1. Introduction

The Ionian and Aegean Seas are located over the northeastern Mediterranean Sea. Their variability of the temperature in the upper-ocean is one of the major hydrological processes that affect both ecosystem and productivity of the broader Mediterranean (Rayner *et al*. 2003; Marino *et al*. 2007). The first observations over these seas were conducted in early-1900 showing significant differences in August between the Aegean (22ºC-25ºC) and the Ionian (24.5ºC-26ºC) Seas (Carapiperis *et al*. 1952). Herein, we focus on the inter-annual variability of the Sea Surface Temperature (SST) from 2008 until today and we examine the existence of statistically significant trends and moreover the formation of Mean Heat Waves (MHWs) both in coastal and offshore regions of the Aegean and Ionian Seas. The year of 2021 is a unique case of prolonged high air temperatures that affected the ocean and biochemical conditions, especially in the northern Aegean Sea.

2. Material and Methods

The data of this study cover the period from January 2008 until August 2021 and are based on the satellite-derived L4 SST dataset that is operationally produced and distributed in near-real time by the Consiglio Nazionale delle Ricerche - Gruppo di Oceanografia da Satellite (CNR-GOS). The SST fields are daily means, and their spatial resolution is 0.01º (Nardelli *et al*. 2013). The computation of the MHWs is based on the methodology introduced by Hobday *et al*. (2016).

3. Results

Both number of MHWs and their duration revealed an annual increasing trend during the 14-year study period (Figure 1) for the entire study domain (34ºN-41ºN, 19ºE-28.5ºE).

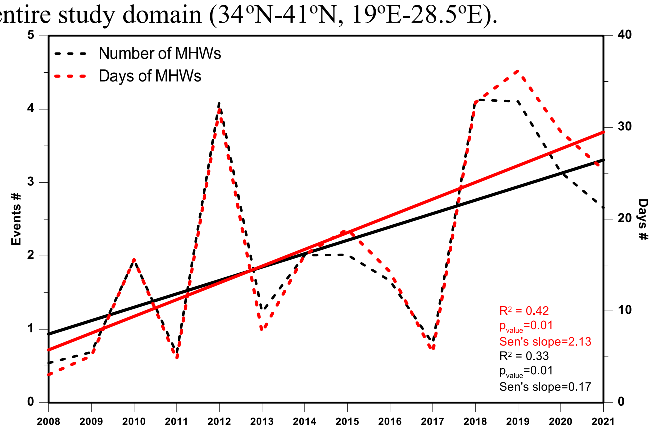

Figure 1. Number of events and duration (days) of MHWs averaged spatially and annually (2008-2021).

The linear trends of both time series are statistically significant based on the Mann-Kendall (MK) 99% significance test (p_{value}=0.01). The highest values were observed during the last four years (2018-2021); 2012 was also a year with a large number of MHWs in agreement with findings by Darmaraki *et al*. (2019) about the broader Mediterranean Sea using a different MHW definition.

The spatial distribution of the total number of MHWs is presented in Figure 2. The largest number of MHWs was observed in the northern Aegean Sea, where the accumulative period characterized as MHWs is more than 300 days; especially the entire northwestern region of the Aegean and Thermaikos Gulf can be characterized as a "hot spot" of MHWs. Smaller areas were also detected in offshore Ionian Sea, while the southeastern Aegean and especially the southern Cretan Sea (Lybian Sea) revealed SST values closer to the climatologic seasonal variability (smaller number and durations of MHWs).

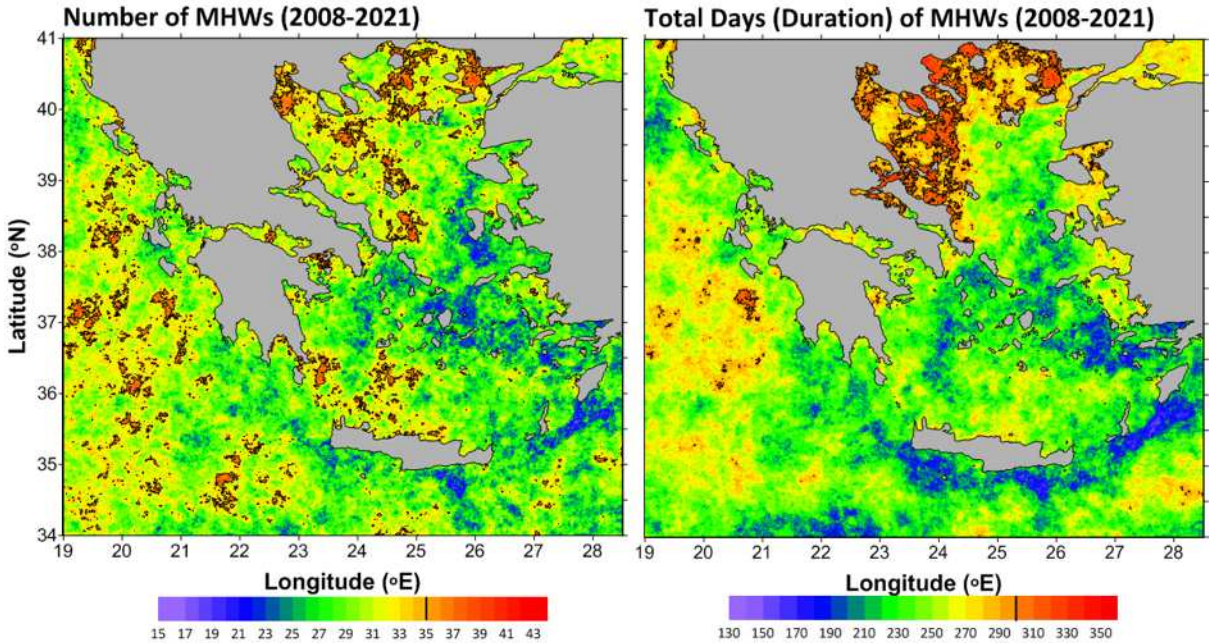

Figure 2. Spatial distribution of the total number of MHWs events (left) and duration (days) (right) as derived from the entire 2008-2021 period. The threshold of 35 events and 300 days in total is marked with a black solid line.

A clear north-south gradient of the mean coastal SST levels was detected for the entire study period (Figure 3) with relatively higher values along the Ionian coastline in comparison with the same latitudes in the Aegean (central Aegean). The southeastern Aegean coasts (e.g., Rhodes Island) revealed the highest mean values. However, the maximum SST were observed in the northeastern Aegean (>30°C) in agreement with the intensity of the MHWs (Figure 2). The lowest maximum values were detected in Cyclades (central Aegean) and along the Lesvos and Chios coastlines while most of the Greek mainland shores exhibit maximum values around 28°C. The highest variance was computed for the northern Thermaikos coasts, where both the maximum (~31°C) and minimum (~9°C) values occurred. On the contrary, very small variance during the entire study period was computed along the coasts of the eastern Aegean islands with relatively high minimum values (~14°C). The maximum values along the northern Aegean coasts (both in mainland and islands) occurred in 2021). The rest of the Aegean coasts revealed maximum SST in 2010, while 2015 was the warmest year for almost all Ionian coasts. On the contrary the coldest years for most of the Greek coasts were 2009, 2012, and 2018. With respect to the interannual SST trends, almost all the coastal areas revealed increasing and statistically significant trends based on the MK test, with largest slopes in the central Ionian and the northern part of the Aegean. The smallest increasing trends were detected in the eastern Aegean Islands, while the coasts of Asia Minor were characterized by slightly milder trends, similar to the rest of the Aegean and Cretan shores.

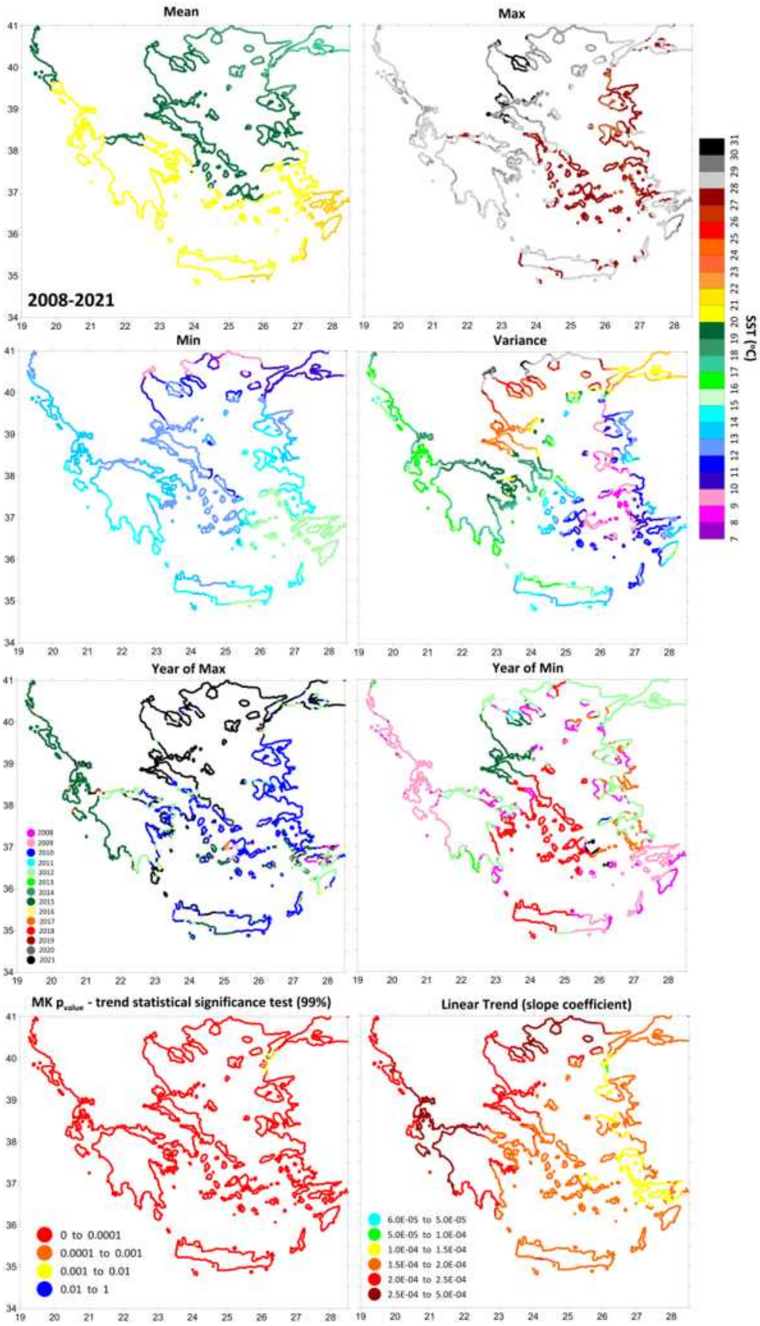

Figure 3. Mean, maximum (max), minimum (min), variance, year of max, year of min, p_{value} (MK test) and slope coefficient of the linear trend of all coastal areas as derived by the entire 2008-2021 period.

The year of 2021 was characterized by a long atmospheric heat wave that began on 28/07 until 14/08 (http://magazine.noa.gr/archives/4560) and affected the SST levels, especially in the northwestern Aegean (25-30 days) coast and Thermaikos Gulf (Figure 4). June and July are characterized by MHWs that lasted between 5 to 15 days. The western Ionian Sea also showed long MHWs during August 2021. All sub-regions of Aegean and Ionian Seas revealed large SST anomalies between July-August of 2021 values and monthly mean values derived from the rest of study period (Figure 5a). Moreover, January, February and March of 2021 also revealed SST anomalies higher than mean monthly values (Figure 5a) and the respective daily values were higher than the monthly 90% percentile derived from the entire study period (Figure 5b). Even the spatially daily mean values of 2021 for both periods (January-February and July-August) varied at the levels of the seasonal 90% percentile derived from the entire study period and area (Figure 5b).

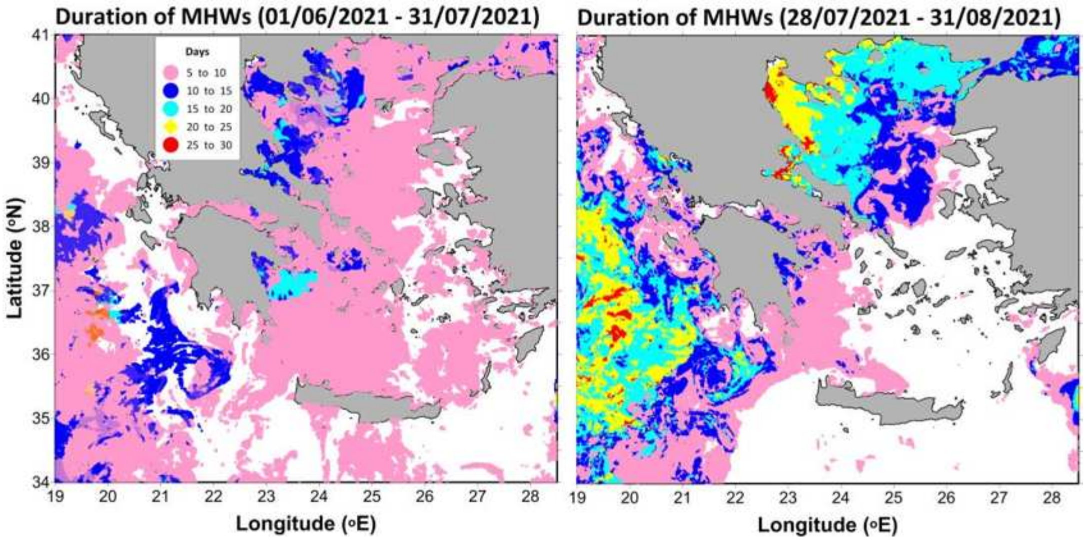

Figure 4. Spatial distribution of the MHWs duration (days) for two summer periods: June-July 2021 (left) and August 2021 (right).

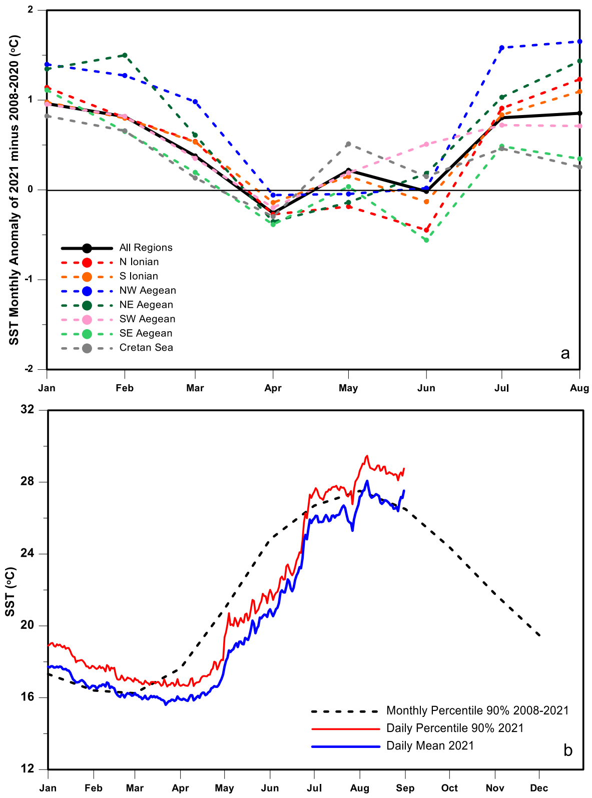

Figure 5. (a) Monthly SST anomaly computed by the difference between the 2021 mean monthly means (January to August) and the respective mean monthly means of the entire 2008-2020 period spatially averaged over each study region. (b) Daily averaged over the entire domain for 2021 and seasonal climatologic values (90% percentile of SST) derived from the entire 2008-2021 period. The dashed curve represents the mean monthly 90% percentile of SST derived from the 2008-2021 period.

4. Conclusions

The analysis based on remote-derived observations of SST showed that climate change effects may increase the number and duration of the MHWs over the Aegean and Ionian Seas during the last 14 years in agreement with previous studies that investigated the SST evolution for previous long-term periods (e.g., 1950-

2008; Skliris *et al*. 2011). The highest number of MHWs were detected over the northern Aegean Sea, with long durations especially in Thermaikos Gulf (northeastern Aegean), where very high maxima were observed. The recent year of 2021 is a characteristic example of significantly high SST levels especially over the northern Aegean and the Ionian Sea associated with long MHWs during August of 2021.

References

Carapiperis L.N. (1952). On the surface temperature of Greek waters. Geofisica pura e applicata 23(1), 153-161.

Darmaraki S., Somot S., Sevault F., Nabat P. (2019). Past variability of Mediterranean Sea marine heatwaves. Geophysical Research Letters 46(16), 9813-9823.

Hobday A.J., Alexander L.V., Perkins S.E., Smale D.A., Straub S.C., Oliver E.C., Benthuysen J.A., Burrows M.T., Donat M.G., Feng M., Holbrook, N.J. (2016). A hierarchical approach to defining marine heatwaves. Progress in Oceanography 141, 227-238.

Nardelli B.B., Tronconi C., Pisano A., Santoleri R. (2013). High and Ultra-High resolution processing of satellite Sea Surface Temperature data over Southern European Seas in the framework of MyOcean project. Remote Sensing of Environment 129, 1-16.

Rayner N.A.A., Parker D.E., Horton E.B., Folland C.K., Alexander L.V., Rowell D.P., Kent E.C., Kaplan A. (2003). Global analyses of sea surface temperature, sea ice, and night marine air temperature since the late nineteenth century. Journal of Geophysical Research: Atmospheres 108(D14), 4407.

Skliris N., Sofianos S.S., Gkanasos A., Axaopoulos P., Mantziafou A., Vervatis V. (2011). Long-term sea surface temperature variability in the Aegean Sea. Advances in Oceanography and Limnology 2(2), 125-139.

IMPLEMENTING A NUMERICAL MODEL FOR THE INVESTIGATION OF THE HYDRODYNAMICS OF SARONIKOS GULF (EASTERN MEDITERRANEAN)

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Abstract
The hydrodynamic circulation of Saronikos Gulf, an Eastern Mediterranean embayment directly impacted by Greece's capital, Athens, is examined through a high-resolution implementation of the Delft3D-FLOW numerical model. An annual cycle is simulated (November 2009 - October 2010), forced with atmospheric data from the ERA5 database, while Copernicus products (Mediterranean Sea Physics Reanalysis dataset) are used to describe the open boundary conditions. The ongoing study presently focuses on (a) calibrating the model and reproducing the seasonal thermohaline conditions and known circulation patterns prevailing in the water body, with emphasis in the vicinity of the port of Piraeus and (b) on setting up and validating a 3D hydrodynamic model, ultimately suitable for water quality modelling. To this end, temperature and salinity field measurements collected monthly by the Hellenic Centre for Marine Research from a network of ten stations, are used for the validation of the model, and simulated velocity fields produced by the model are compared to known current patterns described in the literature. This work is carried out within the context of the EMERGE project, that develops methodologies to evaluate, control and mitigate the environmental impacts of shipping emissions.
Keywords: *Saronikos Gulf, hydrodynamics, simulation, Delft3d-FLOW, EMERGE*
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1. Introduction

Interest in the functioning of the Saronikos Gulf marine ecosystem and its subbasins is ever-increasing, as are the human activities and subsequent pressure affecting it. Located in the Eastern Mediterranean (Figure 1), this semi-enclosed waterbody, is under the influence of the greater metropolitan area of Greece's capital, Athens: intense shipping and maritime transport, industry, fishing, recreation, treated sewage disposal are only some of the activities taking place in its vicinity. Their environmental footprint is superimposed to a wide spatio-temporal spectrum of coastal ocean reaction to various forcing, from fast response to high-frequency meteorological drivers to 'slower' interannual variability. In this ongoing modelling study, the important physical component of water mass circulation in Saronikos Gulf is investigated with high-resolution numerical simulation experiments. It builds on known annual thermohaline conditions and water column processes described in the literature (e.g., Kontoyiannis 2010, HCMR 2011) and aims to contribute to the understanding and quantitative description of circulation processes with high spatio-temporal detail. At the same time, as Saronikos Gulf is a case study area of the EMERGE Horizon2020 project, that develops methodologies to evaluate, control and mitigate the environmental impacts of shipping emissions (https://emerge-h2020.eu/), this work sets the basis for a validated, 3D hydrodynamic model, ultimately suitable for water quality modelling, that is a prerequisite for the success of the project.

2. Material and Methods

2.1. Hydrodynamic modelling

The FLOW module of the Delft3D modelling system is implemented in a 3D, σ-layer configuration for the investigation of the hydrodynamics of the Saronikos Gulf. Delft3D-FLOW has been validated against theoretical, laboratory and real-world applications (Gerritsen *et al.* 2008) and has been implemented in other coastal embayments in the Aegean Sea (Kolovoyiannis *et al*. 2018, Androulidakis *et al*. 2021). Model description and equations can be found in Lesser *et al.* (2004) and Gerritsen *et al.* (2008). An 83 x 101 curvilinear grid was developed, with resolution varying from about 1400m offshore and near the open boundary (Aegean Sea) to less than 300m off Piraeus, that resolves the water exchanges at the straits connecting Elefsis subbasin to Saronikos (Figure 1, left). Bathymetric data from the Hellenic Navy Hydrographic Service, supplemented with digitized data from naval charts, provided the necessary bathymetric information (Figure 1, right). In the vertical, 15 σ-layers discretize the water column. Data for the atmospheric forcing were acquired from the European Centre for Medium-Range Weather Forecasts (ECMWF) ERA5 reanalysis database: hourly air temperature, air pressure, relative humidity, precipitation, cloudiness, net shortwave radiation and x- and y-components of wind velocity on a 0.25° x 0.25° grid were used to quantify the atmosphere-sea interaction and exchange of heat, water and momentum. The Mediterranean Sea Physics Reanalysis dataset (Med MFC, Escudier *et al*. 2021), accommodated by the Copernicus Monitoring Environment Marine Service (CMEMS), provided open boundary data at a resolution of 1/24˚ (ca. 4-5 km): time series of hourly sea surface height and daily temperature and salinity profiles. Astronomical tidal forcing is considered through the amplitude and phase of 13 major tidal constituents provided by the TPXO 7.2 Global Inverse Tide Model. The annual freshwater inflow to the domain is considered at the current stage only for Kifisos river and two intermittent streams flowing into Elefsis bay. Discharge data were drawn from the platform Hypeweb, provided by the Swedish Meteorological and Hydrological Institute (SMHI). Turbulent processes are parameterized with the *k-e* turbulence closure model and a Horizontal Large Eddy Simulation (HLES) approximation. A time step of 0.5 min is used to simulate the annual cycle from November 2009 to October 2010. Model simulations are free (employing no data assimilation or relaxation to climatological or other observed quantities), in a parallel implementation of the Delft3d code on multiple cores.

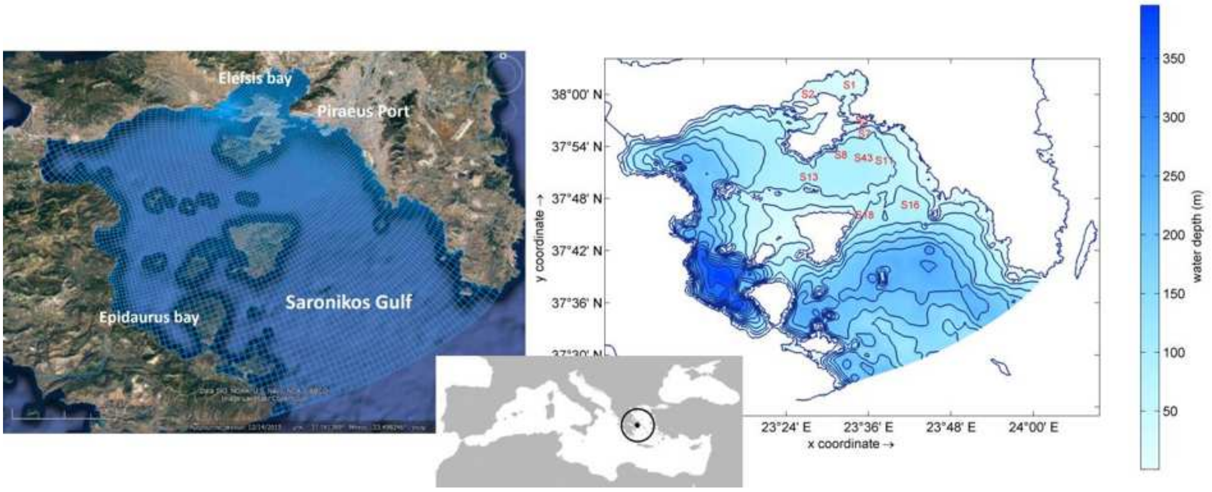

Figure 1. Saronikos Gulf: (left) computational domain and model grid and (right) bathymetry of the study area. The Hellenic Centre for Marine Research network of sampling stations is indicated.

2.2. Field data

The Hellenic Centre for Marine Research (HCMR) has been monitoring the state of the Saronikos Gulf ecosystem through several campaigns in the past. Within this framework, temperature and salinity, among other variables, were sampled monthly from the water column at a network of 10 stations located in the inner Gulf and the Elefsis and Keratsini bays (Figure 1 right) during a sampling campaign covering the November 2009 – October 2010 period. This dataset, which is described in detail in HCMR (2011), is used to assess the skill and validity of the hydrodynamic model.

3. Results

3.1. Hydrodynamic model skill assessment

The capability of the hydrodynamic model to reproduce basic observed features of the Saronikos circulation is assessed both qualitatively and quantitatively. Due to the shallower bathymetry and the relative isolation of Elefsis bay, characteristics that affect its response to the various forcing mechanisms, model results for this subbasin (represented by sampling station S1 in this manuscript) are reported separately, and in parallel with Inner Saronikos Gulf (represented by station S43).

Thermohaline conditions of Inner Saronikos Gulf are reproduced quite well for the 'cold'- mixing period (Dec-Jan-Feb-Mar-Apr and even May) for the whole water column and for all stations located there - S7 to S43 (Figures 2a, b, c, d and 3a, b). In Figures 3c and 3d, we observe that, with the onset of stratification, and for the whole 'warm' period, the model's current configuration produces temperature and salinity profiles that diverge significantly from CTD measurements for the top ~40 m of water column: temperature is underestimated by the model and conversely, salinity is overestimated, forming a simulated, weakly stratified column that permits the mixing of heat further downward and prevents the adequate heating of the top layer. The extend of this divergence suggests that there is a water mass not considered by the model's parameterization, or the boundary conditions (a case of Black Sea Water intrusion in Saronikos, as indicated by the low near-surface salinity measurements, Figure 2c and Figure 3d). Therefore, the validity of the open boundary forcing data provided by the Med MFC model needs to be verified, considering the difficulties in parameterizing water mass exchanges though the Dardanelles strait. During this period, the model tends to successfully reproduce salinity in the deeper part of the water column (below 60 m) while it overestimates temperature (Figures 2a, b, c, d and 3a, b).

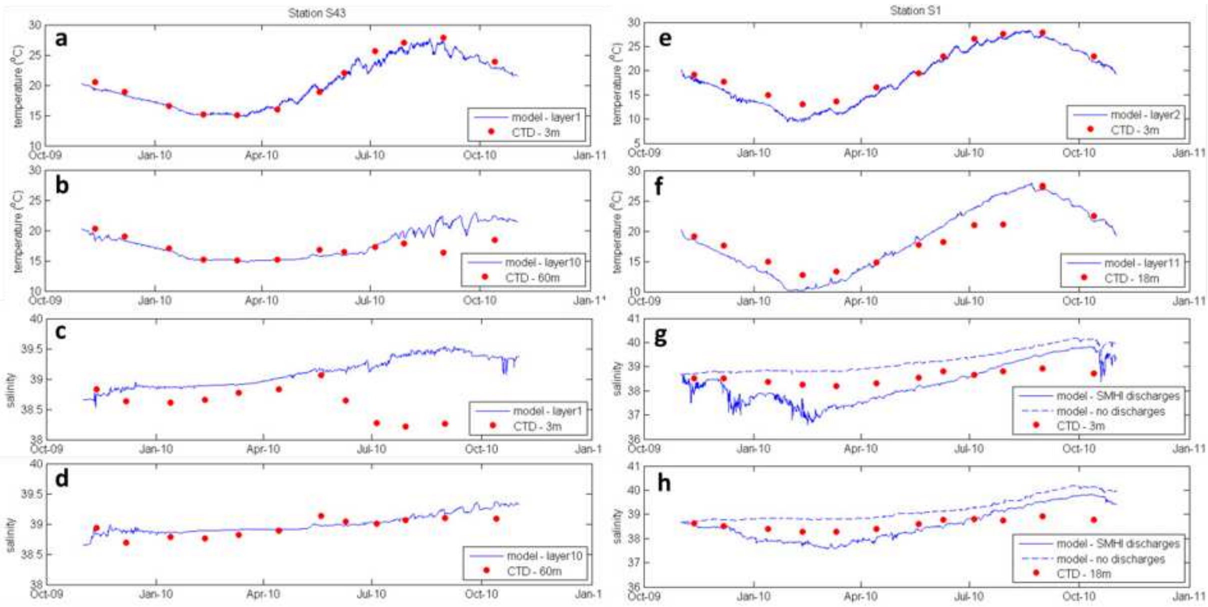

Figure 2. Hydrodynamic model output (solid line) and CTD data (points) for temperature (top two panels, in ºC) and salinity (bottom two panels, in psu) at stations S43 (a, b, c, d) and S1 (e, f, g, h) for the whole simulation period. Results are given for a surface layer (1st and 3rd panel) and a deeper layer (2nd and 4th panel) with depths corresponding to available data.

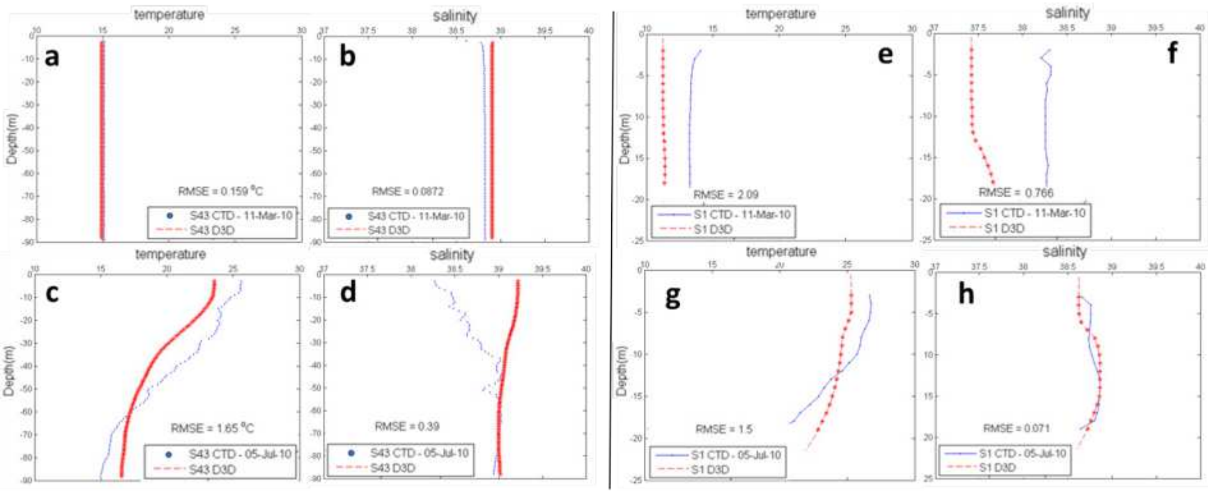

Figure 3. Vertical distribution of hydrodynamic model output (red) and CTD data (blue) for temperature (ºC) and salinity (psu) at stations S43 (a, b, c, d) and S1 (e, f, g, h) for mixing (top panel) and stratification (bottom panel) conditions.

Regarding Elefsis bay, freshwater inflow from the watershed appears to play an important role in regulating salinity, as shown in Figures 2g and h, where simulated salinity without considering any freshwater discharges and with SMHI inflows are presented, along with CTD measurements for near surface and near bottom. In the first case, salinity is overestimated; in the case of SMHI model output usage, it seems that these discharges are overestimated, leading mainly to underestimation of salinity for the 'cold-wet' period. Accurate quantification of these discharges is required for a meaningful simulation of this sub-basin. Although the model captures well the annual cycle, temperatures are underestimated during the 'cold' period (Figures 2e, f and 3e), unlike the open inner Saronikos gulf. May and June are generally reproduced successfully. In station S2 which is located at the west strait connecting Elefsis bay with Saronikos, the colder water mass below 15m is missed by the model in July and August, suggesting a two-layer exchange process not reproduced (not shown here).

3.2. Basin circulation patterns

As presently the model produces realistic results only during mixing conditions for the year 2009 – 2010 (approximately December to April), this section is restricted to this period. The velocity field in the Saronikos Gulf is indeed complex, with meandering currents bounded by coastline and eddy-like structures, both cyclonic and anticyclonic. The barotropic nature of water mass circulation during mixing conditions reported in the literature (Kontoyiannis 2010) is reproduced by the model: as shown in Figure 4, where a snapshot of near surface and near bottom circulation patterns is presented, features such as the low speed (<0.1m/s) cyclonic eddy in the vicinity bounded from Salamina and Aegina islands and the coast of Attica are extending throughout the homogenous water column. This type of cyclonic circulation develops in the area when northerly winds blow, as is the case presented in Figure 4. In the insert windstick plot (Figure 4a), the time series of the wind vector at the location marked with the red circle indicates a strong northerly component. When averaged for the 10-day period presented (March 1st – March 10th), the wind time series produces a mean north-northwest wind of 1.16 m s^{-1}. Furthermore, Figure 4 indicates that water masses are flowing from the western Saronikos basin to the Inner Saronikos basin, following a path from southwest of Salamina to northeast of Aegina and getting entrained into this cyclonic eddy. Similar cyclonic formations can be identified in other parts of the basin (e.g., west of Salamina, west of Aegina), as well as anticyclonic motions (such as the one developed in Epidaurus Bay) and coastal currents.

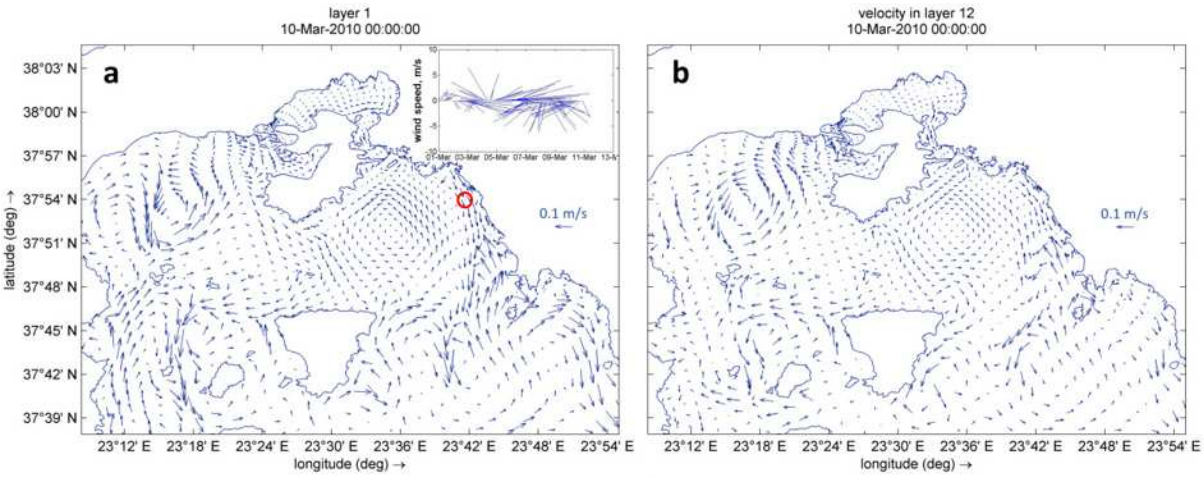

Figure 4. Simulated hydrodynamic circulation at the surface (a) and a near bottom layer (b) of Saronikos Gulf on 10 March 2010 (snapshot during mixing conditions). In Figure 4a, the insert subfigure (up right) presents a time series of the 3-hourly averaged wind velocity vector from March 1st to March 10th at the location marked with the red circle.

In the next stages of numerical experiments, the outflows from the Psittalia Wastewater Treatment Plant will be incorporated, while the meteorological forcing will be provided by a high-resolution implementation of the MEMO atmospheric model on a (coarse) 2 km grid resolution for the wider Saronikos area and a (finer) 500 m grid resolution in the vicinity of Piraeus harbor (up to Aegina Island) and around Salamis Island. These modifications, along with the incorporation of improved bathymetric data at the narrow straits connecting Elefsis bay to Saronikos and freshwater discharges (e.g., calibrated SMHI flows), and if necessary, new open boundary forcing data, are expected to improve simulation capabilities.

Acknowledgements

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References

Androulidakis Y., Kolovoyiannis V., Makris C., Krestenitis Y., Baltikas V., Stefanidou N., Chatziantoniou A., Topouzelis K., Moustaka-Gouni M. (2021). Effects of ocean circulation on the eutrophication of a Mediterranean gulf with river inlets: The Northern Thermaikos Gulf. Continental Shelf Research 221, 104416, https://doi.org/10.1016/j.csr.2021.104416.

Escudier R., Clementi E., Omar M., Cipollone A., Pistoia J., Aydogdu A., Drudi M., Grandi A., Lyubartsev V., Lecci R., Cretí S., Masina S., Coppini G., Pinardi, N. (2020). Mediterranean Sea Physical Reanalysis (CMEMS MED-Currents) (Version 1) set. Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_MULTIYEAR_PHY_006_004_E3R1

Gerritsen H., de Goede E.D., Platzek F.W., van Kester J.A.Th.M., Genseberger M., Uittenbogaard R.E. (2008). Validation document Delft3D-FLOW, a software system for 3D flow simulations. Deltares, pp.163.

HCMR (2011). Monitoring of the Inner Saronikos Gulf ecosystem under the influence of the Psittalia Wastewater Treatment Plant. Final report. Hellenic Centre for Marine Research, Athens, May 2011, pp. 217. In Greek.

Kolovoyiannis V., Zervakis V., Tzoraki O., Mamoutos I. (2018). Salinity shifts in a Mediterranean semi-enclosed coastal basin from the potential construction of a dam in its watershed. Proc. 3rd Int. Congress on Applied Ichthyology & Aq. Environment, Volos, Greece, 8-11 Nov. 2018, p 429-433.

Kontoyiannis H. (2010). Observations on the circulation of the Saronikos Gulf: A Mediterranean embayment sea border of Athens, Greece. J. Geophys. Res. 115, C06029, doi:10.1029/2008JC005026.

Lesser G.R., Roelvink J.A., Van Kester J.A.T.M., Stelling G.S. (2004). Development and validation of a three-dimensional morphological model. Coast. Eng. 51, 883-915.

PRIORITIZATION OF BEACH VULNERABILITY TO SEA LEVEL RISE: THE CASE OF SANTORINI, GREECE

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Abstract
An indicator-based framework was developed to rank beaches, at an island scale, according to their socio-economic significance and vulnerability to sea level rise. The purpose of the analysis is to prioritize/select beaches for detailed assessment of beach erosion risks and design of requisite adaptation measures. The framework was employed for the case study of Santorini Island. Beach erosion risk due to mean and extreme sea level rise under Climate Variability and Change (CV&C) was assessed using cross-shore morphodynamic model ensembles and information (geo-spatial and socioeconomic parameters) that was recorded from readily available historical satellite imagery. Appropriate indicators which are relevant, representative, and measurable were selected and multicriteria approaches were used to optimize indicator weights and to rank the beaches according to their vulnerability to CV&C. This contribution addresses the need for a simple model for managers to employ when planning strategies for the management of touristic beaches under sea level rise.
Keywords: *beach erosion, climate variability and change, morphodynamic models, multi-criteria methods, coastal management*
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1. Introduction

Beach erosion, which is projected to greatly increase under CV&C, poses significant threat to island beaches, as they have limited dimensions and sediment supply (Monioudi *et al.* 2017) and in many cases, constitute major tourism destinations (e.g., Uyarra *et al.* 2005). As a consequence, beach carrying capacity and beach suitability as environments of leisure, might be significantly reduced, resulting to significant international travel expenditure loss and affecting local and national economies (e.g., Scott *et al.* 2012). In addition, the magnitude of the problem and the potential costs of the requisite adaptation measures (e.g., Narayan *et al.* 2016), require response prioritization according to carefully selected criteria which will enable the efficient allocation of the limited, in most cases, human and financial resources. The present work attempts to contribute to the discussion by developing a prioritization framework, which is applied to Santorini, one of the most touristic Greek islands.

2. Material and Methods

2.1 Beach erosion projections

The geo-spatial characteristics (i.e., max. width, area, sediment type), human development features (i.e., accessibility, density of backshore assets) and socio-economic parameters (e.g., beach development, carrying capacity, hotels/restaurants) of all Santorini beaches (30 were identified) were recorded. The spatial characteristics of these beaches were recorded on the basis of the images and other related optical information available in the Google Earth Pro application. The ‘dry’ (subaerial) parts of these beaches were digitized as polygons bounded on their landward side by either natural boundaries (vegetated dunes and/or cliffs) or permanent artificial structures (e.g., coastal embankments, seawalls, roads, and buildings) and on their seaward side by the shoreline. From these polygons, identification of spatial characteristics of the beaches i.e., beach maximum width (BMW), length and area were estimated. Historical changes (Figure 1a) were also studied through the historical imagery available in the Google Earth Pro application. Constraints in the approach can stem from the accuracy/resolution of the (not properly georectified) images and the varying hydrodynamic conditions during the image collection that can affect shoreline delimitation. These may introduce uncertainties which, however, cannot be avoided in regional studies.

Beach retreats under CV&C were projected using 1-D (cross-shore) morphodynamic model ensembles, following the methodology described in Monioudi *et al.* (2017). Specifically, beach retreat (erosion) was assessed with regard to; (a) relative sea level rise (RSLR) and high tide and (b) 1-100 year Extreme Sea Level (ESL, i.e. storm-induced water levels superimposed on RSLR and high tide), projected for the years 2050 and 2100 under the IPCC RCP8.5 scenario. Projections of the RSLR, tide and ESL_{100} specifically for the island of

Santorini were abstracted from the JRC (Joint Research Centre) database (https://data.jrc.ec.europa.eu/collection/liscoast) (Vousdoukas *et al.* 2017). Given the large scale of the application (Island scale), the input data of the models could not be based on in situ measurements. Therefore, the models were set up using a plausible range of environmental conditions (i.e., combinations of different beach slopes, wave conditions and sediment size).

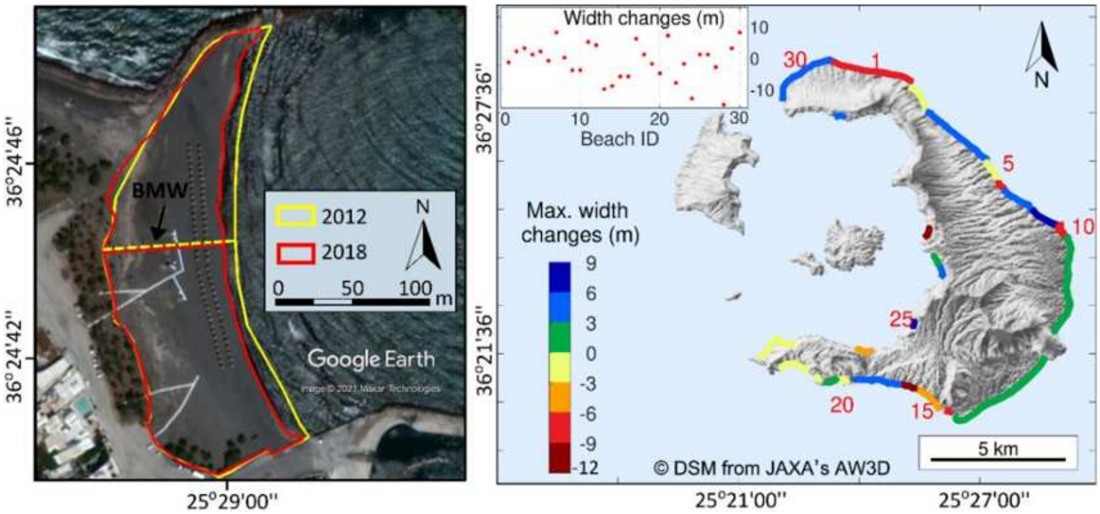

Figure 1. (a) Beach delimitation in the historical imagery (Monolithos beach (ID: 9) is used as an example); (b) historical changes in the recorded BMW. Changes shown are between the years 2012 and 2018.

2.2 Prioritization Framework Using a Multi-Criteria Approach

A prioritization framework was developed to rank beaches, at an island scale, based on their socio-economic importance and vulnerability to CV&C. The first step of the analysis concerned the selection of the most (i.e., the 10 highest ranking beaches) touristic and thus socio-economically important beaches (e.g., Andreadis *et al.* 2021). The Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) multi-criteria method was used due to the large number (30) of alternatives (beaches); according to Zavadskas *et al.* (2016), this method is much less affected by the number of alternatives and criteria (indicators) compared to other available methods. The socio-economic indicators used for this step of the analysis are: (1) the touristic activity, based on the number of hotels and restaurants at and in the vicinity of the beach; (2) beach development/usage, based on the number of recorded umbrellas and sun beds during high season (summer) period; (3) beach "urbanization" which is the density (%) of the backshore infrastructure/assets found immediately (throughout the first line in the backshore zone) behind the beach in relation to the shoreline length; (4) beach accessibility, based on the state of the road (or the absence of, in the case of beaches that can only accessed through the sea) that leads to the beach and the distance from the main road network; (5) Blue Flag awards (in 2021), which are perceived as markers of beach quality by users (Tzoraki *et al.* 2018); (6) the beach area as an indicator of the beach carrying capacity (considering 10 m^2/per person); (7) the sediment type, which is used as an indicator of the beach recreational (hedonic) value, since users mostly prefer sandy beaches. The second step of the analysis involved the prioritization of the selected beaches in terms of their socio-economic significance and their erosion risk under CV&C, using a detailed pair-wise Analytical Hierarchy Process (AHP) multi-criteria approach. For this step, additional to the aforementioned (1-7) indicators, the following were used to describe the beach erosion risk under current (today) conditions: (8) the maximum beach width (BMW), which is a crucial spatial feature, as it does not only control the beach vulnerability to erosion but also the exposure of the backshore infrastructure/assets; (9) the historical trends of erosion/accretion, based on the maximum width changes. The sediment type (indicator 7) also affects the beach erosion potential, whereas the beach "urbanization" (indicator 3) increases the exposure/ impact potential from beach retreat. The AHP procedure was repeated to rank beach vulnerability under future (CV&C) conditions; in these cases, the "width reduction", expressed as a percentage of the current BMW, was used as an indicator instead of the BMW, whereas the "asset exposure" (i.e., whether or not the backshore infrastructure/assets will be impacted under a future ESL_{100}) was used instead of the "historical trends".

3. Results

3.1 Beach erosion

The analysis of the historical imagery showed discernible decreases (erosion) of up to ~12 m in the beach maximum widths (BMWs) for 16 beaches and increases (accretion) of up to ~8 m for 14 beaches (Figure 1b), between the years 2012 and 2018. Concerning the future, due to the different conditions used in the model set ups, the ensembles produced a range of beach erosion projections. For each studied scenario, the 10th and 90th percentile of the projected range were estimated and compared with the maximum recorded beach width, resulting to the indicator "width reduction" (see section 2.2).

Table 1. The 10th and 90th percentile of beach retreat estimates by the model ensembles. Percentages of the beaches that will be retreated/inundated more than 50% of their current BMW and more than their current BMW. Numbers (N) and percentages of beaches where backshore infrastructure and assets are projected to be impacted are also shown.

Sea Level Rise (RCP8.5)			Retreat (R)/ Inundation(I) (m)		R/I to 50 % of max. width (%)	R/I to max. width (%)	Beaches with assets affected	
	Year	(m)					N	%
RSLR + tide	2050	0.30	10th	2.3	0	0	0	0
			90th	8.9	40	0	0	0
	2100	0.84	10th	8.3	23	0	0	0
			90th	24.9	83	67	12	40
ESL_{100}	2050	1.29	10th	15.4	70	20	5	17
			90th	47.8	100	83	17	57
	2100	1.88	10th	27.3	87	70	13	43
			90th	82.1	100	100	22	73

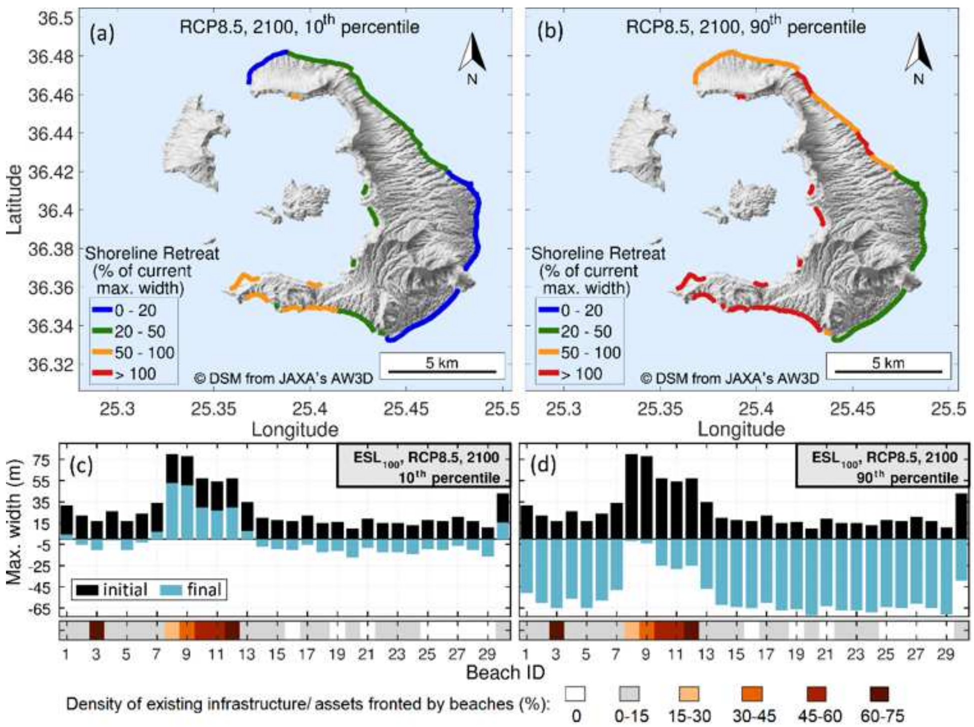

Figure 2. The percentages of the current BMWs of the 30 Santorini beaches projected to be eroded under RSLR and tide based on the (a) 10th and (b) 90th percentile of beach retreat estimates. In the lower panels, the (c) 10th and (d) 90th percentile of the shoreline retreat/inundation under an ESL_{100} are shown together with the recorded density of the backshore assets (as a percentage of the beach length). The current (initial) BMWs (black bars) are compared with those resulting from temporary inundation /retreat (blue bars); negative values indicate total beach inundation.

Under sea level rise of 0.30 m, projected for the year 2050 under RCP8.5, there could be some impacts on the basis of the high (90th percentile) projections of the morphodynamic modelling (Table 1). For the year 2100 under the same scenario, it appears that the selected beaches would be seriously affected due to the projected sea level rise (0.84 m); beach retreat is estimated between 8.3 m and 24.9 m and 67 % of the beaches might see their BMW reduced by up to 100 % (Table 1). Many of these beaches lack the accommodation space to retreat landwards and, thus, will suffer coastal squeeze without appropriate replenishment.

The 100-year ESL (ESL_{100}) in 2050 and 2100 will result in storm beach erosion of up to about 47.8 and 82.1 m, respectively under the RCP8.5 scenario. Substantial impacts are projected as early as 2050 and according to the most conservative projections, about 70 % of all beaches are projected to lose at least 50 % of their current maximum widths and 20 % to be completely eroded under the ESL_{100} (Table 1). In terms of asset exposure, at least 17 % of the beaches presently fronting assets are projected to be overwhelmed during the event. In 2100, impacts could be devastating. Under the RCP8.5 ESL_{100}, 70-100% of all beaches will be completely (at least temporarily) eroded (43–73% of the beaches fronting assets) under the low (10th percentile) and high (90th percentile) projections, respectively (Figure 2). These frontline backshore assets will sustain damages even in the case of a partial (or total) post-storm beach recovery as they are located within the beach erosion-recovery envelop.

3.2 Island Beach Prioritization

For the first stage of the analysis, in order to define the weights (or relative importance) of the indicators (1–7) adopted for the TOPSIS application, AHP was initially used to perform pairwise comparisons based on expert judgments and using a 1–9 scale (Saaty 2008). Adjustments were made to ensure the consistency of the derived pairwise matrix (Consistency Ratio, CR = 0). Following the identification of the possible pairs, suitable weights were assigned to each indicator/criterion using eigenvectors. The selected indicators are not all measured in the same units; thus, vector normalization was used to ensure uniformity and comparability. Then, the TOPSIS method was applied to estimate the preference scores (Figure 3b). The 10 selected beaches with the higher scores are depicted, in red, in Figure 3a and 3b.

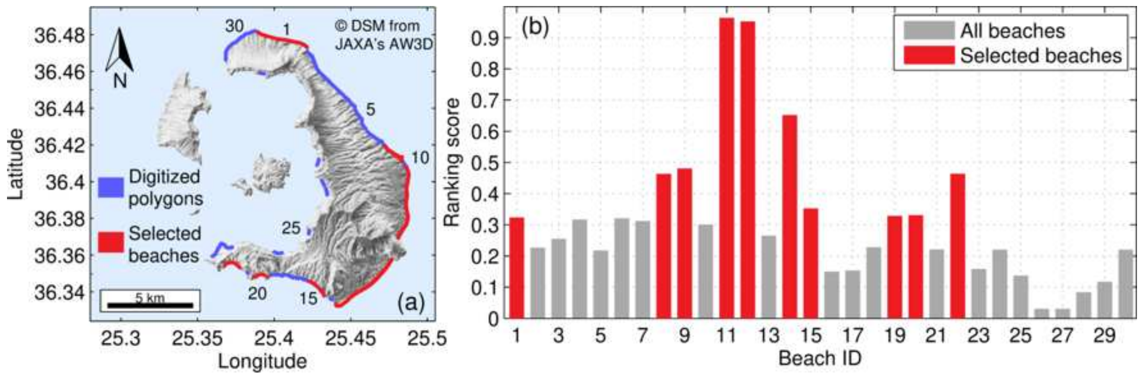

Figure 3. (a) Digitized polygons of Santorini beaches (clockwise beach numbering starting from the north) and selected beaches; (b) ranking scores of all 30 recorded beaches (TOPSIS method) according to their socio-economic significance; the 10 most highly ranked beaches are shown in the red color.

Regarding the second step of the analysis, the AHP multi-criteria approach was applied in order to rank the 10 selected beaches. The weights (or relative importance) of the indicators/criteria (1–9) were defined using the same procedure as in the previous stage. AHP was also used to perform pairwise comparisons among the alternatives (beaches) (matrix consistency was ensured) and then, using the eigenvector method, priority scales were defined for all alternatives and for each indicator/criterion. The final (global) preference score for each alternative (beach) was calculated using the Weighted Product Model (WPM). The whole procedure was repeated 5 times including the current status and the lowest (10th percentile) and highest (90th percentile) and retreat estimates under two IPCC scenarios (Table 2). Kamari beach, followed by Perissa and Vlychada beaches, consistently showed the highest scores, suggesting that these beaches are, the most socio-economically important and vulnerable to beach erosion under CV&C and, thus, should be of highest importance in the list for adaptation measures. It has to be mentioned that the above ranking, depends on the weights assigned to each indicator, which has been based on expert judgment; increasing the number of the interviewed experts will, in turn, adjust the scores.

Table 2. Ranking scores (AHP analysis) of the 10 selected beaches, regarding the beach vulnerability to CV&C. Results are for the current status and future projections under the RCP8.5 emission scenario for the years 2050 and 2100. For beach location see Figure 3a.

	Current	RCP 8.5, 2050		RCP 8.5, 2100	
		10th	90th	10th	90th
1. Baxedes	0.0644	0.0622	0.0726	0.0603	0.0658
8. Monolithos 1	0.0500	0.0551	0.0521	0.0524	0.0574
9. Monolithos 2	0.0739	0.0704	0.0666	0.0681	0.0738
11. Kamari	**0.2510**	**0.2527**	**0.2424**	**0.2455**	**0.2683**
12. Perissa	0.2323	0.2346	0.2251	0.2279	0.2472
14. Vlychada	0.0986	0.0871	0.1017	0.1033	0.0889
15. Eros	0.0662	0.0617	0.0713	0.0722	0.0612
19. Kokkini	0.0422	0.0407	0.0389	0.0395	0.0327
20. Kampia	0.0453	0.0519	0.0495	0.0501	0.0383
22. Mesa Pigadia	0.0761	0.0835	0.0798	0.0807	0.0663

4. Conclusions

The proposed framework can represent the status of island beaches, based on quantifiable geo-spatial and socio-economic characteristics and projections of beach erosion/retreat under different scenarios of mean and extreme sea level rise. It can provide coastal managers and policy makers, better insights on the challenges posed by beach erosion in island settings as well as with a framework for prioritization of adaptation responses and efficient resource allocation.

Acknowledgements

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References

Andreadis O., Chatzipavlis A., Hasiotis T., Monioudi I., Manoutsoglou E., Velegrakis A. (2021). Assessment of and Adaptation to Beach Erosion in Islands: An Integrated Approach. J. Mar. Sci. Eng. 9, 859.

Monioudi I.N., Velegrakis A.F., Chatzipavlis A.E., Rigos A., Karambas T., Vousdoukas M.I., Hasiotis T., Koukourouvli N., Peduzzi P., Manoutsoglou E., Poulos S.E., Collins M.B. (2017). Assessment of island beach erosion due to sea level rise: the case of the Aegean archipelago (Eastern Mediterranean). Nat. Hazards Earth Syst. Sci. 17, 449–466.

Narayan S., Beck M.W., Reguero B.G., Losada I.J., van Wesenbeeck B., Pontee N., Sanchirico J.N. Ingram J.C., Lange J.-M., Burks-Copes K.A. (2016). The Effectiveness, Costs and Coastal Protection Benefits of Natural and Nature-Based Defences. PLoS ONE 11(5), e0154735.

Saaty T.L. (2008). Relative measurement and its generalization in decision making why pairwise comparisons are central in mathematics for the measurement of intangible factors the analytic hierarchy/ network process. Rev. R. Acad. Cien. Serie A. Mat. 102, 251–318.

Scott D., Simpson M.C., Sim R. (2012). The vulnerability of Caribbean coastal tourism to scenarios of climate change related sea level rise. J. Sustain. Tour. 20, 883–898.

Tzoraki O., Monioudi I., Velegrakis A., Moutafis N., Pavlogeorgatos G., Kitsiou D. (2018). Resilience of touristic island beaches under sea level rise: A methodological framework. Coast. Manag 46, 78–102.

Uyarra M.C., Côté I.M., Gill J.A., Tinch R.R.T., Viner D., Watkinson A.R. (2005). Island-specific preferences of tourists for environmental features: implications of climate change for tourism-dependent states. Environ. Conserv. 32, 11–19.

Vousdoukas M.I., Mentaschi L., Voukouvalas E., Verlaan M., Feyen L. (2017). Extreme sea levels on the rise along Europe's coasts. Earth's Future 5(3), 304–323.

Zavadskas E.K., Mardani A., Turskis Z., Jusoh A., Nor K.MD. (2016). Development of TOPSIS Method to Solve Complicated Decision-Making Problems — An Overview on Developments from 2000 to 2015. Int. J. Inf. Technol. Decis. Making 15(03), 645-682.

AUTOMATED 2-D SHORELINE AND WAVE RUN-UP DETECTIONS FROM HIGH FREQUENCY OPTICAL DATA. EXAMPLE FROM AN URBAN PERCHED BEACH: AMMOUDARA, HERAKLEION - CRETE.

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Abstract
Shoreline and wave run-up positions and their changes are crucial morphodynamic parameters for coastal engineering works and coastal zone management. In this contribution, the variability of the shoreline and wave run-up positions of an urban perched beach (Ammoudara, Herakleion - Crete, Greece) is presented for a 4-month energetic wave period. Specialized algorithms capable to detect/record the 2-D shoreline and wave run-up/swash maxima positions with high spatio-temporal resolution, were developed and used in geo-rectified time series of optical images derived from a Beach Optical Monitoring System (BOMS). It was found that the maximum shoreline and wave run-up positions at representative examined cross-shore sections of the beach ranged at 15 m and 5 m, respectively. No significant shoreline changes have been recorded during the 4-month energetic period; however, several beach sections show different patterns of sediment loss or gain. The proposed approach appears to provide an efficient and accurate coastal monitoring tool with high geo-spatial resolution. The latter is extremely important for beach managers, planners and policy makers, as the wave run-up plays a most significant role in the definition of the landward boundary of the Public Maritime Domain, and could define the swash maxima (i.e., the "aigialos" line).

Keywords: *shoreline detection*, *wave run-up*, *swash zone dynamics, coastal video monitoring*, *perched beaches*
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1. Introduction

The positions of the shoreline and the wave run-up (i.e., the time-varying position of the water excursion shoreward), are two morphological parameters of great importance as they form fundamental parameters of the swash zone dynamics. Swash zone is the beach zone where most of human activities are concentrated, and thus, forms a sector of high interest for coastal planners, engineers, and local authorities, while being at the same time one of the most dynamic environments on earth, as morphological changes can be very frequent, characterized by high spatio-temporal variability, being dependent on the hydrodynamic action. The positions of these 2 morphological parameters are crucial factors to be considered for i) the design of coastal works, ii) the prediction of the impacts of changes in the hydrodynamic regime (e.g., storm surges, waves, mean sea level); and iii) the effective design/planning of coastal management schemes (Vousdoukas 2014). At the same time, these morphological parameters form regulatory boundaries. The shoreline position defines the beach carrying capacity (i.e., the number of visitors/tourists that can be hosted simultaneously in a beach), whereas the swash maxima (i.e. the maximum recorded wave run-up) of a beach forms a reference line (defined as the "aigialos line" in Greek) beyond which a 'setback' zone of no further development/constructions are allowed according to the national (Greek Law 2971/2001) and European legislation (e.g., the ICZM Protocol to the Barcelona Convention (Art. 8(2)) and the EU Directive 2014/52/EU).

Nevertheless, the traditional mapping or/and image processing techniques are not able to provide accurate records of high frequency. High-resolution satellite images, which are commonly used for the extraction of such morphological parameters in large scale, are not only characterized by high cost, but also from low temporal coverage, while in many cases images could not be recorded due to physical restrictions (e.g., cloudiness occurring during extreme storminess). On the other hand, repeated topographic records in the field through classic leveling/positioning techniques, require dedicated human efforts especially during extreme storm events. In order to provide predictions of the shoreline and wave run-up positions under specific conditions of hydrodynamic action, coastal engineers tend to develop/use numerical hydro-morphodynamic modeling techniques, based on the mathematical expressions/algorithms used to parameterize the wave-current interactions and the sediment dynamics (e.g., Roelvink *et al*. 2010; Karambas 2012). However, as nearshore hydro-morphological changes are based on complex processes-response mechanisms driving the swash zone, operating at various spatio-temporal scales (Suanez *et al*. 2015), the application of such modeling techniques is not only a complicated task requiring a number of various input parameters measured in the field (topo-bathymetric, granulometric and hydrodynamic data), but are also based on the calibration process (i.e. validation of the model projections/output with the real recorded conditions), requiring the positions of such morphological parameters (normally before and after the simulated hydrodynamic event). In order to simplify this process, coastal engineers commonly use empirical expressions based on previous parameterization efforts. Shoreline movement is estimated through widely accepted methodologies (e.g. the Bruun rule (Bruun 1954) and the EBP method (Dean 1990)), whereas the estimation of wave run-up excursion is based on theoretical formulae that suggest that the excursion is controlled by the incident wave energy and the nearshore seabed slope (e.g., Holman 1986; Stockdon *et al*. 2006) Over the recent years, emphasis has been given to the development of image processing algorithms/techniques, capable to record/monitor with high accuracy specific coastal features of interest on specialized optical datasets deriving from coastal video monitoring systems (e.g., Vousdoukas *et al*. 2010; Vousdoukas 2014; Velegrakis *et al*. 2016).

2. Methodology

Ammoudara is a microtidal perched beach (i.e. fronted by a natural submerged reef, Gallop et al 2012), with a length of 6.1 km and ranging widths (between 15 and 70 m). The main morphological characteristic of this perched beach is the submerged fragmented reef (with varying crest depths between 1.5 – 0.2 m) located at the eastern sector of the beach and covering a length of 3.7 km (partially shown in Figure 1a). The reef is consisting of beachrock formations, providing evidence of the old position of the shoreline, which is now submerged. It has been estimated that significant erosion has taken place over the last 60 years. Since 1960, the retreat (erosion) has been estimated as 0 - 60 m, with the longer retreats being evident at the eastern sector of the beach (Alexandrakis *et al*. 2013). However, today the beachrock reef appears to provide effective protection against beach erosion, acting as an offshore breakwater, effectively reducing the wave energy (Velegrakis *et al*. 2016).

A Beach Optical Monitoring System (BOMS - http://www.vousdoukas.com/index_video.html) was installed at the seaward roof of the Herakleion Olympic Stadium at an elevation of 26 m (Figure 1a). The BOMS consists of a station PC and 3 PointGrey FLEA-2 video cameras, set to obtain high resolution optical data (hourly 10-minute videos consisting of 3,000 images/frames, captured at the beginning of each hour during daylight). All images are corrected for lens distortion and furthermore processed by using standard photogrammetric methods and accurate positions of Ground Control Points (GCPs), collected with a Differential GPS (Topcon Hipper RTK-DGPS). The georectified/UTM-projected imagery of each hourly 10-min burst (3,000 snapshots/frames) are furthermore processed in order to generate high resolution time-stack images of the cross-shore position of the shoreline (TIMEX images, expressed as the "mean state" of the swash-backwash movement) and the swash maxima (IMMAX images – see Figure 1c) together with other optical products (for details see Velegrakis *et al*. 2016). For the purpose of this study, TIMEX and IMMAX datasets of a highly energetic (in terms of wave action) winter period have been extracted, covering a period of 168 days (14/11/2013 – 30/04/2014). However, due to video system downtime and specific cases continuous and concurrent morphodynamic and hydrodynamical information was not available for 13 days.

Two automated detectors following a similar concept were developed/used in order to record the shoreline and the wave run-up positions on each hourly extracted TIMEX and IMMAX image respectively. Both detectors are based on an algorithm using a localized kernel that progressively grows along the TIMEX and IMMAX digital image, following the high intensity zone along the feature of interest (Velegrakis *et al*. 2016; Chatzipavlis *et al*. 2019). The fully automated detectors have been tested/used by a single human operator, performing shoreline and wave run-up detections for 2002 images. When needed, manual corrections/digitization have been applied to the detectors automated output with the use of a specifically developed software/programming code. Regarding the accuracy of the records, this tends to decrease with the distance from the camera due to the increasing pixel footprint. Thus, in order to obtain records of high accuracy, detections from the proximal beach stretch (700 m long – Figure 1a) were considered in this analysis. For this section of the beach, the pixel footprint and the accuracy of detections have been estimated at about 0.25 m. The maximum shoreward movement of each cross-shore point of all the hourly detected/recorded shoreline and wave run-up lines has been extracted in order to investigate the longterm morphological behavior of the monitored section of the beach. In addition, a reference line was set/used in order to estimate the shoreline and wave run-up variability for the monitored period. For the purpose of this study the movement/variability of 7 equally spaced (every 100 m) profiles was taken into consideration (Figure 1b and 1c).

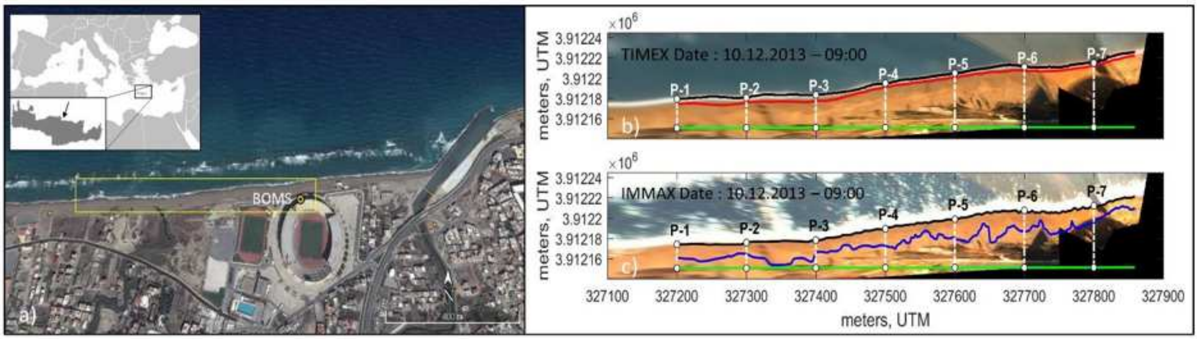

Figure 1. (a) The eastern section of Ammoudara beach and the area of high accuracy/resolution isolated from the BOMS products. (b) Example of a single shoreline detection (black line) on the plotted TIMEX image of a stormy hour; red line shows the shoreward maxima of all cross-shore shoreline points for the whole monitoring period. (c) Example of a single wave run-up detection (black line) on the plotted IMMAX image of a stormy hour; wave run-up global maxima (i.e., the "aigialos line for the monitored period - blue line). The 7 selected cross-shore points and the reference line used to estimate the distance of the selected points are also evident.

3. Results

Shoreline changes of the monitored period were found to be insignificant both when the total and the selected 7 cross-shore shoreline points are examined (Figures 1b, 1c and Figure 2–upper panel respectively). The maximum recorded shoreline variability was found to be about 5 m for the case of P-1 located at the westernmost margin of the monitored area. Cross-shore points P-1, P-2 and P-3 located at the western sector of the monitored area were found to have slightly larger long-term variability when compared to those of the eastern sector (P-4, P-5, P-6 and P-7). When it comes to the wave run-up variability of all cross-shore profiles for all records/detections (i.e., the aigialos line for the monitored period), this follows a more dynamic pattern compared to the shoreline variability. There are beach sections where the wave run-up range was found to be about 20 m (at x = 327350 m and x = 327660 m – Figure 1c), whereas in relatively close points the wave run-up range was found to be lower (e.g., 11 m at P-2, x = 327300 m and 14 m x = 327700 m). When the long-term wave run-up variability of the 7 selected cross-shore profiles is examined (Figure 2–lower panel), it is found that the points of the western sector of the examined area are slightly more sensitive to morphological changes compared to those of the eastern sector. Points located at P-1, P-2 and P-2 were found to range between 12 and 15 m, whereas P-4, P-5, P-6 and P-7 were found to have ranges of 11-13 m.

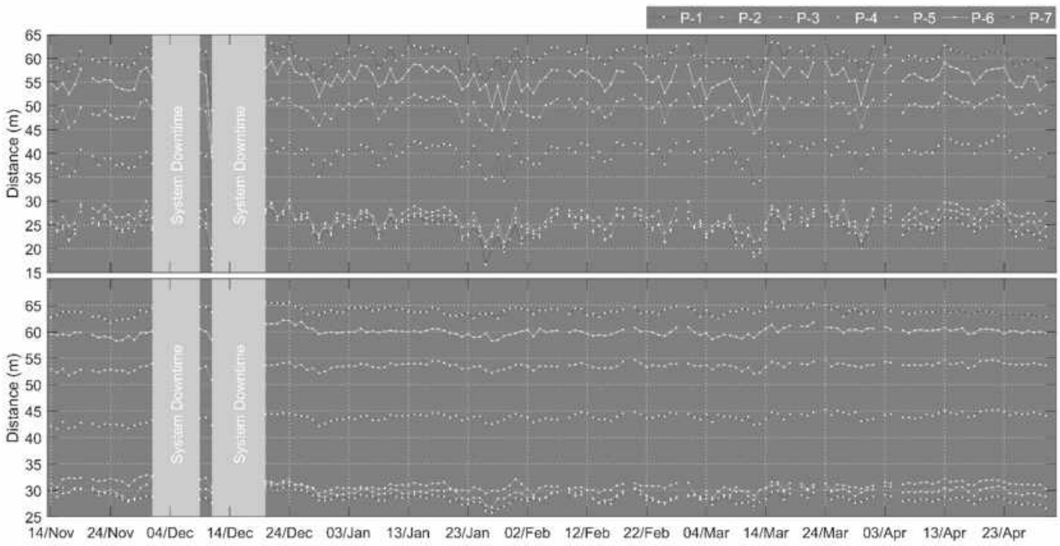

Figure 2. Variability of the distance between the reference line and (a) the daily maxima wave run-ups; and (b) the daily mean shorelines at the 7 selected cross-shore profiles in Ammoudara beach.

4. Conclusions

Shoreline and wave run-up positions of the selected monitored area in Ammoudara beach were found to have different behavior. Shoreline variability was found to be small (erosion or accretion), supporting the findings of previous studies that the submerged reef provides effective protection against beach erosion (e.g. Alexandrakis *et al*. 2013; Velegrakis *et al*. 2016). On the other hand, wave run-up variability was found to have a more dynamic tendency showing different patterns at different beach cross-shore profiles. The cross-shore profiles located at the western sector of the examined area were found to have a slightly larger wave run-up range; moreover, neighbouring cross-shore profiles were found to notable differences in the excursion range (as evident for instance at the sector between P-5 and P-6, Figure 1c). The latter could be attributed to changes in nearshore slopes that control the wave excursion.

The developed/tested methodology was able to provide an efficient and powerful tool for coastal engineers, planners and the local authorities, the method, which is based on state-of-the art image processing techniques was found to provide fast and high-frequency records of shoreline and wave run-up positions. The former can reveal beach morphological trends, whereas the latter are of extreme importance as they can accurately define (in a long-term) the “aigialos” line; both crucial parameters for coastal planning.

References

Alexandrakis G., Ghionis G., Poulos S.E. (2013). The Effect of beach rock formation on the morphological evolution of a beach. The case study of an Eastern Mediterranean beach: Ammoudara, Greece. Journal of Coastal Research SI 69, 47-59.

Bruun P. (1954). Coast erosion and the development of beach profiles. Technical Memorandum, vol. 44, Beach Erosion Board, Corps of Engineers, 82 pp.

Chatzipavlis A., Tsekouras G.E., Trygonis V., Velegrakis A.F., Tsimikas J., Rigos A., Hasiotis Th., Salmas C. (2019). Modeling beach realignment using a neuro-fuzzy network optimized by a novel backtracking search algorithm. Neural Computing and Applications 31, 1747–1763. doi: 10.1007/s00521-018-3809-2.

Dean R.G. (1990). Equilibrium beach profiles: characteristics and applications Journal of Coastal Research 7, 53-84.

Gallop S.L., Bosserelle C., Eliot I., Pattiaratchi C.B. (2012). The influence of limestone reefs on storm erosion and recovery of a perched beach. Continental Shelf Research 47, 16-27.

Holman R.A. (1986). Extreme value statistics for wave run-up on a natural beach. Coastal Engineering 9(6), 527–544. doi: 10.1016/0378-3839(86)90002-5.

Karambas Th. V. (2012). Design of detached breakwaters for coastal protection: development and application of an advanced numerical model. Proceedings of the 33rd International Conference on Coastal Engineering 2012, 1(33), sediment.115. doi:10.9753/icce.v33.sediment.115.

Roelvink D., Reniers A.J.H.M., Van Dongeren A., Van Thiel de Vries J., Lescinski J., McCall R. (2010). XBeach model description and manual. Unesco-IHE Institute for Water Education, Deltares and Delft University of Technology. Report June, 21, 2010.

Stockdon H.F, Holman R.A, Howd P.A., Sallenger J.A.H. (2006). Empirical parameterization of setup, swash, and runup. Coastal Engineering 53(7), 573–588. doi: 10.1016/j.coastaleng.2005.12.005.

Suanez S., Blaise E., Cancouet R., Floc’h F. (2016). Empirical Parameterization of Wave Runup and Dune Erosion during Storm Conditions on a Natural Macrotidal Beach Serge. Journal of Coastal Research 75, 932-936. doi: 10.2112/SI75-187.1.

Velegrakis A.F., Trygonis V., Chatzipavlis A.E., Karambas T., Vousdoukas M.I., Ghionis G., Monioudi I.N., Hasiotis T., Andreadis O., Psarros F. (2016). Shoreline variability of an urban beach fronted by a beachrock reef from video imagery. Natural Hazards 83(1), 201–222. doi: 10.1007/s11069-016-2415-9.

Vousdoukas M.I., Ferreira P.M., Almeida L.P., Ferreira O., Psaros F. (2010). Coastal video monitoring system optimization using computational intelligence methods. Paper presented at the Maritime Rapid Environmental Assessment conference, Lerici, Italy, 18-22 October 2010.

Vousdoukas M.I. (2014). Observations of wave run-up and groundwater seepage line motions on a reflective-to-intermediate, meso-tidal beach. Marine Geology 35, 52-70. doi:10.1016/j.margeo.2014

UAS IMAGE QUALITY EVALUATION

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Abstract
Unmanned aerial systems (UASs) are widely used in the acquisition of high-resolution imagery in the coastal environment. The efficiency of the UAS surveys deals with many limitations (mostly related to the prevalent environmental conditions) in marine applications. The reliability and quality of the acquired imagery depend on several environmental parameters. This study proposes an image quality equation which uses four environmental variables and estimates the quality of UAS imagery in coastal areas. The image quality estimates are based on the sunglint presence, sea surface texture, water turbidity, and image naturalness. These variables have been proven to significantly affect the image quality and the reliability of the retrieved information. The sunglint effect and the waves affect mostly the sea-surface conditions preventing the visibility of the seabed, while high turbidity levels fog the water and image distortions alter the shapes of the objects captured in the imagery. The equation was applied in imagery acquired in different coastal areas and environmental conditions by different types of UAS. We acquired UAS images in optimal and non-optimal conditions, according to a UAS data acquisition protocol, to evaluate the estimates of the equation. The evaluation of the image quality estimates was performed by visual inspection along with correlation with the prevalent environmental conditions. The results showed that 80% of the imagery acquired in optimal conditions had a better image quality in comparison to their estimates.
Keywords: *UAS imagery, image quality, UAS data acquisition protocol, coastal environment*
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1. Introduction

Unmanned Aerial Systems (UASs) are widely used in the acquisition of geospatial information in marine applications. In recent years, UAS have been used in a variety of applications for mapping (Papakonstantinou *et al.* 2017; Topouzelis *et al.* 2017a), monitoring (Papakonstantinou *et al*. 2016; Topouzelis *et al.* 2017b), and management (Papakonstantinou *et al.* 2019) of the coastal and marine environment. UAS as a close-range remote sensing tool is ideal for such applications. However, the collection of reliable information in a coastal and marine environment is challenging due to several limitations related to weather conditions (e.g., wind speed, precipitation probability), oceanographic parameters (e.g., turbidity levels), and sea-state conditions (e.g., wavy sea surface) (Doukari *et al*. 2019). These limitations that might prevail in the area during a UAS survey affect the quality of the produced geoinformation (e.g., orthophoto-maps) (Doukari *et al.* 2021).

The need for the collection of high-resolution data in the marine environment and the constant update of existing knowledge led to the necessity of a UAS data acquisition protocol. The purpose of the protocol was to provide solutions to UAS limitations in marine mapping and monitoring by proposing effective techniques for flight planning and by making rules of environmental parameters and thresholds that are appropriate for UAS data acquisition. The UAS data acquisition protocol consists of three main categories: (i) Morphology of the area, (ii) Environmental conditions, (iii) Flight parameters and contributes to the identification of the optimal data acquisition conditions (Doukari *et al.* 2019). In this study, we used the theoretical information of the UAS data acquisition protocol to acquire UAS imagery in different environmental conditions and evaluated their quality using an image quality equation. The equation considers four factors (namely, sunglint presence, image texture, turbidity levels, and image naturalness) and estimates the image quality.

2. Material and Methods

2.1. Image Quality Equation

The proposed Image Quality Equation (IQE) consists of four variables: sunglint, waves, turbidity, image distortions. These variables correspond to sea-surface and water quality parameters and image quality factors that affect the most the sea-surface conditions and the reliability of the imagery. The sunglint effect and the waves are the parameters that affect the sea-surface, preventing in many cases the visibility of the seabed. The sunglint effect usually appears as very bright areas on the sea-surface while the waves affect the texture of the sea-surface. The turbidity affects the water clarity which in high levels can reduce the amount of light reaching the sea bottom resulting in inadequate lighting of the marine habitats. Image distortions like a blur and lack of naturalness affect the reliability of the acquired imagery and alter the information captured in the imagery. For the calculation of these variables, we used RGB-based indices and statistics to quantify the effect of the parameters on the image quality. The processes were performed using R and Python packages. The IQE is given by equation (1):

$$IQE = x_1 + x_2 + x_3 + x_4 \qquad (1)$$

where x_1= the number of brighter pixels, x_2= the number of pixels with high turbidity levels, x_3= the variability of the image, x_4= the BRISQUE - image quality estimation.

The values of each variable are converted to an ascending number (from 1 to N_i, where i is the number of images) and their summary is calculated per image. The calculated summaries constitute the overall quality accuracies of each image; the lower the overall accuracy number, the higher the quality of the image.

Sunglint effect: The sunglint effect on an aerial image is one of the most common issues of remotely sensed data. Sunglint is captured as light spots or extended mirror patches on the sea-surface depending on its condition. For the sunglint detection, we used a Brightness Index (BI – equation 2) (Yamashita & Yoshimura, 2008; Letu *et al.* 2014) to isolate the brighter pixels of the images which correspond to the sunglint pixels. For the isolation of the brighter pixels, we used the interquartile range (IQR) known as the Tukey fences method (Tukey 1977).

$$BI = (Red + Green + Blue) / 3 \qquad (2)$$

where Red is the digital number (DN) of the red channel, Green is the DN of the green channel, and Blue is the DN of the blue channel.

Turbidity levels: The Normalized Difference Turbidity Index (NDTI – equation 3) was used to calculate the turbidity levels of the acquired imagery. The NDTI has been used in water quality applications (Elhag *et al*. 2019), classification of ponds (Lacaux *et al.* 2007), and extraction of wetlands (Subramaniam & Saxena, 2011), using high-resolution satellite imagery. The values of the NDTI vary from -1 to 1, with higher values representing higher levels of turbidity. An adaptive threshold was used on 0.5-1 values of the NDTI images, to isolate the high turbid pixels and calculate their amount in each image. A better-quality image is assumed to be the image with a smaller number of high turbid pixels.

$$NDTI = (Red - Green) / (Red + Green) \qquad (3)$$

where, Red is the DN of the red channel, and Green is the DN of the green channel.

Image Texture: The Grey Level Co-occurrence Matrix (GLCM) method was used as one of the most common methods in image quality assessment (Gadkari 2004; Albregtsen 2008; Hall-Beyer 2017), to measure the texture of the images (Pan *et al.* 2020). An R package was used for the calculation of the statistics: mean, variance, homogeneity, contrast, entropy, dissimilarity, second moment, and correlation of the red and green band of our images, derived from grey-level co-occurrence matrices. Moreover, a Principal Component Analysis (PCA) was used to reduce the number of the texture bands. Four PCAs explained the 90% variability of the texture features. Statistical variability indicators (i.e., standard deviation, coefficient of variation) were used to examine the reliability of the results. It is assumed that the sea-surface is smoother when the variability of the texture features is lower.

Image naturalness: The Blind/Referenceless Image Spatial QUality Evaluator (BRISQUE) is a natural scene statistic-based model in the spatial domain which provides a distortion-independent measure of image quality. The model is built based on statistical regularities observed in natural scenes (Ruderman 1994). BRISQUE was performed using a python library on the acquired images. It calculates the no-reference image quality score for each image by comparing it to a default model based on natural-scene images with similar distortions. The image quality score ranges from 0 to 100, with smaller scores corresponding to more natural imagery. The BRISQUE seems to perform well in image quality assessment if you are not interested in the exact type of image distortion.

3. Results and Discussion

We examined the performance of the image quality equation on UAS imagery acquired in different days and environmental conditions. The acquisition times were separated into optimal and non-optimal according to a UAS data acquisition protocol (Doukari *et al.* 2019), which suggests the optimal values of environmental conditions like wind speed, air temperature, sun elevation angle, etc. The dataset of images acquired in both optimal and non-optimal conditions enhanced the information on the conditions that affect the quality of the UAS images in the coastal environment. The evaluation of the quality estimates was performed by visual inspection and correlation with the prevalent environmental conditions. We used a subset of imagery to perform the equation, in which the 80% of images acquired in optimal conditions had better quality estimates.

The images with higher quality estimates were acquired in times that the wind speeds were lower than 3m/s, the sun elevation angles were between the proposed angles (25-45 degrees) for the avoidance of sunglint presence and the sky was clear. The images with lower estimates were acquired in higher wind speeds and sun elevation angles higher than 45 degrees, while the sky was partly to fully cloudy. Considering these states, the sea-surface condition and the illumination of the seabed are the parameters that mostly affect the image quality.

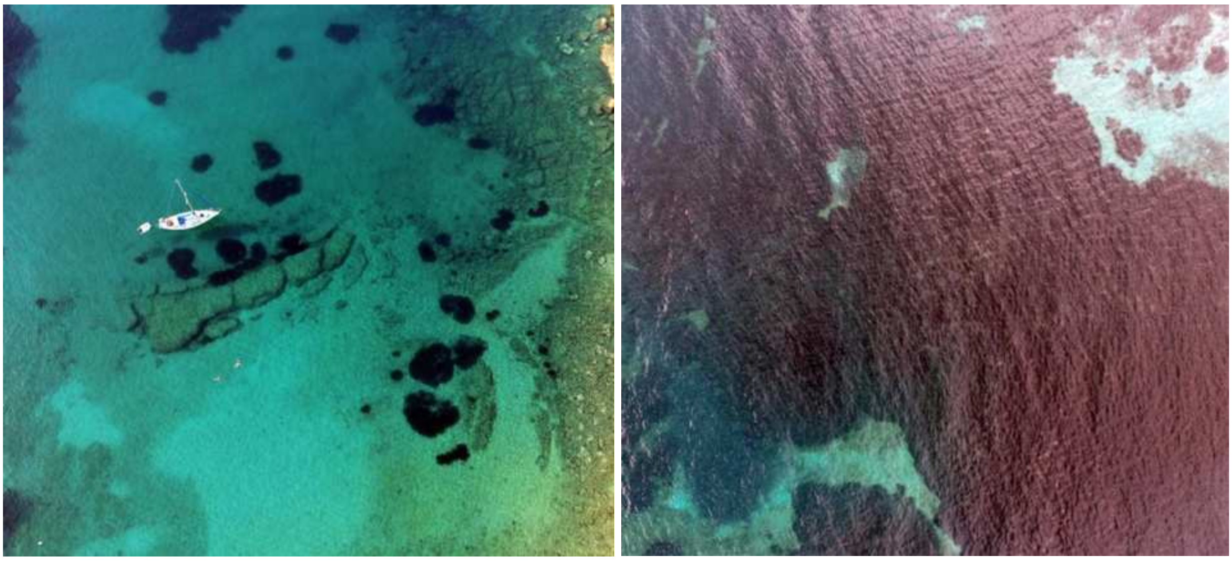

Figure 1. The higher-quality image (left) and the lower-quality image (right) of the subset, according to the image quality equation.

In Figure 1 the higher-quality and the lower-quality images, according to the estimates of the image quality equation, are presented; the quality estimate of the first image is 7 and of the second image is 21. As mentioned before, the lower the quality estimate, the higher the image quality. The first image acquired in optimal conditions, has a calm sea-surface without a sunglint presence, the water clarity is good, and the color contrasts of the image allow the distinction of the different habitats. The second image acquired in non-optimal conditions, presents wave patterns on sea-surface with sunglint areas which create brightness variations, altering the shapes of the marine habitats. The evaluation of the UAS imagery proved that the UAS surveys conducted on optimal acquisition times result in high-quality images. UAS as a widely used tool in high-resolution mapping at coastal areas, combined with the proposed equation, results in the acquisition of high-quality imagery, overcoming the UAS limitations in the coastal environment.

References

Albregtsen F. (2008). Statistical Texture Measures Computed from Gray Level Coocurrence Matrices. Image Processing Laboratory Department of Informatics University of Oslo, pp. 1–14.

Doukari M., Batsaris M., Papakonstantinou A., Topouzelis K. (2019). A Protocol for Aerial Survey in Coastal Areas Using UAS. Remote Sensing 11(16), 19. doi: 10.3390/rs11161913.

Doukari M., Katsanevakis S., Soulakellis N., Topouzelis, K. (2021). The Effect of Environmental Conditions on the Quality of UAS Orthophoto-Maps in the Coastal Environment. ISPRS International Journal of Geo-Information 10(1), 18. doi: 10.3390/ijgi10010018.

Elhag M., Gitas I., Othman A., Bahrawi J., Gikas P. (2019). Assessment of water quality parameters using temporal remote sensing spectral reflectance in arid environments, Saudi Arabia. Water 11(3), 556. doi: 10.3390/w11030556.

Gadkari D. (2004). Image Quality Analysis Using GLCM. 120 p. https://stars.library.ucf.edu/etd/187.

Hall-Beyer M. (2017). GLCM Texture: A Tutorial v. 3.0. 75 p. http://dx.doi.org/10.11575/PRISM/33280

Lacaux J.P., Tourre Y.M., Vignolles C., Ndione J.A., Lafaye M. (2007). Classification of ponds from high-spatial resolution remote sensing: Application to Rift Valley Fever epidemics in Senegal. Remote Sensing of Environment 106(1), 66–74. doi: 10.1016/j.rse.2006.07.012.

Letu H., Nagao T.M., Nakajima T.Y., Matsumae Y. (2014). Method for validating cloud mask obtained from satellite measurements using ground-based sky camera. Applied Optics 53(31), 7523-7533. doi: 10.1364/ao.53.007523.

Pan H., Gao P., Zhou H., Ma R., Yang J., Zhang X. (2020). Roughness Analysis of Sea Surface from Visible Images by Texture. IEEE Access 8, 46448–46458. doi: 10.1109/ACCESS.2020.2978638.

Papakonstantinou A., Doukari M., Stamatis P., Topouzelis K. (2019). Coastal Management using UAS and High-Resolution Satellite Images for Touristic Areas. IGI Global Journal 10(1), 54–72. doi: 10.4018/IJAGR.2019010103.

Papakonstantinou A., Topouzelis K., Doukari M. (2017). UAS close range remote sensing for mapping coastal environments. Proc. SPIE 10444, Fifth International Conference on Remote Sensing and Geoinformation of the Environment (RSCy2017), 1044418. https://doi.org/10.1117/12.2278988

Papakonstantinou A., Topouzelis K., Pavlogeorgatos G. (2016). Coastline Zones Identification and 3D Coastal Mapping Using UAV Spatial Data. ISPRS International Journal of Geo-Information 5(6), 75. doi: 10.3390/ijgi5060075.

Ruderman D.L. (1994). The statistics of natural images. Network: Computation in Neural Systems 5(4), 517–548. doi: 10.1088/0954-898X_5_4_006.

Subramaniam S., Saxena M. (2011). Automated algorithm for extraction of wetlands from IRS Resourcesat Liss III data, Int. Arch. Photogramm. Remote Sens. Spatial Inf. Sci., XXXVIII-8/W20, 193–198. https://doi.org/10.5194/isprsarchives-XXXVIII-8-W20-193-2011.

Topouzelis K., Papakonstantinou A., Doukari M., Stamatis P., Makri D., Katsanevakis S. (2017a). Coastal habitat mapping in the Aegean Sea using high resolution orthophoto maps. Proc. SPIE 10444, Fifth International Conference on Remote Sensing and Geoinformation of the Environment (RSCy2017), 1044417. https://doi.org/10.1117/12.2279140.

Topouzelis K., Papakonstantinou A., Doukari M. (2017b). Coastline Change Detection Using Unmanned Aerial Vehicles and Image Processing Techniques. Fresenius Environmental Bulletin 26(9), 5564-5571.

Tukey J.W. (1977). Exploratory data analysis. In Reading Mass.: Addison-Wesley Pub. Co. doi: 10.1007/978-3-319-39264-6_15.

Yamashita M., Yoshimura M. (2008). Development of Sky Conditions Observation Method using Whole Sky Camera. Journal of the Japan society of photogrammetry and remote sensing 47(2), 50–59. doi: 10.4287/jsprs.47.2_50.

SURFICIAL SEDIMENT DISTRIBUTION IN THE SEMI-ENCLOSED GULF OF KALLONI (LESVOS ISL)

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Abstract

A big number of surficial sediment samples was collected in the Gulf of Kalloni in Lesvos Island. Granulometric classes and statistical grain-size parameters were extracted, and a series of maps were produced showing the spatial distribution of the individual sediment fractions and the statistical parameters. The results show that the Gulf of Kalloni is actually a sink for fine-grained (muddy) sediments, whereas the strait connecting the gulf with the open sea is covered by sandy sediments due to winnowing caused by seabed currents. The distribution of the granulometric fractions revealed that streams deliver specific grain-sizes into the gulf. Locally, these sediments are transported and redistributed due to the general hydrodynamic regime. Bivariate plots of statistical grain-size parameters distinguished 3 main sediment groups deposited along the strait, off the coastline around the gulf and in the central and deeper part of the gulf. The results of this effort may contribute to the coastal zone management of this semi-enclosed system, which is considered of high ecological importance.

Keywords: *granulometric analysis*, *statistical grain-size parameters, spatial distribution, scatter plots*, *Lesvos Isl.*

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1. Introduction

Semi-enclosed coastal systems are usually sinks of fine-grained material stemming from terrestrial inputs, unavoidably enriching surficial sediments and water masses with organic material, nutrients, trace metals etc, mainly due to human activities developing over the drainage systems. Thus, locally, hypoxia or eutrophication might occur (Newton *et al*. 2014). Surficial and subsurface deposits in these systems are the carriers of sedimentary records and their study is of crucial importance for the analysis of the system dynamics. This has become more important when considering the system response to climate change and its contribution in integrated coastal zone management. Within this context this paper examines a big data set of surficial samples from the Gulf of Kalloni, aiming to expand and complete existing surficial sediment distribution towards shallower waters but also to investigate the sources of separate sediment sub-fractions deposited into the gulf.

The Gulf of Kalloni is a shallow (< 20 m) land-locked embayment located at the southwestern part of Lesvos Island (NE Aegean Sea), connected through a narrow strait with the open sea (Figure 1). The gulf is surrounded mainly by volcanic rocks, although sedimentary and plutonic formations also occupy the wider drainage system (Katsikatsos *et al.* 1986). A number of small ephemeral rivers are the main sediment inputs, especially during the rainy periods, the most important being Tsiknias, Vouvaris, Potamia and Mylopotamos draining into the northern part of the gulf (Figure 1). During winter, sudden pulses of nutrient-rich inputs from the watershed can create harmful algal blooms (Tsirtsis *et al.* 2008). Wind-forced and tidal currents drive surface water circulation (Millet & Lamy 2002, Petalas *et al.* 2020). Debenay *et al.* (2005) observed a complex wind-driven circulation varying seasonally and affecting spatial patterns and composition of macrobenthic species and foraminiferal fauna assemblages. The gulf is a sink for fine-grained sediments, since mud seems to be the main matrix, occupying mainly the deepest part, where also increased organic carbon values have been observed (Tassos 1977; Anagnostou *et al.* 1998; Gavriil & Angelidis, 2005). Mineralogical and geochemical analysis of the surficial sediments at the eastern part of the gulf showed that the main minerals are quartz, feldspar, montmorillonite, illite, kaolinite, calcite, Mg-calcite and aragonite, whose spatial variation depends on the depositional environment and onshore geology (Kelepertsis & Chatsidimitriadis 1983).

A great variety of bivalve molluscs occupy the gulf being also a main biological resource and a major fishing target for local fishermen (Voultsiadou *et al.* 2010). Due to the intense biogenic production calcium carbonate content attains relatively big values all over the gulf, especially towards shallower waters (Tassos 1983). The seafloor has an irregular morphology mainly at the central part of the gulf, related to low-relief mounds of a biogenic origin (oyster build-ups) (Chronis *et al.* 2014).

2. Materials and Methods

Seventy four (74) sediment samples were collected deeper than ~5 m depth with a Van Veen grab (Figure 1). Grain size analysis included 69 samples, since 5 samples collected over the reef structures contained only oysters and pieces of biogenic assemblages with no supporting matrix. Visual observation of all the grab samples revealed sediments loaded with abundant biogenic fragments (coarser than sand) in a fine-grained matrix. During laboratory sample treatment, the coarser (> 1 mm) biogenic fragments were removed prior to granulometric analysis. Wet-sieving was used for the separation of fines from coarser sediments. The pipette method was applied for the grain-size analysis of the fine sediment fraction and dry sieving for the coarse fraction (Folk 1980). Small biogenic fragments <1 mm), wherever possible were removed from the sieves. The statistical grain-size parameters were extracted by Gradistat in phi (Ø) s (Blott & Pye 2001). Comparison of the Folk's graphic and moment methods showed a very good correlation (99.5% for mean size, 94% for standard deviation (sorting), 88.4% for skewness and 68% for kyrtosis), thus the results of the graphic method were finally selected for plotting and interpretation of the sediment distribution using ArcMap 10.1.

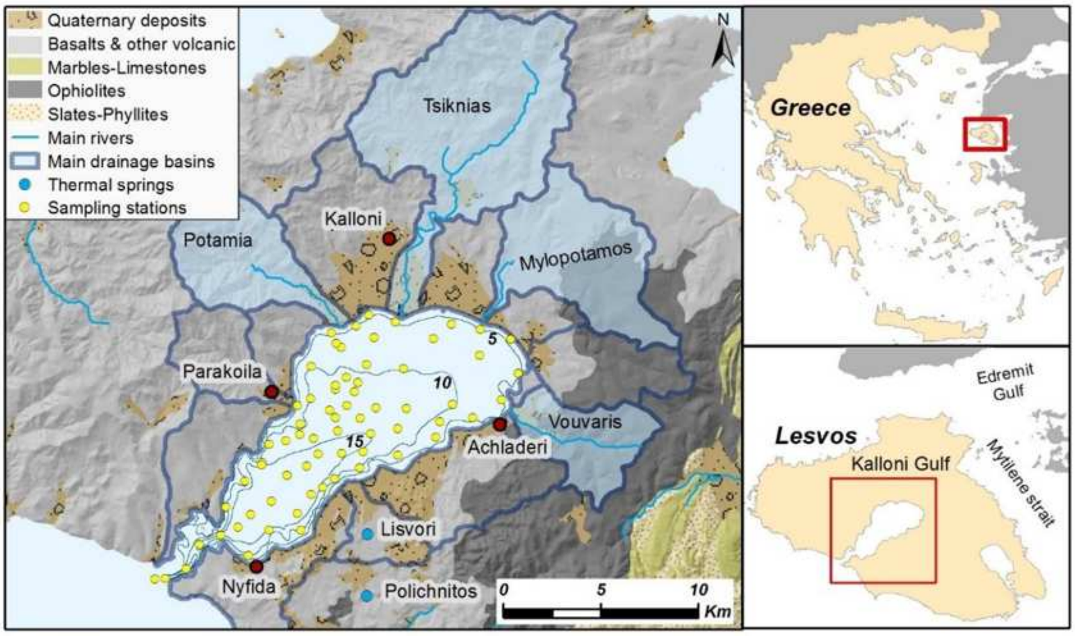

Figure 1. Location of Lesvos Island and the Gulf of Kalloni, of the major drainage basins and rock formations and of the collected sediment samples.

3. Results

Grain-size analysis revealed that the surficial sediments range from sand to mud with most of the samples characterized as muds in the Folk (1980) ternary diagram. Clay is the most abundant grain-size class with a mean content exceeding 50% (Table 1). Regarding the sand distribution it resembles the mean size and mirrors silt (and mud) spatial coverage. Clay does not show a specific coverage pattern, although maximum values are observed towards the shallower parts to the north and northwest.

Table 1. Main fractions percentages and statistical grain-size parameters of the sediment samples.

	Sand (%)	Silt (%)	Clay (%)	Mud (%)
Min	0.33	0.0	0.0	0.07
Max	99.93	56.34	73.68	99.67
Mean	15.04	28.74	52.30	81.04
	Mean size (Ø)	**Sorting (Ø)**	**Skewness**	**Kurtosis**
Min	0.97	0.66	-1.61	0.20
Max	9.24	4.04	0.89	3.22
Mean	7.40	2.09	-0.54	0.63

Mean size has an average of 7.4 Ø (Table 1) implying to very fine sediments almost all over the gulf. Coarser sediments are mainly found along the strait in the gulf entrance and closer to the coastline, usually off the mouth of the main streams (Figure 2). It becomes obvious that the hydrodynamic regime along the strait is responsible for non-deposition of the finer fractions, whereas temporal inputs from the surrounding drainage system results to the coarser distribution close to the shoreline. Sorting varies from 0.66 to 4.0 Ø (Table 1) suggesting moderately well to very poorly sorted sediments. Best sorting values are observed along the strait (Figure 2), whereas worst sorting is detected towards the shallower waters in good correlation with the sand fraction, suggesting a wide range of sand sub-fractions in the sediment samples. Skewness (mean -0.54) distribution resembles sorting with higher values along the strait and around the gulf (Figure 2) due to the high content in the 3 and 4 Ø sub-fractions, whilst at the deeper (central part) of the gulf the coarser fractions of silt (5 and 6 Ø) appear to be abundant in relation to other fine-grained sub-fractions. Kurtosis (0.2 to 3.22) has lower values in the wider part of the gulf (Figure 2) suggesting that the sediments are not unimodal.

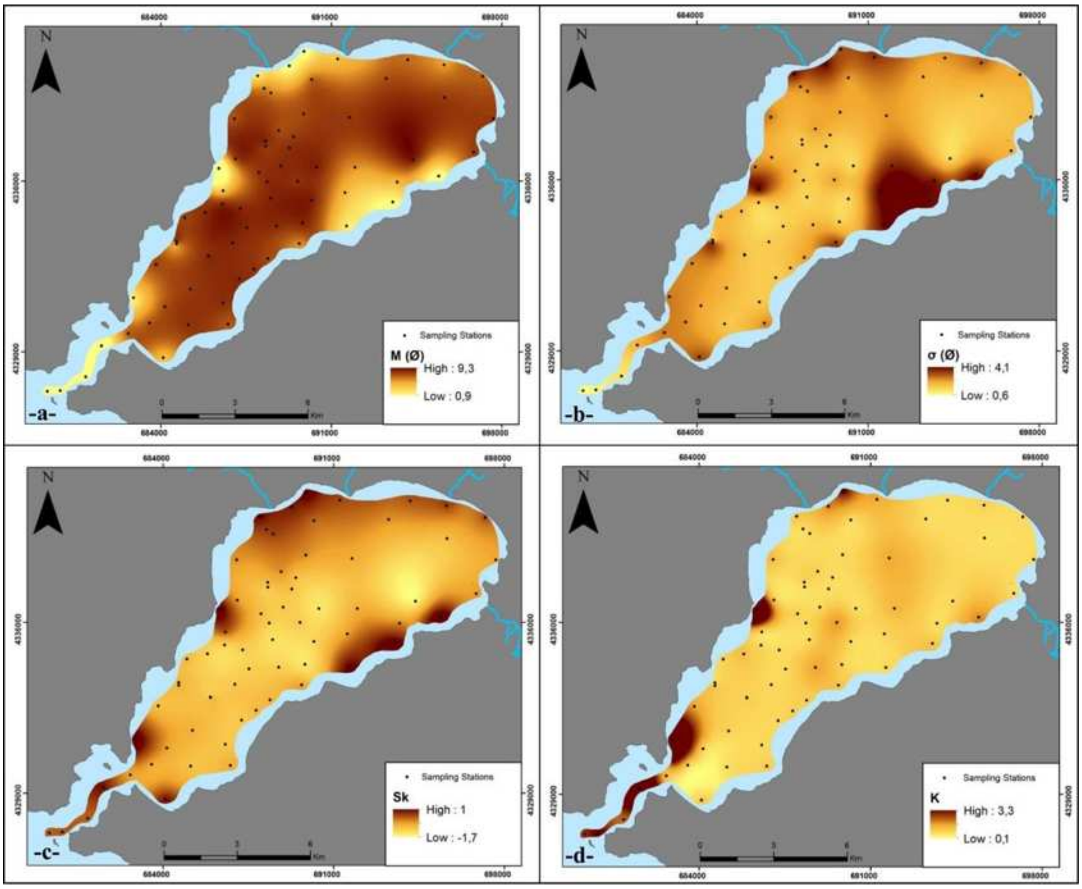

Figure 2. Spatial distribution of the statistical grain-size parameters (M: mean size, σ: sorting, Sk: skewness, K: kurtosis).

The sand and silt sub-fractions spatial mapping (Figure 3) also show evidence of the main sediment inputs and their distribution due to hydrodynamics. More specifically, the coarser sand fractions (0 and 1 Ø) are detected along the strait and off Potamia river to the northwest, whilst 2 and 3 Ø are mainly found at the eastern part of the gulf and locally off river mouths. The finer sandy fraction (4 Ø) is mainly observed in the gulf entrance and the inner part of the strait. The coarser silt fractions (5 and 6 Ø) appear to be more abundant at the northern part, off river mouths, whereas the finer fractions (7 and 8 Ø) do not show a specific pattern (Figure 3).

Scatter plots of the statistical grain-size parameters in relation with the spatial sediment distribution, helped to categorize the gulf deposits into three groups (Figure 4). Group A is attributed to the coarser sediments detected along the strait and locally closer to the coastline. They are moderate to poorly sorted and in the K-Sk bivariate plot they are grouped into two subgroups with different characteristics. Group B show silty sediments that were collected mainly at shallower waters off the coastline, being very poorly sorted. They are coarse skewed to very fine skewed and very platykurtic to mesokurtic (one sample being leptokurtic), suggesting that they are consisting of more than one grain sub-population. Group C is related to the finer sediments (clays) collected at the central and deeper part of the gulf. These deposits are poorly sorted and according to the K-Sk scatter plot they are platykurtic to very platykurtic and very coarse skewed indicative of at least bimodal deposits.

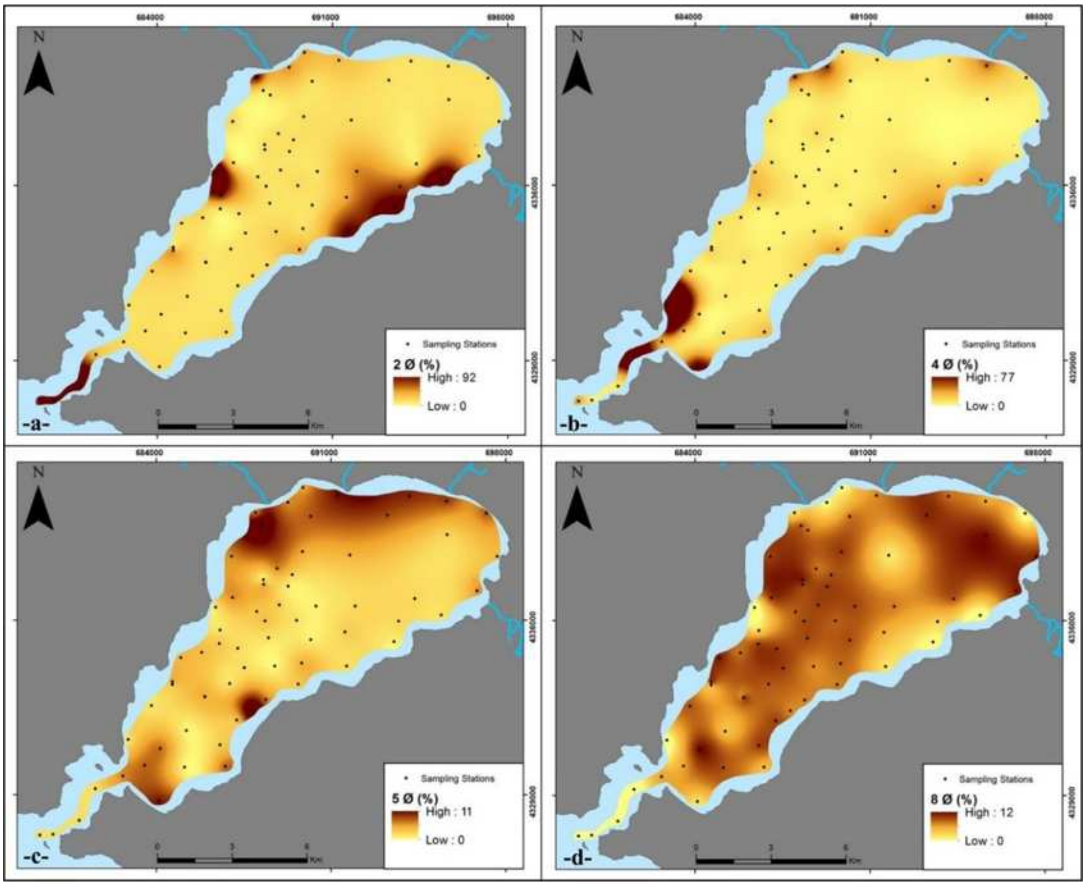

Figure 3. Spatial distribution of representative sand and silt sub-fractions in Ø units.

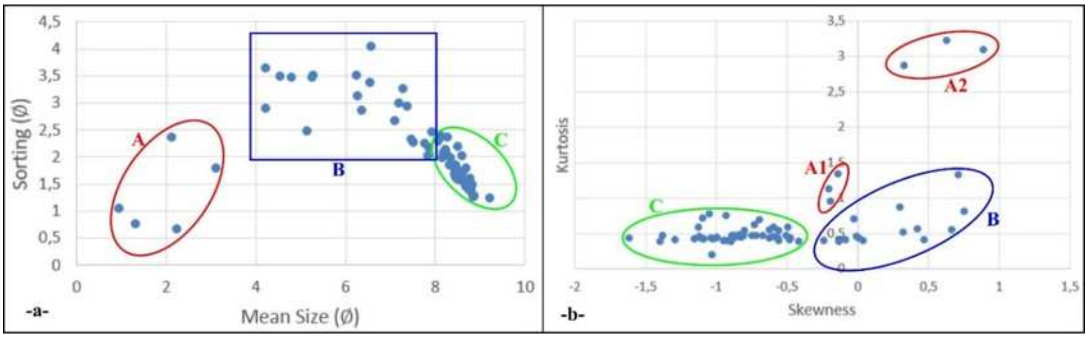

Figure 4. Scatter plots of (a) sorting vs mean size and (b) kurtosis vs skewness for the Kalloni sediments.

4. Discussion and Conclusions

The Gulf of Kalloni, a semi-enclosed system of high ecological importance, represents a sink for fine-grained sediments delivered by the local ephemeral drainage system. However, the precise amount of sediments delivered in the gulf is not known in the absence of dedicated hydrological surveys. Surficial sediment analysis revealed sandy sediments (i) along the strait connecting the gulf with the open sea, related to winnowing caused by seabed currents, and (ii) towards the shallower waters, spotted mainly off the mouth of the small rivers. Beside sand, rivers also deliver silty sediments. The wave climate and the water circulation (Mamoutos *et al.* 2018) is responsible for transportation of sediment and its distribution in the shallow waters of the gulf. The currents, although relatively weak, contribute to the dispersion and deposition of the finer (clayey) material almost all over the gulf. The dominance of poorly and very poorly sorted sediments stems from the abundance of the small-size biogenic fragments but also to the variety of the grain sub-fractions throughout the gulf. Scatter plots of the statistical grain-size parameters revealed the presence of 3 main sediment groups deposited along the strait, off the coastline around the gulf and in the central part of the gulf. This work complements previous efforts for the study of the surficial deposits in the Gulf of Kalloni and is expected to contribute to the integrated coastal zone management of this high ecological value semi-enclosed system.

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References

Anagnostou C., Kaberi H., Karageorgis A., Krassakopoulou E. (1998). Environmental characterization of Kalloni Gulf, Lesvos Island, Greece: International Conference "Protection and Restoration of the Environment IV", Halkidiki, Macedonia, Greece, 1-4 July 1998. pp. 315–322.

Blott S.J., Pye, K. (2001). Gradistat: a grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surface Processes and Landforms 26, 1237–1248.

Chronis A, Hasiotis T, Lowag J., (2014). Relationship between gas-bearing Sediments and Biogenic Mounds (Student Research Project in Hydrography). Hydro International 18(8), 22-25.

Debenay J.P., Millet B., Angelidis M.O. (2005). Relationships between foraminiferal assemblages and hydronamics in the gulf of Kalloni, Greece. Journal of Foraminiferal Research 35(4), 327–343.

Folk R.L. (1980). Petrology of the sedimentary rocks, 2nd edn. Hemphill Publishing Company, Austin.

Gavriil A. M., Angelidis M. O. (2005). Metal and organic carbon distribution in water column of a shallow enclosed Bay at the Aegean Sea Archipelago: Kalloni Bay, island of Lesvos, Greece. Estuarine, Coastal and Shelf Science 64(2-3), 200–210. doi:10.1016/j.ecss.2005.02.015.

Katsikatsos, G., Migiros G., Triantafyllis E., Mettos A. (1986). Geological Structure of Internal Hellenides (E. Thessaly–SW Macedonia, Euboea–Attica–Northern Cyclades Islands and Lesbos) - Geological and Geophysical Research. Special Issue, Institute of Geology and Mineral Exploration: Athens, 191–212.

Kelepertsis A. E., Chatsidimitriadis E. (1983). Factors controlling the mineralogy and geochemistry of the recent surface sediments of the Kalloni Gulf, Lesvos Island, Greece. Geologische Rundschau 72(1), 167–190.

Mamoutos I., Petalas S., Sampatakaki A., Dimitrakopoulos A., Zervakis V. (2018). H high-resolution hydrodynamic simulation of Lesvos semi-enclosed embayment Kalloni Gulf: Preliminary results. 3rd International Congress on Applied Ichthyology & Aquatic Environment, 8-11 November 2018, Volos, Greece, pp. 435-439.

Millet B., Lamy N. (2002). Spatial Patterns and Seasonal Strategy of Macrobenthic Species Relating to Hydrodynamics in a Coastal Bay. J. Rech. Oceanographique 27, 30-42.

Newton A., Icely J., Cristina S., Brito A., Cardoso A.C., Colijn F., Riva S.D., Gertz F., Hansen J.W., Holmer M., Ivanova K., Leppäkoski E., Canu D.M., Mocenni C., Mudge S., Murray N., Pejrup M., Razinkovas A., Reizopoulou S., Pérez-Ruzafa A., Schernewski G., Schubert H., Carr L., Solidoro C., Viaroli P., Zaldívar J.M. (2014). An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. Estuarine, Coastal and Shelf Science 140, 95-122.

Petalas S., Mamoutos I., Dimitrakopoulos A., Sampatakaki A., Zervakis V. (2020). Developing a pilot operational oceanography system for an enclosed basin. Journal of Marine Science and Engineering 8, 336, doi:10.3390/jmse8050336.

Tassos S.T. (1977). Marine Holocene sediments of Kalloni Bay, Lesvos Island, Greece. In: Proceedings of the VI Colloquium on the geology of the Aegean region, Institute of Geological and mining research, Athens, Greece, pp. 367-374.

Tassos S. (1983). Marine Holocene foraminifera and ostracoda from Kalloni Bay, Lesvos Island, Greece. Bollettino della Societa Paleontologica Italiana 22(1-2), 135-144.

Tsirtsis G., Spatharis S., Sampatakaki A., Spyropoulou A. (2008). Thresholds of terrestrial nutrient loading for the development of eutrophication episodes in a coastal embayment in the Aegean Sea. Transit. Waters Bull. 3, 25-37.

Voultsiadou E., Koutsoubas D., Achparaki M. (2010). Bivalve mollusc exploitation in Mediterranean coastal communities. Journal of Biological Research 13, 35-45.

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HUMAN DISTURBANCE ALTERS TERRITORIAL BEHAVIOR OF THE GHOST CRAB *Ocypode cursor* (LINNAEUS, 1758)

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Abstract
Human activities increasingly influence species in various ecosystems. Species often develop new strategies or alter their behavior to deal with these forces. Sandy shores are one of the ecosystems that experience the most severe human disturbance due to their services such as tourism. Ghost crabs are widespread bioindicator species to examine the consequences of those human activities for sandy shores. Previous research has mostly focused on variations in the population demographics of these species under various levels of human disturbance, and changes in their behaviors are often ignored. Therefore, here I investigate whether these common bioindicator species alter their territorial behavior under human influence. I measured the inter-burrow distance of ghost crabs and the opening diameter of those neighbor burrows at four sites that differ in the level of human disturbance. I found that the distance between neighbor burrows increased with the level of human disturbance and the difference in burrow density did not play a significant role in these variations. I further found that differences in the size of the neighbor burrows were an important determinant of the distance between two burrows, suggesting that smaller individuals stay away from the burrows owned by larger crabs. This study concludes that human disturbances alter territorial behavior of ghost crabs. Overall, the results of this study suggest that intra-specific interactions of species under human disturbance should more closely be monitored by managers and scientists in order to develop useful management plans.
Keywords: *Behavior, intra-specific interaction, inter-burrow distance, sandy beach, Turkey*
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1. Introduction

Human disturbance has influenced many species in various ecosystems over the last century. Coastal regions, in particular, experience severe human disturbance due to their multiple ecosystem services such as tourism, fishing, and boating (Davenport & Davenport 2006). These forces are expected to be higher in the future for these regions as the human population is growing faster in the coastal regions compared to other ecosystems (Vitousek *et al*. 1997). Therefore, understanding the potential consequences of human disturbances for species inhabiting these regions is important for having sustainable ecosystems in the future.

Bioindicator species are widely used to collect data for determining the results of the human disturbances on populations, communities, and ecosystems (Solomon *et al*. 2003). These data are often collected observing population demographics of bioindicator species. Bioindicator species often reduce their population densities and individual body sizes under human disturbance (Carignan & Villard 2002). Yet, behavioral aspects of the human disturbances are often missing in those studies (Costa *et al*. 2021).

Ghost crabs are widespread bioindicators for human disturbance on the sandy shores (Schlacher *et al*. 2016; Gül & Griffen 2018; Bal *et al*. 2021). Similar to other bioindicator species, ghost crabs show lower population density and individual body sizes at the sites influenced by human activities; therefore, studies have often focused on their population demographics (Schlacher *et al*. 2016). Furthermore, ghost crabs alter their daily activities and behaviors as a response to human disturbance (Gül & Griffen 2019a). For example, ghost crabs create smaller burrows (Gül & Griffen, 2018) in order to save energy under human influence (Gül & Griffen 2019a). Moreover, ghost crabs are territorial species (Lucrize & Schlacher 2014) and this behavior determines their distribution on sandy shores (Gül & Griffen 2019b). However, variations in the strength of territorial behavior of ghost crabs under human disturbance are not known. Therefore, here I examined ghost crab populations across sites that differ in the level of human disturbance. I hypothesized that ghost crabs would show stronger territorial behavior under the human influence as they become more aggressive due to shortage of high-quality food (Gül & Griffen 2020).

2. Materials and Methods

Study sites: Sandy shores in Turkey were closed due to the COVID-19 pandemic from January to June 2021. They were reopened beginning on the 1st June 2021, and I took advantage of this reopening to conduct this study. I visited four sandy shores on the Mediterranean coast of Turkey at the end of July (Figure 1). These four sites were chosen because of the differences in the level of human disturbance they experienced. One site (Resort) is next to hotels that are mostly the destinations of international tourists. The other one (Kadriye) is a public beach that is freely accessed by locals, domestic and international tourists. The third beach (Nigit 1) is a public beach that has a free access by cars and people and is mostly visited by local residents. The last beach (Nigit 2) is next to Nigit 1, but it has limited access, therefore experiences a relatively low level of human disturbance.

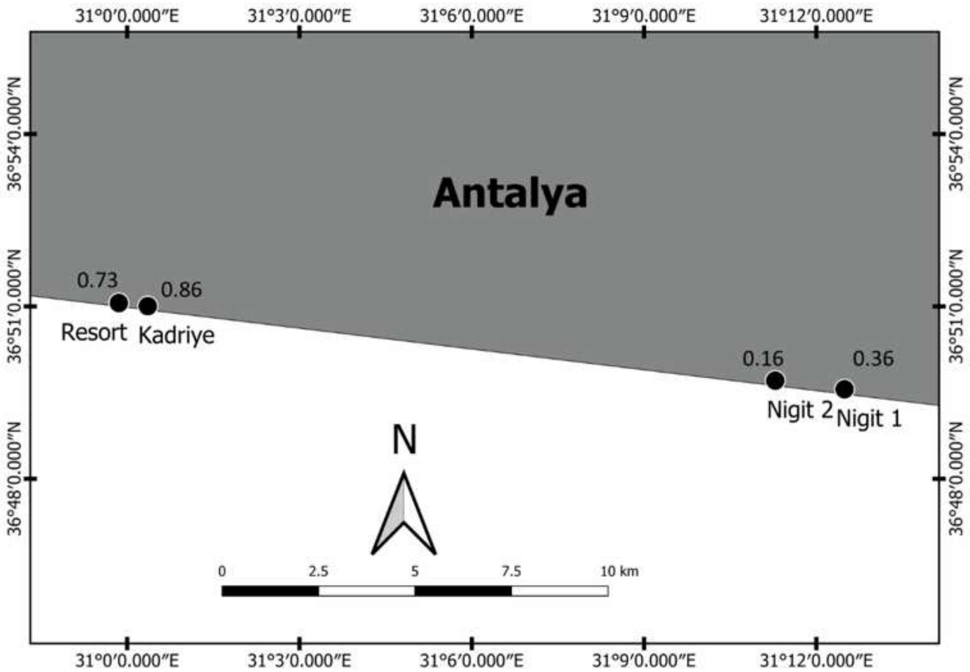

Figure 1. Study sites on the Mediterranean coast of Turkey. Numbers next to the study sites indicate the urbanization index (UI) values.

I categorized the study sites using the urbanization index (UI) modified from González *et al*. (2014). To this end, I collected data on (1) frequency of visitors, (2) number of vehicles on the sand, (3) proximity of the sites to the urban centers, (4) buildings on the sand and dunes, (5) frequency of beach cleaning, and (6) infrastructures such as parking lots, water, restrooms, etc. Then, I summed the values obtained from those observations and divided them by the maximum value (i.e., 30 for 6 variables) (Table 1).

Table 1. Site-specific urbanization indicators and urbanization index (UI) values.

Study sites	Buildings on the sand	Visitor frequency	Vehicles on the sand	Beach cleaning	Proximity to urban centers	Infrastructures	UI
Resort	4	4	2	4	5	3	0.73
Kadriye	4	5	2	5	5	5	0.86
Nigit 1	0	3	4	0	2	2	0.36
Nigit 2	0	1	2	0	2	0	0.16

Data collection: To determine the inter-burrow distance, I set up three replicate quadrats (10×20 m) at each site parallel to the sea. As ghost crabs may have multiple burrows or they may frequently abandon their burrows, I used the burrow resetting method (BRM) (Pombo & Turra 2019). After setting up the quadrats I covered all burrows. After 24 hours, I revisited the same quadrats and examined the active burrows (i.e., open burrows). I measured the burrow opening diameters as a proxy for the crab size (e.g., carapace width, Türeli *et al*. 2009) using a digital Vernier caliper and measured the distance between two burrows that were the closest to each other using a metallic ruler. I measured the burrow density in each site applying the same quadrat and BRM techniques; however, I used larger quadrats (20×20 m). To understand whether inter-burrow distance varied between sites that differ in the level of human disturbance, I employed a general linear model (GLM) in which the distance was treated as a dependent variable, and urbanization index, burrow density, and difference between the size of neighbor burrows were treated as response variables.

As the average body sizes were different between sites with various levels of human disturbance, I removed the effects of burrow opening diameters by regressing the neighbor burrow size difference against the inter-burrow distance (Packard & Boardman, 1999). I then applied another GLM model in which I treated the standardized residuals obtained from regression as dependent variables and urbanization index as the response variable.

3. Results

I found that the degree of human disturbance had a significant influence on the inter-burrow distance (GLM, $t = 2.832$, $p = 0.0055$, Figure 2a). Specifically, inter-burrow distance of ghost crabs in Nigit 2 was lower than inter-burrow distance in Kadriye (Tukey HSD, $z = -5.05$, $p < 0.001$) and in Resort (Tukey HSD, $z = -4.896$, $p < 0.001$). Similarly ghost crabs in Nigit 1 had shorter inter-burrow distance compared to their conspecifics in Kadriye (Tukey HSD, $z = -3.256$, $p = 0.0045$), and in Resort (Tukey HSD, $z = -2.735$, $p = 0.0018$). No significant difference was found between Nigit 1 and Nigit 2 (Tukey HSD, $z = 2.225$, $p = 0.067$), and Resort and Kadriye (Tukey HSD, $z = -0.945$, $p = 0.344$) in terms of inter-burrow distance. Surprisingly, these variations in the inter-burrow distance between sites were not influenced by the burrow density (GLM, $t = -0.388$, $p = 0.698$) and the interaction between urbanization index and density (GLM, $t = -1.516$, $p = 0.132$). Differences between the sizes of two neighbor burrows was an important determinant for the inter-burrow distance (GLM, $z = 2.21$, $p = 0.0293$, Figure 2b), but not the interaction between the urbanization index and size difference (GLM, $z = 1.601$, $p = 0.112$).

When I controlled for the burrow size difference between the neighbor burrows, the inter-burrow distance in Nigit 2 was significantly shorter compared to the inter-burrow distance in Kadriye (Tukey HSD, $z = -5.046$, $p < 0.001$) and in Resort (Tukey HSD, $z = -4.028$, $p = 0.0002$). Similarly, ghost crabs created burrows closer to each other in Nigit 1 compared to the crabs in Kadriye (Tukey HSD, $z = -3.277$, $p = 0.0041$) and in Resort (Tukey HSD, $z = -3.926$, $p = 0.0108$). No differences in inter-burrow distance between Nigit 2 and Nigit 1 (Tukey HSD, $z = -2.094$, $p = 0.1088$), and between Kadriye and Resort (Tukey HSD, $z = 1.632$, $p = 0.1083$) were found (Figure 2c).

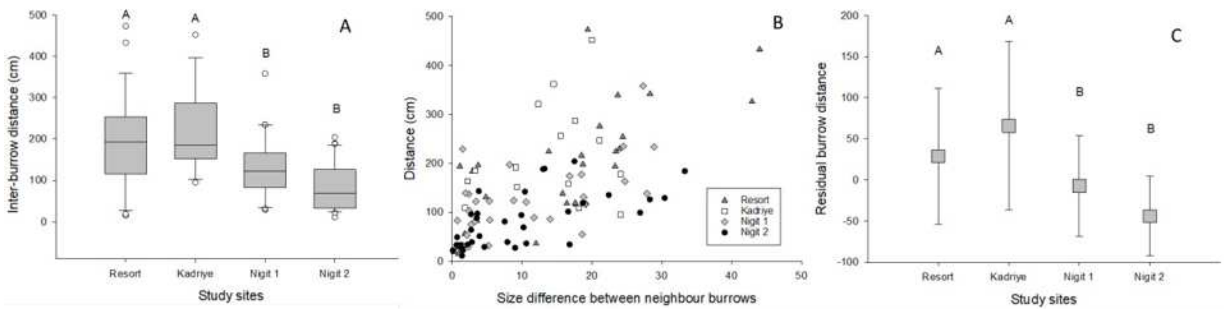

Figure 2. Variation in the inter-burrow distance (±S.D.) of *Ocypode cursor* (a) across sites that differ in the level of human disturbance, (b) with relation to the size difference between the neighbor burrows, (c) and variation in residual inter-burrow distance (±S.D.), after accounting for the difference between the size of the neighbor burrows, between study sites with various levels of human disturbance.

4. Discussion

I have shown that *O. cursor* alter their territorial behavior under human influence. I have further shown that these variations in the territorial behavior are determined by the differences between the neighbor burrow sizes. Surprisingly, differences in the burrow density due to the levels of human disturbance were not an important factor for observed variations in the territorial behavior of the crabs between sites. As territoriality is an important factor influencing the distribution (Gordon 1997) and agonistic behavior in species (Milner *et al*. 2010), changing strength of this behavior is likely to shift ecology and physiology of the ghost crabs.

Variations in the inter-burrow distance as a proxy for the changing territorial behavior between sites with various level of human disturbance were found in this study. Ghost crabs at disturbed sites consume a lower quality of food compared to their conspecifics at pristine sites and this variation is directly influenced by the degree of human disturbance, resulting a more aggressive personality in the ghost crabs at the sites with human disturbance (Gül & Griffen 2020). Therefore, the findings of this study suggest that ghost crabs become more aggressive territorialists and they exclude their conspecifics from larger areas due to this more aggressive behavior. Findings the positive relationship between the size differences between the neighbor burrows and the inter-burrow distance may provide further evidence for this hypothesis, as crabs may willingly become a part of agonistic encounters when they are more aggressive (Mowles *et al*. 2012) and smaller individuals mostly loose these encounters and/or may become food for the larger individuals (Gül & Griffen 2020; Tiralongo *et al*. 2020).

Density and average body sizes of ghost crabs are inversely related to the degree of human disturbance on sandy shores (Schlacher *et al*. 2016; Gül & Griffen 2018). Therefore, a longer inter-burrow distance might be expected at disturbed sites as crabs can have more available space due to lower density. On the other hand, as larger crabs more frequently exist at low-impacted sites, the longer distance between a larger and smaller burrow is logically expected. However, here I have found that the inter-burrow distance (i.e., territorial behavior) is not density-dependent. Further, it is not a consequence of the size differences between neighbor burrows, as the results, after accounting for the crab size, show that this behavior is directly related to the human disturbance.

Overall, this study shows that ghost crabs become stronger territorialists at disturbed sites and the strength of this behavior is influenced by the difference between the size of the burrow owner crabs, suggesting that human disturbance alter the strength of the intra-specific interactions, and therefore these alterations must be closely investigated.

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References

Bal M., Yapan B.Ç., Özkan K. (2021). The response of Tufted Ghost Crab, *Ocypode cursor*, populations to recreational activities in an urbanized coast with small-scale protected zones. Zoology in the Middle East 67(1), 32–41.

Carignan V., Villard M.A. (2002). Selecting indicator species to monitor ecological integrity: A review. Environmental Monitoring and Assessment 78(1), 45–61.

Costa L.L., Soares-Gomes A., Zalmon I.R. (2021). Burrow occupation rates and spatial distribution within habitat of the ghost crab *Ocypode quadrata* (Fabricius, 1787): Implications for impact assessments. Regional Studies in Marine Science 44, 101699.

Davenport J., Davenport J.L. (2006). The impact of tourism and personal leisure transportation coastal environments: a review. Estuarine, Coastal and Shelf Science 67(1–2), 280–292.

González S.A., Yáñez-Navea K., Muñoz M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. Marine Pollution Bulletin 83(1), 265–274.

Gordon D.M. (1997). The population consequences of territorial behaviour. Trends in Ecology and Evolution 12(2), 63–66.

Gül M.R., Griffen B.D. (2018). Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. PLoS One 13(12), e0209977.

Gül M.R., Griffen B.D. (2019a). Burrowing behavior and burrowing energetics of a bioindicator under human disturbance. Ecology and Evolution 9, 14205–14216.

Gül M.R., Griffen B.D. (2019b). Combined impacts of natural and anthropogenic disturbances on the bioindicator *Ocypode quadrata* (Fabricius, 1787). Journal of Experimental Marine Biology and Ecology 519, 151185.

Gül M.R., Griffen B.D. (2020). Diet, energy storage, and reproductive condition in a bioindicator species across beaches with different levels of human disturbance. Ecological Indicators 117, 106636.

Lucrezi S., Schlacher T.A. (2014). The ecology o ghost crabs-a review. Oceanography and Marine Biology - Annual Review 52, 201–256.

Milner R.N.C., Booksmythe I., Jennions M.D., Backwell P.R.Y. (2010). The battle of the sexes? Territory acquisition and defence in male and female fiddler crabs. Animal Behaviour 79(3), 735–738.

Mowles S.L., Cotton P.A., Briffa M. (2012). Consistent crustaceans: the identification of stable behavioural syndromes in hermit crabs. Behavioral Ecology and Sociobiology 66, 1087–1094.

Packard G.C., Boardman T.J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comparative Biochemistry and Physiology B 122, 37–44.

Pombo M., Turra A. (2019). The burrow resetting method, an easy and effective approach to improve indirect ghost-crab population assessments. Ecological Indicators 104, 422–428.

Schlacher T.A., Lucrezi S., Connolly R.M., Peterson C.H., Gilby B.L., Maslo B., Olds A.D., Walker S.J., Leon J.X., Huijbers C.M., Weston M.A., Turra A., Hyndes,G.A., Holt R.A., Schoeman D.S. (2016). Human threats to sandy beaches: a meta-analysis of ghost crabs illustrates global anthropogenic impacts. Estuarine, Coastal and Shelf Science 169, 56–73.

Solomon M., Jaarsveld A.S.V., Biggs H.C., Knight N.H. (2003). Conservation targets for viable species assemblages? Biodiversity and Conservation 12, 2435–2441.

Tiralongo F., Messina G., Marino S., Bellomo S., Vanadia A., Borzì L., Tibullo D., Stefano A.D., Lombardo B.M. (2021). Abundance, distribution and ecology of the tufted ghost crab *Ocypode cursor* (Linnaeus, 1758) (Crustacea: Ocypodidae) from a recently colonized urban sandy beach, and new records from Sicily (central Mediterranean Sea). Journal of Sea Research 156, 101832.

Türeli C., Duysak Ö., Akamca E., Kiyagi V. (2009). Spatial distribution and activity pattern of the ghost crab, *Ocypode cursor* (L., 1758) in Yumurtalik Bay, North-Eastern Mediterranean-Turkey. Journal of Animal and Veterinary Advances 8(1), 165–171.

Vitousek P.M., Mooney H.A., Lubchenco J., Melillo J.M. (1997). Human domination of the Earth’s ecosystems. Science 277, 494–499.

MOLECULAR IDENTIFICATION OF A PARASITIC NEMATODE FOUND IN THE DIGESTIVE SYSTEM OF A STRANDED MEDITERRANEAN MONK SEAL (*Monachus monachus*) IN THE AREA OF PAGASITIKOS GULF

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Abstract
The aim of the current study was the molecular identification of a parasitic nematode found in the digestive system of a stranded Mediterranean monk seal (*Monachus monachus*) in the area of Pagasitikos Gulf in 2019.The body of the seal was transferred to the Laboratory of Hydrobiology and Ichthyology for a post-mortem examination. Analysis of the stomach contents revealed the presence of a nematode. DNA extraction of the parasites was conducted and PCR amplification of the cytochrome oxidase subunit I (COI) mitochondrial gene was implemented. Sequencing analysis showed that the nematodes that were found in the stomach contents of the seal belonged to the *Pseudoterranova* genus and presented a 96% similarity to the *Pseudoterranova bulbosa* nematode species. A bootstrap tree (10,000 replicates) was produced with the use of the Kimura -2- parameter (K2P). From the constructed phylogenetic tree a clear genetic connection between our parasitic nematode named *Pseudoterranova*_sp_COI and *Pseudoterranova bulbosa* was established which indicates the first time a parasitic nematode as such, to be found in the waters of the Mediterranean Sea and also in the stomach of a *Monachus monachus*.
Keywords: *Monachus monachus, parasitic nematode, molecular identification*
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1. Introduction

The Mediterranean monk seal (*Monachus monachus*) belongs to the Phocidae family and is considered as an endangered species. The biggest population is found in the eastern side of the Mediterranean Sea. In Greece the species is being mainly found at the Marine National Park of Alonissos which is a restricted area for the protection of the species. The Mediterranean monk seal is considered to be an opportunistic predator because it is able feed on a lot of different marine species such as prey that mainly consists of benthopelagic fish of the Congridae, Gadidae, Sparidae and Scorpaenidae families and of cephalopods of the Octopodidae and Sepiidae families (Salman *et al.* 2001; Karamanlidis *et al*. 2011; Pierce *et al.* 2011; Kıraç & Ok 2019). The fact that its prey varies makes the Mediterranean monk seal prone to parasitic infections as the species that it feeds upon work as paratenic hosts for such parasites. Most of the time these parasites are transferred through consumption of an intermediate or paratenic host that carries infective third-stage larvae (L3) and inhabit the gastric, intestinal, ectopic, and gastrointestinal tracts as well as the heart and the lungs of the final host (Mattiucci *et al.* 2018). The Mediterranean monk seal acts as a host to a variety of parasites that reside in different parts of its body and consequently affect different aspects of its wellbeing. The parasites that have been detected in the body of the seal belong to the species *Acanthocheilonema spirocauda* which is a heartworm, *Anisakis pegreffii, Contracaecum osculatum, Leishmania infantum* and individuals that belong to the genus *Diphyllobothrium* that are mainly found in the gastric and intestinal tracts and the ectoparasite *Lepidophthirus piriformis* that resides in the fur of the animal (Campana-Rouget 1955; Schnapp *et al.* 1962; Blagoveshtchensky 1966; Mackiewicz 1988; Papadopoulos *et al.* 2010).

2. Materials and Methods

During the anatomy of the stomach of the Mediterranean monk seal two individuals resembling a parasitic nematode (Figure 1) were detected and afterwards preserved in a 70% ethanol solution. DNA extraction was carried out with EXTRACTME DNA TISSUE KIT which is mainly used in extracting animal tissue. After the DNA extraction a Master Mix was created for the specimens Parasite 1 (Π1) and Parasite 2 (Π2). For the Master Mix was used 25 μl 1X PCR Buffer, $MgCl_2$ 10 μl $MgCl_2$ (25mM), 1 μl of dNTPs (10mM), 7.5 μl of each forward and reverse primer, 1 μl Taq polymerase (KAPA Taq) and distilled water up to 50 μl. A PCR was then optimized for a number of 35 cycles and annealing temperature at 50 ^{o}C.

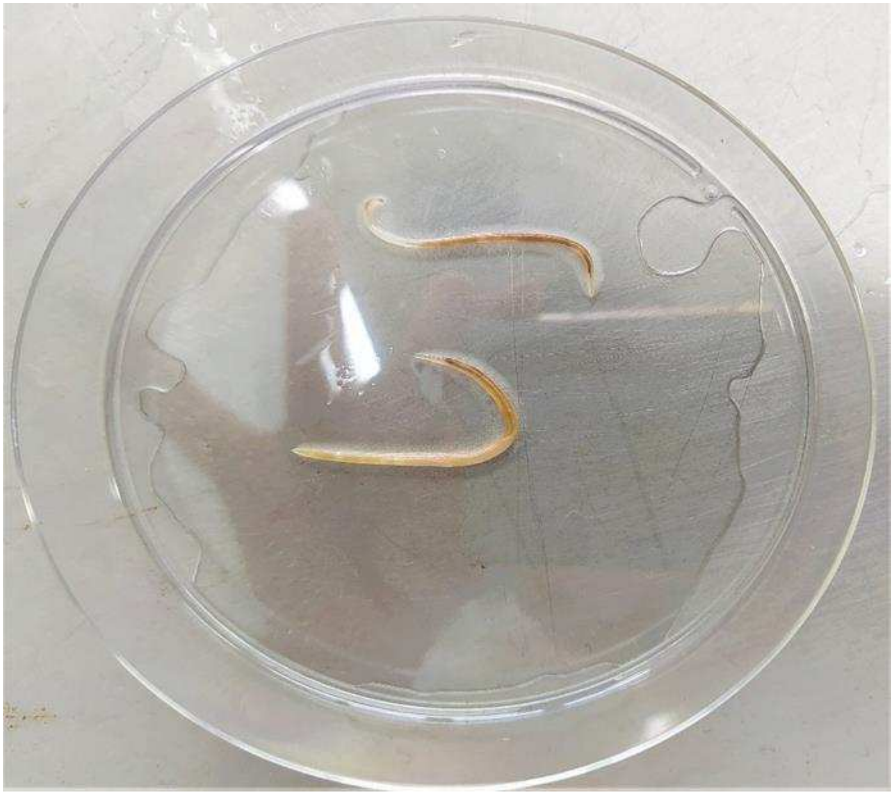

Figure 1. The two parasitic nematodes that were found in the stomach contents of the Mediterranean monk seal.

3. Results

The PCR products were resolved using agarose gel 1.2%. PCR products were then analyzed in an ABI 3730 automated sequencer. A Maximum Likelihood (ML) phylogenetic tree was constructed with 10,000 repeats. An *Anisakis physeteris* sequence was used as outgroup. The sequences were aligned by ClustalW by the program MEGA-X and were analyzed by Bootstrap and with the Kimura -2- parameter (K2P). The results from the molecular analysis showed a 96% similarity between the specimen *Pseudoterranova* sp. COI and the nematode species *Pseudoterranova bulbosa* indicating a close evolutionary path. The evolutionary relationships between the specimens and the other species of *Pseudoterranova* sp are shown in the phylogenetic tree that was constructed (Figure 2). Between the specimens *Pseudoterranova* sp. COI and *Pseudoterranova bulbosa* a monophyletic relationship was established as for the specimen *Pseudoterranova* sp. COI and the rest of the *Pseudoterranova* species a polyphyletic relationship was established. The aim of the current study was to investigate with the help of DNA barcoding the identity of the nematode species that was found in the stomach contents of the Mediterranean monk seal and answer the question of whether or not this specific nematode has an evolutionary connection to Mediterranean monk seal's diet.

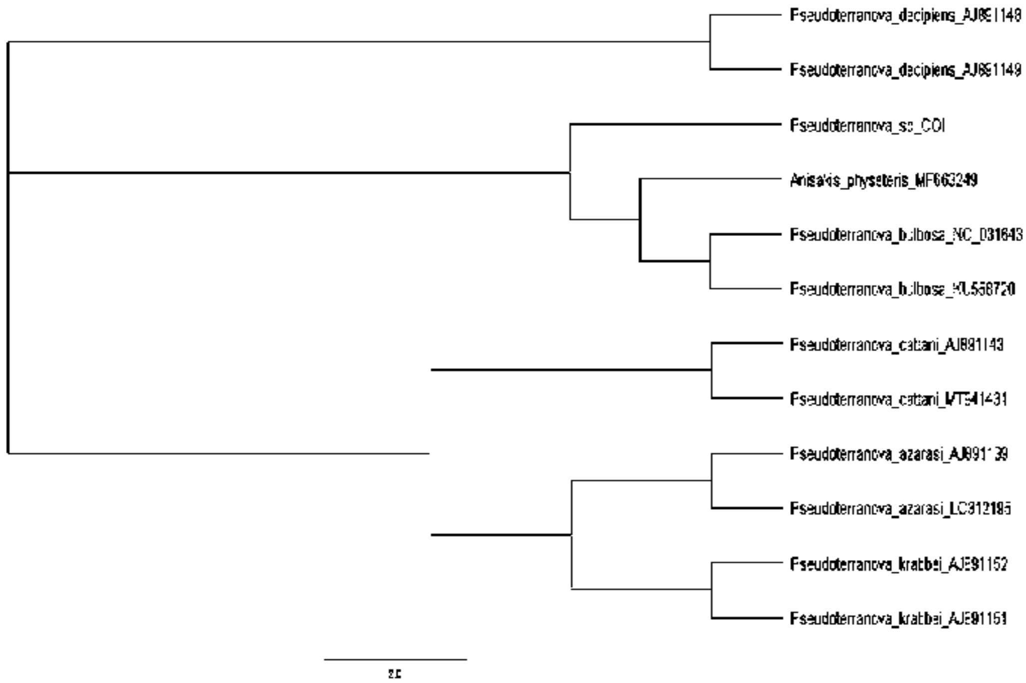

Figure 2. Maximum Likelihood (ML) phylogenetic tree.

4. Discussion

The close evolutionary relationship between the specimen species and *Pseudoterranova bulbosa* indicates the possibility of the species *Pseudoterranova bulbosa* being present in the Mediterranean Sea. This assumption generates the question of, how this particular species came to be into the stomach of a Mediterranean monk seal. *P. bulbosa* mainly resides in the depths of the northern parts of the Atlantic, Pacific Ocean and Japan (Paggi *et al.* 1991). The bearded seal (*Erignathus barbatus*) is considered the main final host of this specific parasite while benthic and benthopelagic flatfish act as intermediate hosts (Paggi *et al.* 1991, 2000; Brattey & Davidson 1996); Mattiucci *et al.* 1998). The life cycle of *P. bulbosa* is similar to the life cycles of other *Pseudoterannova* individuals as its stages of growth use different hosts such as copepods, amphipods and teleost fish. In the possible presence of *P. bulbosa* in the Mediterranean many questions arise regarding its appearance. One possible explanation for the appearance of *P. bulbosa* in the stomach of the Mediterranean monk seal is that it was transferred through consumption of an infected intermediate host. *Gadus morhua* could be considered responsible for this particular transfer as it has been found to be infected by this particular parasite and it also resides in the Mediterranean Sea (Jørgensen *et al.* 2008; Morey *et al.* 2012). It is widely known that fish constitute a vital part in the development and dissemination of the nematode parasites as they provide a shelter and ideal conditions for them to grow (McClelland 1995). In addition, the parasitic nematodes that reside into cold waters are not particularly selective to their intermediate hosts (Holems 1990) a behaviour that helps them infect whichever host comes their way (Alt *et al.* 2019). Most parasites are selective only for their final hosts. Another explanation for the presence of *P. bulbosa* in the Mediterranean could be that of Climate change as it affects the distribution of the fish in the marine ecosystem changing biogeography and biodiversity. Climate change can also cause change in the food web which can lead to fish searching for new feeding grounds and spreading the parasites to new hosts (Steele 1998, 2004; Hsieh *et al.* 2005; Jiao 2009). The melting of the ice can also provide new migration paths for marine mammals from northern to southern regions making them susceptible to new parasitic infections. On that note, it is possible that *P. bulbosa* was transferred from another marine mammal through feces, containing parasitic eggs that were deposited on the sea bed and were passed on to an intermediate host. *Globicephala melas* has been recorded to carry out long migratory trips from the North Atlantic to the Mediterranean Sea. Such behaviour can be attributed to other marine mammal species and can lead to the distribution of parasites to new ecosystems and hosts (Abend & Smith 1999). The appearance of *Pseudoterranova bulbosa* and *Pseudoterranova* sp. COI can be explained from an evolutionary standpoint. The use of the species *Anisakis physeteris* as an outgroup contributes in showing a close evolutionary relation between *P. bulbosa* and *Pseudoterranova* sp. COI. *Anisakis physeteris* is a nematode species with a global distribution as it can be found in the Atlantic, Mediterranean and South African waters. The main intermediate hosts of this specific parasite are fish belonging to the Kogiidae family. As final hosts marine mammals that belong to the Physeteridae family specifically the species *Physeter macrocephalus* are considered the main host of the parasite (Hermosilla *et al.* 2018). This parasite has also been detected in other marine mammals such as *Ziphius cavirostris* and the pinniped *Arctocephalus pusillus* (Stewardson, & Fourie 1998; Mattiucci *et al*. 2001). *Anisakis physeteris* has been detected in fish and cephalopods that constitute part of the prey of *Physeter macrocephalus* and *Monachus monachus* such as *Merluccius merluccius*, *Scomber scombrus*, *Phycis phycis*, *Phycis blennoides, Illex coindetii, Histioteuthis bonnellii* and *Histioteuthis reversa* (Mattiucci *et al*. 2001; Farjallah *et al*. 2008; Palomba *et al*. 2021). Considering this information and the fact that *Anisakis physeteris* and *Pseudoterranova* sp. COI have a close common ancestor we could assume that the two species followed a parallel evolution pattern. The cause for their divergence could be that individuals of the same species back then, would infect hosts who possessed different biological characteristics and resided in different ecosystems. This assumption could explain how this two species came to be so closely related yet so geographically isolated.

References

Abend A.G., Smith T.D. (1999). Review of distribution of the long-finned pilot whale (*Globicephala melas*) in the North Atlantic and Mediterranean. NOAA technical memorandum NMFS-NE: 117.

Alt K.G., Kochmann J., Klimpel S., Cunze S. (2019). Improving species distribution models of zoonotic marine parasites. Scientific Reports 9(1), 1–10.

Blagoveshtchensky D., (1966). New forms of lice (Siphunculata) parasites of pinnipeds and hares. Entomological Review URSS 45, 806–813.

Brattey J., Davidson W.S. (1996). Genetic variation within *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) from Canadian Atlantic marine fishes and seals: characterization by RFLP analysis of genomic DNA. Canadian Journal of Fisheries and Aquatic Sciences 53(2), 333–341.

Campana-Rouget Y., A.E.B. (1955). Une nouvelle espece d'Anisakis chez un phoque Mediterranean. Annales de Parasitologie 30, 477–480.

Farjallah S., Slimane B.Ben., Busi M., Paggi L., Amor N., Blel H., Said K., D'Amelio S. (2008). Occurrence and molecular identification of Anisakis spp. from the North African coasts of Mediterranean Sea. Parasitology Research 102(3), 371–379.

Hermosilla C., Hirzmann J., Silva L.M.R., Brotons J.M., Cerdà M., Prenger-Berninghoff E., Ewers C., Taubert A. (2018). Occurrence of anthropozoonotic parasitic infections and faecal microbes in free-ranging sperm whales (*Physeter macrocephalus*) from the Mediterranean Sea. Parasitology Research 117(8), 2531–2541.

Holmes J.C. (1990). Helminth communities in marine fishes. In: "Parasite communities: patterns and processes", Esch G.W., Bush A.O., Aho J.M (eds). Springer, p. 101–130.

Hsieh C., Glaser S.M., Lucas A.J., Sugihara G. (2005). Distinguishing random environmental fluctuations from ecological catastrophes for the North Pacific Ocean. Nature 435(7040), 336–340.

Jiao Y. (2009). Regime shift in marine ecosystems and implications for fisheries management, a review. Reviews in Fish Biology and Fisheries 19(2), 177–191.

Jørgensen C., Dunlop E.S., Opdal A.F., Fiksen Ø. (2008). The evolution of spawning migrations: State dependence and fishing-induced changes. Ecology 89(12), 3436–3448.

Karamanlidis A.A., Kallianiotis A., Psaradellis M., Adamantopoulou S. (2011). Stomach contents of a subadult Mediterranean monk seal (*Monachus monachus*) from the Aegean Sea. Aquatic Mammals 37(3), 280–283.

Kıraç C., Ok M. (2019). Diet of a Mediterranean monk seal *Monachus monachus* in a transitional post-weaning phase and its implications for the conservation of the species. Endangered Species Research 39, 315–320.

Mackiewicz J.S. (1988). Cestode transmission patterns. Journal of Parasitology 74, 60–71.

Mattiucci S., Paggi L., Nascetti G., Ishikura H., Kikuchi K., Sato N., Cianchi R., Bullini, L. (1998). Allozyme and morphological identification of Anisakis, Contracaecum and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). Systematic Parasitology 40(2), 81–92.

Mattiucci S., Paggi L., Nascetti G., Abollo E., Webb S. C., Pascual S., Cianchi R., Bullini L. (2001). Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae)s. International Journal for Parasitology 31(1), 9–14.

Mattiucci S., Cipriani P., Levsen A., Paoletti M., Nascetti G. (2018). Molecular epidemiology of Anisakis and anisakiasis: an ecological and evolutionary road map. Advances in Parasitology 99, 93–263.

McClelland G. (1995). Experimental infection of fish with larval sealworm, *Pseudotertanova decipiens* (Nematoda, Anisakinae), transmitted by amphipods. Canadian Journal of Fisheries and Aquatic Sciences 52(S1), 140–155.

Morey G., Morales-Nin B., Riera F., Grau, A. Geffen A.J., Pérez-Mayol S., Chang M.Y., Grau A.M. (2012). Atlantic cod *Gadus morhua* in the Mediterranean: A surprising immigrant. Marine Ecology Progress Series 467, 277–280.

Paggi L., Nascetti G., Cianchi R., Orecchia P., Mattiucci S., D'amelio S., Berland B., Brattey J., Smith J.W., Bullini L. (1991). Genetic evidence for three species within *Pseudoterranova* decipiens (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Seas. International Journal for Parasitology 21(2), 195–212.

Paggi L., Mattiucci S., Gibson D.I., Berland B., Nascetti G., Cianchi R., Bullini L. (2000). *Pseudoterranova decipiens* species A and B (Nematoda, Ascaridoidea): nomenclatural designation, morphological diagnostic characters and genetic markers. Systematic Parasitology 45(3), 185–197.

Palomba M., Mattiucci S., Crocetta F., Osca D., Santoro M. (2021). Insights into the role of deep-sea squids of the genus *Histioteuthis* (Histioteuthidae) in the life cycle of ascaridoid parasites in the Central Mediterranean Sea waters. Scientific Reports 11(1), 7135.

Papadopoulos E., Loukopoulos P., Komnenou A., Androukaki E., Karamanlidis A. A. (2010). First report of *Acanthocheilonema spirocauda* in the Mediterranean monk seal (Monachus monachus). Journal of Wildlife Diseases 46(2), 570–573.

Pierce G.J., Hernandez-Milian G., Santos M.B., Dendrinos P., Psaradellis M., Tounta E., Androukaki E., Edridge A. (2011). Diet of the monk seal (*Monachus monachus*) in Greek Waters. Aquatic Mammals 37(3), 284–297.

Schnapp B., Hellwing S., Ghizelea G. (1962). Contributions to the knowledge of the Black Sea seal (*Monachus monachus*) Herm. Travaux Du Museum d'Histoire Naturelle ''Gr. Antipa, 3, 383–400.

Steele J.H. (2004). Regime shifts in the ocean: reconciling observations and theory. Progress in Oceanography 60(2–4), 135–141.

Stewardson C.L., Fourie H.J. (1998). Endoparasites of the cape fur seal *Arctocephalus pusillus pusillus* from the Eastern Cape coast of South Africa. Transactions of the Royal Society of South Africa 53(1), 33–51.

DENSITY AND RECRUITMENT OF A NEW INVASIVE SPECIES FOR THE TURKISH COAST OF THE BLACK SEA

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Abstract
Invasive species have become an important concern for the ecology due to their influence on biodiversity, local economy, and ecosystem services. The Black Sea, similar to many other ecosystems around the world, is under attack of invasive species, and this deterioration is expected to be stronger in the future due to human disturbances and climate change. Populations of a new invader for the Turkish coast of the Black Sea, Pacific oyster (*Crassostrea gigas*, Thunberg, 1793) have recently been reported on different parts of the Turkish coast. However, to better understand the potential influences of the Pacific oyster, further information about the population structures and relations with other species are urgently needed. Therefore, here we examined the density and recruitment of the Pacific oyster with relation to three most abundant species in Fatsa port, Ordu, Turkey. We found that the Pacific oyster had a moderate density and recruitment compared to other species. We also found that the densities and the recruitment rates of the Pacific oyster were inversely related to the densities and the recruitments of the Mediterranean mussel and barnacle but not of the tubeworm (*Vermiliopsis* spp.), suggesting that there is strong competition between the Pacific oyster and other two species. Overall, this study states that the Pacific oyster has the potential to dominate the Turkish coast of the Black Sea, and thus a management plan to reduce its impacts is urgently needed.
Keywords: *Balanus*, *Crassostrea gigas, inter-specific interactions, native species, Mediterranean mussel*
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1. Introduction

The main purpose of ecology is to understand the spatio-temporal distribution of organisms. As species continue to be introduced in new ecosystems, invasive species have become a major subject in ecology (Sax *et al*. 2005). When a species is introduced to a new ecosystem, it is important to understand its potential influence to protect this whole system from loss of its functioning. Therefore, invasive species should closely be monitored as they have become the major force that has altered biodiversity, function, and services in many ecosystems around the world (Fei *et al*. 2014).

The Black Sea has faced many issues due to invasive species. Various invasive species have caused different problems in the Black Sea, including loss of fisheries from the 1980s to the beginning of the 2000s, reductions in mussel beds and organisms associated with these beds, and near extinction of some native species (Kideys 2002; Sağlam *et al*. 2011). Low biodiversity in the Black Sea has historically been considered as the main reason for these invasions (Paavola *et al*. 2005); however, climate change, without doubt, is another obvious causation (Micu & Todorova 2009). Furthermore, alterations in the structures and topography by human activities such as land-filling may play another important role in the recent increase of the number of invasions (Beyazli & Aydemir 2008).

The Pacific oyster has been used for aquaculture in seventy countries in the world because of its commercial importance and high capability of adaptation to various environmental conditions (Ruesink *et al*. 2005). The species has been introduced to the Crimean coast of the Black Sea for a similar purpose in the 1980s (Zolotarev 1996). Reports showed that this species has established breeding populations on the north coasts of the Black Sea (Micu 2004). The species has also been reported in the Sea of Marmara, the Aegean Sea, and the Mediterranean Sea (Acarli *et al*. 2017; Gökçek *et al*. 2020). However, populations of Pacific oyster (*C. gigas*) on the east coast of the Black Sea were reported just a couple of years ago, suggesting a late adaption to this region (Aydın & Gül 2021).

Populations of the Pacific oyster (*C. gigas*) have recently been reported in various localities on the Turkish coast of the Black Sea (Aydın & Gül 2021). However, no further information is known including density, recruitment, and interactions with native benthic invertebrates, which are highly important in terms of understanding potential influence of this species in the region. Therefore, density and recruitment of the Pacific oyster with relation to three most abundant species, the Mediterranean mussel (*Mytilus galloprovincialis*), barnacle (*Balanus* spp.), and the tubeworm (*Vermiliopsi*s spp.) were investigated.

2. Materials and Methods

We took advantage of an abandoned fishing vessel (33 m × 7 m, L×W) to determine the density and recruitment of the invasive Pacific oyster (*C. gigas*) associated with three benthic invertebrates on the Turkish coast of the Black Sea. We considered the most abundant benthic organisms for this study including the Mediterranean mussel (*Mytilus galloprovincialis*), barnacle, *Balanus* spp., Pacific oyster (*Crassostrea gigas*), and tubeworm (*Vermiliopsis* spp.). This fishing vessel stayed in the water half-submerged for over a year in the Fatsa port, Ordu, Turkey (41º02'30'' N, 37º29'17'' E). For the density investigation, we sampled 5 square quadrats (40 cm X 40 cm, 16 dm^2) on the single side of the fishing vessel on a single day (11th June 2020). We chose the sampling day in June for density investigation because this time of the year was just before the recruitment period of these organisms. The samples were collected by Scuba diving. We removed all organisms in the quadrat using a metal scraper into a bag. Then, we brought the samples to the laboratory for further investigations. In the laboratory, we visually identified the species and counted the number of organisms. We ignored some rare organisms such as bryozoans and tunicates.

For recruitment, which refers to new settlers on the bare substrate, we investigated the same quadrats that we had cleared during density investigation. We set up identical square quadrats after 5 months. We took pictures of the organisms settled on the same bare substrate and examined the pictures visually for species identification and counting the number of organisms.

We applied two separate Generalized Linear Mixed-Effects Model (GLMM) with Poisson distributions to understand whether the density and recruitment of the species were different from each other. In the models, either density or recruitment of species were treated as response variables. Species were treated as fixed factors and the quadrats were treated as random factors in both models. The models were followed by Tukey's multiple comparison tests to determine the pairwise difference between species. All statistical analyses were run using R (version 3.6.2).

3. Results

We found that the density of the organisms significantly varied between species. The density of the species varied between 0.75±0.5 (S.D.) ind/dm^2 (*Vermiliopsis* spp.) and 22.23±3.87 (S.D.) ind/dm^2 (the Mediterranean mussel) (Figure 1a). The density of *C. gigas* was 1.18±0.36 (S.D.) ind/dm^2. The multiple comparison test results showed that the densities of the native species and barnacles were positively related to each other (Table 1). However, the density of Pacific oysters and the density of other species were inversely related to each other except *Vermiliopsis* spp. (Table 1).

Table 1. Results of Tukey's HSD multiple comparison test to determine pairwise differences in the density and recruitment between species.

	Density		Recruitment	
Comparison	*Z*- values	*P*-values	*Z*- values	*P*-values
Barnacle- *Vermiliopsis* spp.	12.346	<0.001	5.49	<0.001
Mussel- *Vermiliopsis* spp.	25.835	<0.001	21.149	<0.001
Oyster- *Vermiliopsis* spp.	2.788	0.0053	2.765	0.0057
Mussel- Barnacle	28.003	<0.001	26.773	<0.001
Oyster- Barnacle	-10.935	<0.001	-3.151	0.0032
Oyster- Mussel	-27.837	<0.001	-24.475	<0.001

The results of species recruitments were similar to their densities. The average recruitment of the species varied between 0.33±0.16 (S.D.) ind/dm^2 (*Vermiliopsis* spp.) and 20.38±5.96 (S.D.) ind/dm^2 (the Mediterranean mussel) (Figure 1b). The rate of recruitment in the Pacific oyster (*C. gigas*) was 0.65±0.51 ind/dm^2 (Figure 1b). The recruitment rates of the native species and barnacle were positively related to each other and inversely related to the recruitment rate of *C. gigas* (Table 1).

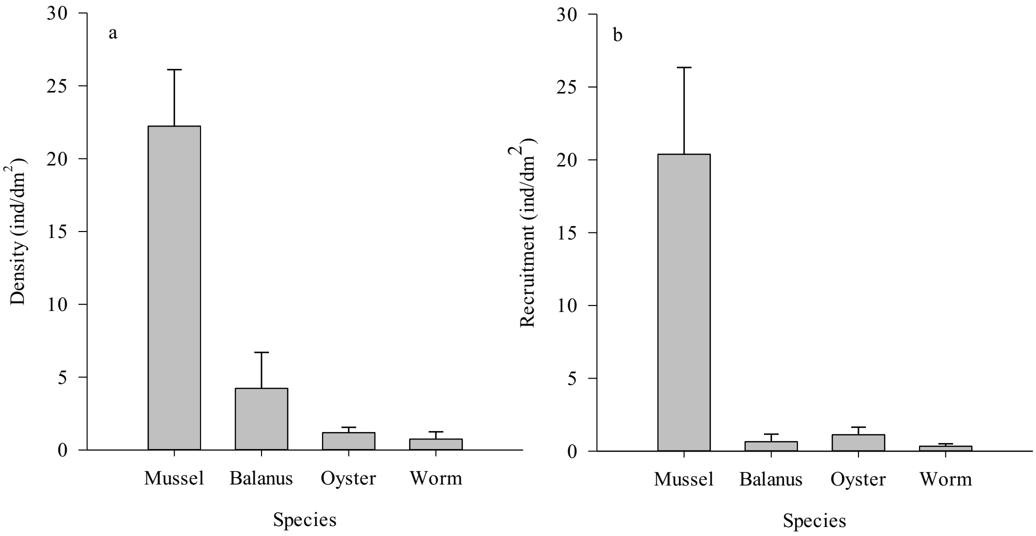

Figure 1. Density (a) and recruitment (b) (mean±S.D.) of the study species on the Turkish coast of the Black Sea.

4. Discussion

We have shown that the densities and the recruitments of the native species and barnacle are positively related to each other on the Turkish coast of the Black Sea. However, both the density and the recruitment of invasive *C. gigas* were inversely related to the density and the recruitment of the native species and barnacle. As benthic organisms often compete with each other for space on their limited substrates (Sax *et al*. 2005), an increase in the number of these species is likely to affect the strength of this inter-specific behavior and their ecology.

The Mediterranean mussel (*M. galloprovincialis*) was found to be the most abundant species in this system. Further, this species had the highest recruitment rate during our study period. The Turkish coast of the Black Sea is known to be dominated by large mussel beds; even though mussel populations have been detrimentally crashed by an invasive species (*Rapana venosa*) (Kasapoglu *et al*. 2011). The Mediterranean mussels host many benthic invertebrates by providing substrate for some to attach and shelter for the mobile ones (Çınar *et al*. 2020). The results showed that the densities of the native species and barnacle were positively related to each other, suggesting that these organisms use mussels as a highly suitable substrate to settle.

We found inverse relationships between the density of the invasive Pacific oyster (*C. gigas*) and the densities of mussel and barnacle, suggesting there is a strong competition between these species and the invasive oyster. These types of inter-specific interactions are widely seen all around the world (Rodriguez 2006). This finding brings a question of interest of how mussel density is positively related to other species and is inversely related to the invasive oyster. A potential explanation for these variations in the inter-specific interactions between native and invasive species can be the resistance created by mussels against the invasive species on the limited substrate. On the other hand, these resistance mechanisms could be traits that have long been adapted by native species and invasives that entered the system previously (Byers 2002). This idea may explain why recruitment rates of the species followed a similar pattern with their densities.

We have surprisingly found a positive interaction between the invasive Pacific oyster and *Vermiliopsis* spp in terms of their densities and the recruitment rates. The Pacific oyster has been cultured on the Crimean coast of the Black Sea. On the Crimean coast, many different Polychaete species from Serpulidae family have been reported and *Vermiliopsis* spp is a species of the same family. Therefore, it is reasonable to conclude that the Pacific oyster has been adapted to conditions that are provided by *Vermiliopsis* spp and uses this advantage on introduction to the Turkish coast, which is called selection regime modification (SRM) (Byers 2002).

Given these findings, an important question of why it took more than 40 years for the invasive Pacific oyster to arrive on the Turkish coast must still be answered. This "late arrival" probably neither has a simple answer nor is a consequence of the change in a single variable. Therefore, an urgent study is needed to investigate how the Pacific oyster has adapted to the Turkish coast and what the potential consequences of its introduction are. Without those studies, any idea would not be more than speculation. The most reasonable one is the changes in the coastal structures. Turkey has long fulfilled the Black Sea coast with large rocks and soil to gain more land for a living (Beyazli & Aydemir 2008). These fulfilling operations might change the structures of the coastal populations, topography, and biodiversity, and therefore might provide a suitable environment for the Pacific oyster (Tyrrell & Byers 2007). Another potential explanation could be an indirect influence of Rapa whelk (*Rapana venosa*). The Rapa whelk has destroyed many populations in the Black Sea including the Mediterranean mussel since its first introduction in 1947 (Kasapoglu *et al*. 2011). As a result of the detrimental influence of the Rapa whelk, the biological resistance provided by the Mediterranean mussel might decline, and therefore the Pacific oyster might find an opportunity to invade into the Turkish coast.

References

Acarli S., Vural P., Gündüz F. (2017). Meat yield and condition index of invasive species Pacific oyster (*Crassostrea gigas*, Thunberg 1793) in Bandirma Bay (Marmara Sea, Turkey). International İskenderun Bay Symposium, İskenderun, Turkey (2017), pp. 120.

Aydın M., Gül M.R. (2021). Presence of the Pacific oyster (*Crassostrea gigas* Thunberg, 1793) in the Black Sea. Journal of Anatolian Environmental and Animal Sciences 6(1), 14–17.

Beyazli D., Aydemir Ş. (2008). Landfilling with mixed wastes: Environmental effects of wastes and their management in the Eastern Black Sea region of Turkey. Indoor and Built Environment 17(2), 92–102.

Byers J.E. (2002). Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos 97, 449–458.

Çınar M.E., Bakir K., Öztürk B., Doğan A., Açık Ş., Kirkim F., Dağli E., Kurt G., Evcen A., Koçak F., Bitlis B. (2020). Spatial distribution pattern of macroinvertebrates associated with the black mussel *Mytilus galloprovincialis* (Mollusca: Bivalvia) in the Sea of Marmara. Journal of Marine Systems 211, 103402.

Fei S., Phillips J., Shouse M. (2014). Biogeomorphic impacts of invasive species. Annual Review of Ecology Evolution and Systematics 45, 69–87.

Gökçek E.Ö., Acarli S., Karahan B., Vural P., Baştanlar E.K. (2020). First molecular record of the alien species Pacific oyster (*Crassostrea gigas*, Thunberg 1793) in the Marmara Sea, Turkey. Marine Science and Technology Bulletin 9, 23–31.

Kasapoglu N., Duzgunes E., Saglam N.Eç, Saglam H. (2011). Alien species and their impacts in the Black Sea. V. Internatıonal conference aquaculture & fishery, Belgrade, Serbia, (2011). pp. 256–260.

Kideys A.E. (2002). Fall and rise of the Black Sea ecosystem. Science 297, 1482–1484.

Micu D., Todorova V. (2009). Early signs of “mediterranization” and climate change in the Black Sea. In: “Climate forcing and its impacts on the Black Sea marine biota”, Briand F (eds). CIESM Workshop Monographs, No: 39, p. 103–104.

Paavola M., Olenin S., Leppäkoski E. (2005). Are invasive species most successful in habitats of low native species richness across European brackish water seas? Estuarine, Coastal and Shelf Science 64(4), 738–750.

Rodriguez L.F. (2006). Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biological Invasions 8, 927–939.

Ruesink J.L., Lenihan H.S., Trimble A.C., Heiman K.W., Micheli F., Byers J.E., Kay M.C. (2005). Introduction of non-native oysters: Ecosystem effects and restoration implications. Annual Review of Ecology Evolution and Systematics 36, 643–689.

Sağlam N.E., Kesici U.Y., Akdoğan P. (2011). Karadeniz’deki bazı istilacı türler ve Karadeniz ekosistemi üzerine olan etkileri (*in Turkish*). Eğirdir Su Ürünleri Fakültesi Dergisi 7(1), 25–38.

Sax D.F., Stachowicz J.J, Gaines S.D. (2005). Species invasions, insights into ecology, evolution and biogeography. Sinaeur Press, Sunderland, MA.

Tyrrell M.C., Byers J.E. (2007). Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology 342, 54–60.

Zolotarev V. (1996). The Black Sea ecosystem changes related to the introduction of new mollusc species. Marine Ecology 17, 227–236.

ASSESSMENT OF INVASIVENESS POTENTIAL OF *Synaptula reciprocans* (ECHINODERMATA: SYNAPTIDAE) BY THE AQUATIC SPECIES INVASIVENESS SCREENING KIT

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Abstract
Risk screening of alien *Synaptula reciprocans* was carried out using the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the South Aegean Sea as the Risk Assessment (RA) area. Basic Risk Assessment (BRA) and Climate Change Assessment (CCA) scores were determined as 15.5 and 12.0, respectively. *S. reciprocans* exhibited a low invasion potential when only BRA score was considered. However, considering the projected climate change, BRA+CCA score suggested a high invasion potential (27.5). In the predicted climate change scenarios, it is crucial to monitor the invasiveness and associated impacts of *S. reciprocans* in the Mediterranean Sea.
Keywords: *AS-ISK, biological invasion, southern Aegean Sea*
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1. Introduction

Synaptula reciprocans (Forsskål, 1775) is an Indo-Pacific originated species. It is also found in the sublittoral zone of the tropical coasts of the Red Sea. It entered the Mediterranean Sea via Suez Canal in the 1960s and, in nearly 50 years, spread to many parts of the eastern Mediterranean, e.g., Israel (Clark & Rowe 1971), Cyprus (Cherbonnier 1986), Syria (Bitar *et al.* 2003), Lebanon (Zibrowius & Bitar 2003) and has recently reached the south Aegean Sea (Cinar *et al.* 2006; Okus *et al*. 2007; Ragkousis *et al*. 2017; Mahdy *et al*. 2019).

Full risk assessments, starting with risk screening and identification practices, are used to perform all management models, especially to predict the potential invasiveness of an alien species entering a new environment and then its potential impacts (Daehler *et al*. 2004). Risk identification and screening modules are based on a synthesis of information on the bio-ecological and developmental, undesired characteristics of the target non-native species, and the risk assessment area (Pheloung *et al.* 1999). These applications can be very useful in the rapid and inexpensive identification of invasiveness of alien species (Baker *et al.* 2007). Additionally, risk screenings play a very important role in recognizing the lack of reliable data in the literature (Copp *et al*. 2009). This also helps determine management and research priorities for target species. The Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp *et al*. 2016) that has been developed to incorporate the 'minimum requirements' (Roy *et al*. 2014) for the assessment of species with regard to the recent EU Regulation on the prevention and management of the introduction and spread of invasive alien species (European Commission, 2014). This risk assessment tool allows early detection of the invasion potentials and strategies of alien species, providing an opportunity for effective management and early intervention of these species.

There are many studies on the invasiveness potential of alien marine invertebrate species with risk identification tools (AS-ISK) (e.g., Zenetos & Galanidi 2017; Clarke *et al.* 2020; Killi *et al.* 2020; CIRCABC, 2021; Stasolla *et al*. 2021; Ruykys *et al*. 2021; Vilizzi *et al*. 2021). Notably, there has been no evidence-based study of the invasion potential of *S. reciprocans*, which has expanded its distribution to the Southern Aegean coasts. The aim of this study is to identify invasiveness risk of *S. reciprocans* in the south Aegean Sea using the Aquatic Species Invasive Screening Kit (AS-ISK).

2. Materials and Methods

The Aquatic Species Invasiveness Screening Kit (AS-ISK v1) (Copp *et al*. 2016) was used to identify potentially invasive species with respect to the RA area (Bilge *et al*. 2019). AS-ISK consists of 55 questions in total. The first 49 questions cover the biogeographical/historical (Section 1) and biological/ ecological (Section 2) aspects of the species under assessment, and these comprise the Basic Risk Assessment (BRA); the remaining six questions require the assessor to predict how future climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal, and impact, and these represent Section 3 of the Climate Change Assessment (CCA). For each question, the assessor must provide a response, justification and level of confidence, and the screened species eventually receives both a BRA and a BRA + CCA (composite) score (respectively ranging, theoretically, from −20 to 68 and from −32 to 80). AS-ISK scores <1 suggest that the species is unlikely to become invasive in the RA area, and is therefore classified as 'low risk'; on the contrary, higher score values categorise the species as posing either a 'medium risk' or a 'high risk' of becoming invasive. Distinction between medium and high-risk levels depends upon definition of a 'threshold' value, which is typically obtained through RA area-specific 'calibration' provided the availability of a representative sample size (i.e., number of screened species). Finally, the ranked levels of confidence (1 = low; 2 out of 10 chances; 2 = medium, 5 out of 10; 3 = high; 8 out of 10; 4 = very high; 9 out of 10) associated with each question-related response in AS-ISK mirror the confidence rankings recommended by the International Programme on Climate Change (Metz *et al.* 2005; Copp *et al.* 2016; Bilge *et al*. 2019).

Prior to risk screening of *S. reciprocans*, literature on biogeography (AS-ISK Section 1) and biology/ecology (AS-ISK Section 2) (internet databases, articles, thesis and project final reports) of the species were searched and Basic Risk Assessment (BRA) scores were obtained. For the Climate Change Assessment (CCA) part (AS-ISK Section 3), the Köppen-Geiger climate classification was used (Peel *et al*. 2007). For the Climate Change (CCA) questions, two sources related to possible future climate scenarios were used; Demir *et al*. (2008) and www.mgm.gov.tr/FILES/iklim/iklimdegisikligi-project2015.pdf. Remarkably, both sources estimate a 0.5–1°C increase in air temperatures over the next 50-year period. The increase in water temperature was also determined by a relationship existing between air temperature (Ta) and water temperature (Tw); (Erickson & Stefan 1996); Tw = 3.47 + 0.898Ta.

Using AS-ISK v1 (available at https://www.cefas.co.uk/nns/tools/), the south Aegean Sea was identified as the Risk Assessment (RA) area. As each response in AS-ISK for a given species is allocated a confidence category (1= low; 2=medium; 3=high; 4=very high), a confidence factor (CF) was computed as:

$$\Sigma(CQi)/(4 \times 55)\ (i = 1, \ldots, 55)$$

where CQ*i* is the certainty for question *i*, 4 is the maximum achievable value for certainty (i.e. 'very certain') and 55 is the total number of questions comprising the AS-ISK tool. The CF therefore ranges from a minimum of 0.25 (i.e. all 55 questions with certainty score equal to 1) to a maximum of 1 (i.e. all 55 questions with certainty score equal to 4).

3. Results and Discussion

As a result of the risk screening of *S. reciprocans*, the BRA score in the south Aegean Sea was calculated as 15.5 (Table 1).

Table 1. AS-ISK (v1) scoring output for *S. reciprocans* in the southern Aegean Sea

Statistics	Scores
BRA Score	15.5
BRA+CCA Score	27.5
Score partition	
A. Biogeography/Historical	3.5
1. Domestication/Cultivation	-2.0
2. Climate, distribution and introduction risk	1.0
3. Invasive elsewhere	4.5
B. Biology/Ecology	12.0
4. Undesirable (or persistence) traits	4.0
5. Resource exploitation	5.0
6. Reproduction	1.0
7. Dispersal mechanisms	0.0
8. Tolerance attributes	2.0
C. Climate change	12.0
9. Climate change	12.0
Confidence	
BRA+CCA	0.4
BRA Score	0.4
CCA Score	0.5

In the research conducted by Vilizzi *et al.* (2021) for global aquatic organisms, the BRA threshold value for marine invertebrates was determined as 15.1 and the BRA+CAA value as 14.25. The BRA+CCA score was calculated as 27.5 in the current study, and according to the score determined by Vilizzi *et al.* (2021), it is clear that this sea cucumber species has a high invasion potential compared to their CAA value. Considering both CCA and BRA scores, the most important factors that increase the AS-ISK score are high climate adaptability, flexibility in the use of food resources, and high invasion potential elsewhere, while the factors that lower the scores are; the lack of data on the species in aquaculture, distribution mechanism, tolerance and reproduction (Table 1).

BRA score indicated a low invasiveness potential for *S. reciprocans*, and the most influential factors in this score were resource exploitation (5.0), invasiveness in other areas (4.5), and undesirable (or persistence traits) features (4.0) (Table 1). Even if our BRA score (15.5) was slightly higher than the threshold score ever calculated for marine invertebrate groups (MIGs) as 15.1 (Vilizzi *et al.* 2021), it is not surprising as the biology and ecology of the species are not fully known (i.e. only very little data is available). This alien sea cucumber species has settled and formed dense populations on almost all Mediterranean coasts and is already occur in the RA. Unfortunately, inter and intraspecific interactions of this species, which can live in different habitats, and potential ecological impacts on the marine ecosystem are unknown.

Our BRA+CAA score (27.5) showed that *S. reciprocans* had a high invasive potential and it was considerably higher than the score calculated for MIGs as 14.25 (Vilizzi *et al.* 2021). Current climate models predicted that the water temperature will increase by 0.5-1°C in the next 50 years (Peel *et al*. 2007; Demir *et al*. 2008 and www.mgm.gov.tr/FILES/iklim/iklimdegisikligi-project2015.pdf.), and therefore, it is likely that the distribution of *S. reciprocans* will further expand in the future climatic conditions. However, it appears quite difficult to provide risk management recommendations unless the lack of available data on this species is completed.

References

Baker R.H.A., Black R., Copp G.H., Haysom K.A., Hulme P.E., Thomas M.B., Brown A., Brown M., Cannon R.J.C., Ellis J., Ellis E., Ferris R., Glaves P., Gozlan R.E., Holt H., Howe L., Knight J.D., MacLeod A., Moore N.P., Mumford J.D., Murphy S.T., Parrott D., Sansford C.E., Smith G.C., St-Hilaire S., Ward N.L. (2007). The UK Risk Assessment Scheme for all Non-Native Species. Neobiota 7, 46–57.

Bilge G., Filiz H., Yapici S., Tarkan A.S., Vilizzi L. (2019). A risk screening study on the potential invasiveness of Lessepsian fishes in the south-western coasts of Anatolia. Acta Ichthyologica et Piscatoria 49, 23–31.

Cherbonnier G. (1986). Holothuriens de la Méditerranée et du nord de la mer Rouge. Bulletin du Muséum National d'Historie Naturelle 8, 43-46.

Bitar G., Dupuy de la Grandrive R., Foulquie M. (2003). Second mission relating to the development of marine protected areas on Syrian coasts. Technical Report, 40 pp.

CIRCABC (2021). EU Collaborative and Information Resources Centre for Administration, Businesses and Citizens. https://circabc.europa.eu (Accessed: 06.10.2021).

Cinar M.E., Bilecenoglu M., Ozturk B., Can A. (2006). New records of alien species on the Levantine coast of Turkey. Aquatic Invasions 1, 84–90.

Clark A.M., Rowe F.E.W. (1971). Monograph of shallow-water Indo-West Pacific echinoderms. British Museum, Natural History, London, 238 pp.

Clarke S.A., Vilizzi L., Lee L., Wood L.E., Cowie W.J., Burt J.A., Mamiit R.J., Hassina A., Davison P.I., Fenwick G.V., Harmer R., Skóra M.E., Kozic S., Aislabie L.R., Kennerley A., Le Quesne W.J.F., Copp G.H., Stebbing P.D., (2020). Identifying potentially invasive non-native marine and brackish water species for the Arabian Gulf and Sea of Oman. Global Change Biology 26, 2081–2092.

Copp G.H., Vilizzi L., Mumford J., Fenwick G.V., Godard M.J. (2009). Calibration of FISK, an Invasive-Ness Screening Tool for Non-Native Freshwater Fishes. Risk Analysis 29, 457–467.

Copp G.H., Vilizzi L., Tidbury H., Stebbing P.D., Tarkan A.S., Moissec L., Goulletquer Ph. (2016). Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. Management of Biological Invasions 7, 343–350.

Daehler C.C., Denslow J.S., Ansari S., Kuo H.C. (2004). A risk assessment system for screening out invasive pest plants from Hawaii and other Pacific Islands. Conservation Biology 18, 360–368.

Demir İ., Kılıç G., Coşkun M. (2008). Sayfa 365–373. PRECIS Bölgesel İklim Modeli ile Türkiye İçin İklim Öngörüleri: HadAMP3 SRES A2 Senaryosu IV. Atmosfer Bilimleri Sempozyumu, Bildiriler Kitabı. İTÜ Uçak ve Uzay Bilimleri Fakültesi Meteoroloji Mühendisliği Bölümü, 25–28 Mart 2008, İstanbul, Türkiye (in Turkish).

Erickson T.R., Stefan H.G. (1996). Correlations of Oklahoma stream temperatures with air temperatures. University of Minnesota, St. Anthony Falls Laboratory, Project Report No. 398.

European Commission (2014). Turkey progress report. *http://ec.europa.eu/enlargement/pdf/key_documents/2014/20141008-turkey-progress-report_en.pdf*.
Forsskål P., Niebuhr C. (1775). Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium / quae in itinere orientali observavit Petrus Forskål. Post mortem auctoris edidit Carsten Niebuhr. Adjuncta est materia medica kahirina atque tabula maris Rubri geographica. Hauniæ [Copenhagen]:ex officina Mölleri.[alternative spelling Forskål is also correct], available online at https://www.biodiversitylibrary.org/page/2088059.
Killi N., Tarkan A.S., Kozic S., Copp G.H., Davison P.I., Vilizzi L. (2020). Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea. Marine Pollution Bulletin 150:110728.
Mahdy A., Hamdy A. Omar Saif A.M., Nasser Khaleid F., Abd El-Wakeil, Ahmad H., Obuid-Allah (2019). Community structure of Echinoderms in littoral zone of the Red Sea Coast of Egypt. Egyptian Journal of Aquatic Biology 22(5), 483–498.
Okuş E., Altıok H., Yüksek A., Yılmaz N., Yılmaz A.A., Karhan S., Demirel N., Müftüoğlu E., Demir V., Zeki S., Kalkan E., Taş, S. (2007). Biodiversity in western part of the Fethiye Bay. Journal of the Black Sea / Mediterranean Environment 13, 19–34.
Peel M.C., Finlayson B.L., McMahon T.A. (2007). Updated World Map of Köppen-Geiger Climate Classification. Hydrology Earth Systems Sciences 11, 1633–1644.
Pheloung P.C., Williams P.A., Halloy S.R. (1999). A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. Journal of Environmental Management 57, 239–251.
Ragkousis M., Marmara D., Filiz H., Uyan U., Tuncer S., Romanidis-Kyriakidis G., Giovos I. (2017). The northward expansion of *Synaptula reciprocans* (Echinodermata) in the Mediterranean Sea. Journal of the Black Sea / Mediterranean Environment 23, 209–215.
Roy H.E., Peyton J., Aldridge D.C., Bantock T., Blackburn T.M., Britton R., Clark P., Cook E., Dehnen-Schmutz K., Dines T., Dobson M., Edwards F.O., Harrower C., Harvey M.C., Minchin D., Noble D.G., Parrott D., Pocock M.J.O., Preston C.D., Roy S., Salisbury A., Schönrogge K., Sewell J., Shaw R.H., Stebbing P., Stewart A.J.A., Walker K.J. (2014). Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Global Change Biology 20(12), 3859–3871.
Ruykys L., Ta K.A.T., Bui T.D., Vilizzi L., Copp G.H. (2021). Risk screening of the potential invasiveness of non-native aquatic species in Vietnam. Biological Invasions 23, 2047–2060.
Stasolla G., Tricarico E., Vilizzi L. (2021). Risk screening of the potential invasiveness of non-native marine crustacean decapods and barnacles in the Mediterranean Sea. Hydrobiologia 848, 1997–2009.
Vilizzi L., Copp G.H., Hill J.E., *et al*. (2021). A global-scale screening of non-native aquatic organisms to identify potentially invasive species under current and future climate conditions. Science of the Total Environment 788, 147868.
Zenetos A., Galanidi M., (2017). EU Non-native Species Risk Analysis – Risk Assessment for *Rapana venosa* (Valenciennes, 1846). Technical note prepared by IUCN for the European Commission.
Zibrowius H., Bitar G. (2003). Invertébrés marins exotiques sur la côte du Liban. Lebanese Science Journal 4, 67–74.

OCCUPANCY ESTIMATION OF ALIEN, CRYPTOGENIC, AND NEONATIVE SPECIES IN PELOPONNESE (GREECE)

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Abstract

The occupancy of 18 alien, cryptogenic and neonative target species along the coastline of Peloponnese was estimated based on a repetitive presence-absence visual survey, conducted in August 2020. Specifically, 35 rocky-reef sites were surveyed; at each site three 15-min visual surveys were conducted in the upper infralittoral zone (0–5 m), by the same observer, recording the presence or absence of the target species. The data collected were used to jointly estimate occupancy and detectability through a maximum likelihood approach, thus avoiding detectability biases (i.e. due to false absences). Fifteen of the target species were detected in at least one site, i.e. the alien fishes *Fistularia commersonii*, *Pterois miles*, *Siganus luridus*, *Siganus rivulatus*, and *Stephanolepis diaspros*, the alien invertebrates *Conomurex persicus*, *Diadema setosum*, *Percnon gibbesi*, *Pinctada imbricata*, and *Synaptula reciprocans*, the alien macroalgae *Asparagopsis taxiformis* and *Stypopodium schimperi*, the neonative fish *Enchelycore anatina*, the cryptogenic invertebrate *Oculina patagonica*, and the cryptogenic macroalga *Ganonema farinosum*. *Siganus rivulatus* had the highest occupancy (~85.6%), followed by *P. gibbesi* (~78.8%), *S. luridus* (~77.4%), and *G. farinosum* (~67.1%). The other target species (i.e. the alien fishes *Lagocephalus sceleratus* and *Sargocentron rubrum* and the alien macroalga *Caulerpa cylindracea*) were not detected at any station, although they have been reported before in Peloponnese. The complete absence of *C. cylindracea*, despite being considered among the most invasive alien species in the Mediterranean is remarkable, indicating a ‘boom-and-bust’ invasion pattern in the region, probably being affected by intense herbivory by both native and alien herbivores.

Keywords: *Biological invasions, detectability, Mediterranean, occupancy estimation, visual underwater survey*

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1. Introduction

The Mediterranean Sea is a hotspot of biological invasions, with almost 1,000 alien or cryptogenic species having been recorded, of which >650 species are considered as established (Zenetos & Galanidi 2020). Furthermore, neonative species (*sensu* Essl *et al*. 2019), i.e. eastern Atlantic species expanding their range, most probably due to climate change, and entering into the Mediterranean Sea, also contribute to the continuous modification of Mediterranean biota (Evans *et al*. 2020).

Presence-only records of alien/cryptogenic/neonative species are useful to evaluate changes in distribution range and establishment success in new regions. However, they are insufficient to assess population status. Occupancy, defined as the probability of presence in a sampling unit, is a low-cost state variable that can allow such assessments. Occupancy is easier to estimate than population density or abundance and is adequate for large-scale monitoring, in particular in studies of biological invasions (Katsanevakis *et al*. 2011a; Issaris *et al*. 2012). When based on repetitive sampling and the application of a maximum-likelihood framework that accounts for imperfect detectability, unbiased occupancy values can be estimated (MacKenzie *et al*. 2006).

The aim of the present study was to estimate the occupancy of alien, cryptogenic and neonative species in the shallow zone of rocky reefs along the coastline of Peloponnese, and thus provide an assessment of their status and a baseline for future evaluations of the dynamics of biological invasions in the region.

2. Materials and Methods

The occupancy of 18 targeted species was investigated in August 2020, through a snorkelling visual survey conducted in the upper infralittoral zone (0–5 m) of rocky reefs along the coastline of Peloponnese (Greece), applying the field protocol proposed by Issaris *et al*. (2012). Specifically, 35 sites were surveyed; disproportionally less sites were surveyed in the western and northern coasts due to the scarcity of reefs (Fig. 1; Table 1). At each site, three 15-min snorkelling surveys were conducted by the same observer, recording the presence/absence of the targeted species. Photographic and video footages were also taken to document the records.

Table 1: Sampling sites (as in Figure 1), their geographical coordinates (in decimal degrees) and sampling dates.

Site	Latitude (N)	Longitude (E)	Sampling date	Site	Latitude (N)	Longitude (E)	Sampling date
S1	37.839567	23.062711	2/8/2020	S19	36.681483	22.82852	10/8/2020
S2	37.836799	23.126953	2/8/2020	S20	36.800386	22.619632	10/8/2020
S3	37.758523	23.131717	1/8/2020	S21	36.590226	22.472262	11/8/2020
S4	37.631865	23.162702	1/8/2020	S22	36.429423	22.487247	11/8/2020
S5	37.582646	23.348332	3/8/2020	S23	36.544627	22.388554	12/8/2020
S6	37.532236	23.483558	3/8/2020	S24	36.638598	22.378841	12/8/2020
S7	37.295836	23.201473	4/8/2020	S25	36.809786	22.296156	13/8/2020
S8	37.532704	22.899315	5/8/2020	S26	36.928537	22.138975	13/8/2020
S9	37.531797	22.920579	5/8/2020	S27	36.752383	21.919022	14/8/2020
S10	37.51286	22.738422	6/8/2020	S28	36.913757	21.690182	14/8/2020
S11	37.424628	22.768842	6/8/2020	S29	37.088789	21.579716	15/8/2020
S12	37.188361	22.908864	7/8/2020	S30	37.639219	21.306101	15/8/2020
S13	36.971198	22.995059	7/8/2020	S31	38.103791	21.348445	16/8/2020
S14	36.78526	23.087068	8/8/2020	S32	38.172115	22.314059	17/8/2020
S15	36.737409	23.045865	8/8/2020	S33	38.143059	22.380176	18/8/2020
S16	36.685875	23.039815	8/8/2020	S34	38.009394	22.755306	18/8/2020
S17	36.433751	23.11342	9/8/2020	S35	37.944926	22.932367	19/8/2020
S18	36.46412	22.983407	9/8/2020				

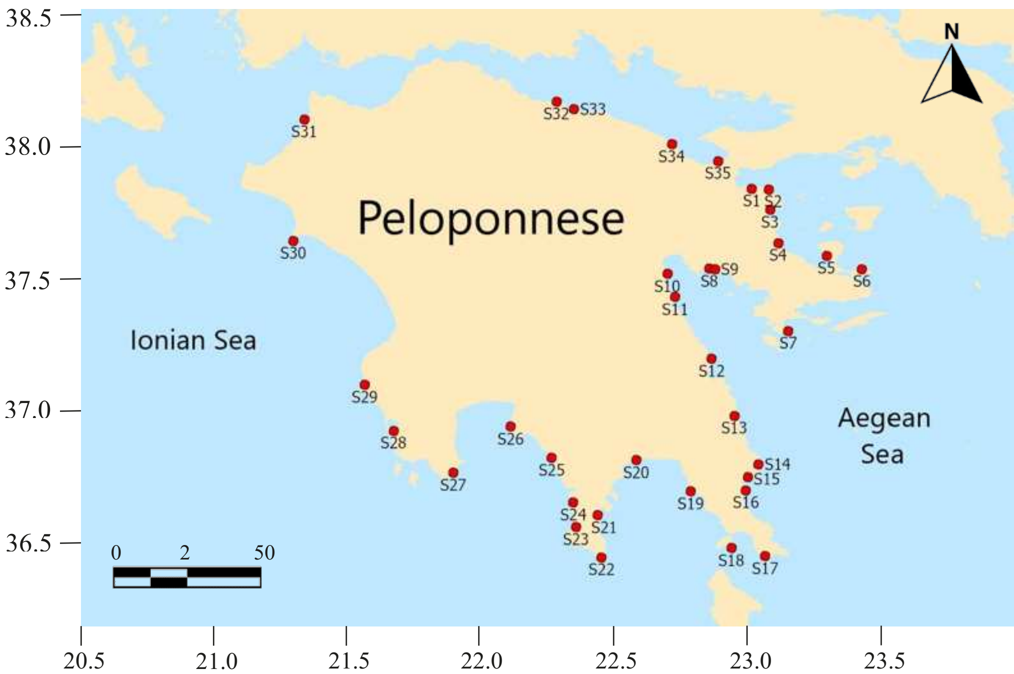

Figure 1. Sampling sites of the occupancy survey in Peloponnese (numbers corresponding to localities reported in Table 1).

The resulting presence/absence data for each site and survey were used to jointly estimate the occupancy and detectability of each species by applying the single-season occupancy model of MacKenzie *et al*. (2006). The software PRESENCE v10.7 (Hines 2006) was used for all estimations except for *S. diaspros*, *F. commersonii*, *E. anatina*, and *S. reciprocans* due to insufficient data; in this case the naïve estimate of occupancy is given, i.e. the number of sites with detection divided by the total number of sites.

Specifically, occupancy ψ was jointly modelled with the probability of detection p. Two stochastic processes affect the detection of each target species at a site (MacKenzie *et al*. 2006). A site might be either occupied (with probability ψ) or unoccupied (with probability $1 - \psi$) by the target species. In the latter case, the target species will not be detected. If the site is occupied, the observer will either detect the target species (with probability p) or not (with probability $q = 1 - p$). The probability of each detection history can be expressed as a function of ψ and p. For example, the probability of the detection history H_i=101 (denoting that the site i was surveyed three times, with the species being detected during the first and third surveys but not by the second) would be $Pr(H_i = 101) = \psi p_1 q_2 p_3 = \psi p(1-p)p = \psi p^2(1-p)$. For sites where the species is not detected during any of the three surveys, there are two possibilities, either the species is present but never detected (a 'false absent') or the species is genuinely absent. Thus, $Pr(H_i=000) = \psi q_1 q_2 q_3 + (1-\psi) = \psi(1-p)^3 + (1-\psi)$. By deriving such expressions for each of the N=35 observed detection histories for each species, the likelihood of the data for each species can be estimated as $L(\psi, p|H_1, H_2, \dots, H_{35}) = \prod_{i=1}^{35} \Pr(H_i)$; ψ and p can then be estimated with standard maximum likelihood techniques (MacKenzie et al. 2006).

3. Results and Discussion

Fifteen of the target species were detected in at least one site, i.e. the alien fishes *Fistularia commersonii*, *Pterois miles*, *Siganus luridus*, *Siganus rivulatus*, and *Stephanolepis diaspros*, the alien invertebrates *Conomurex persicus*, *Diadema setosum*, *Percnon gibbesi*, *Pinctada imbricata radiata*, and *Synaptula reciprocans*, the alien macroalgae *Asparagopsis taxiformis* and *Stypopodium schimperi*, the neonative fish *Enchelycore anatina*, the cryptogenic invertebrate *Oculina patagonica*, and the cryptogenic macroalga *Ganonema farinosum*. The other target species (i.e. the alien fishes *Lagocephalus sceleratus* and *Sargocentron rubrum* and the alien macroalga *Caulerpa cylindracea*) were not detected at any station, although they have been reported before in Peloponnese according to the Ellenic Network on Aquatic Invasive Species (ELNAIS; Zenetos *et al*. 2015). This is indicative of their very low occupancy in the upper infralittoral zone of reef habitats in the region.

Siganus rivulatus had the highest occupancy (~85.6%), followed by *P. gibbesi* (~78.8%), *S. luridus* (~77.4%), and *G. farinosum* (~67.1%) (Table 2). All four species were very abundant, exhibiting invasive character at many sites and dominating local communities. Among the detected species, *F. commersonii*, *E. anatina*, and *S. reciprocans* had the lowest occupancy as they were observed at only one site each.

In particular, the high occupancy and abundance of the two siganids is worrying as they may have severe impacts on rocky reefs due to overgrazing (Katsanevakis *et al*. 2014). The two species were found to dominate fish communities at many of the surveyed sites, as previously reported from other localities of the southern Aegean Sea (e.g. Thessalou-Legaki *et al*. 2012; Gerovasileiou *et al*. 2017; Sini *et al*. 2019). At such high abundances they can cause a regime shift from algal forests, such as those formed by perennial macroalgae of *Cystoseira sensu lato*, to rocky barrens (Sala *et al*. 2011), with significant impacts on biodiversity and ecosystem services (Katsanevakis *et al*. 2014).

Percnon gibbesi is a predominantly herbivorous crab that has expanded rapidly in the Mediterranean after its introduction in 1999 (Katsanevakis *et al*. 2011b). Although there are no reported impacts of this species on biodiversity and ecosystem services (Katsanevakis *et al*. 2014), the species can become very abundant locally and also contribute to the overgrazing of macroalgal forests by other native and alien herbivores.

There are no reported impacts of the cryptogenic red alga *Ganonema farinosum* on biodiversity or ecosystem services in the Mediterranean Sea (Verlaque *et al*. 2015). Nevertheless, the species was found in the present survey to be very abundant at some stations, dominating macroalgal communities, in particular in overgrazed reefs. It seems that the species is favored by overgrazing, as it is probably avoided by both native and alien grazers and thus benefits from the release of competition by other macroalgae. Similar cases of high abundance of *G. farinosum* in overgrazed reefs have been reported from Crete (Gerovasileiou *et al*. 2017), Karpathos and Chalki islands (Thessalou-Legaki *et al*. 2012), and Zakynthos (Dimitriadis *et al*. 2021).

Remarkable is the complete absence of *C. cylindracea*, despite being considered among the most invasive alien species in the Mediterranean (Katsanevakis *et al*. 2014, 2016) and being recorded at high densities in the region in the past. Katsanevakis *et al*. (2016) ranked it as the species with the highest cumulative impact on marine habitats in the Mediterranean Sea. In the past, the species used to be very abundant in shallow rocky reefs in the Mediterranean (e.g. Katsanevakis *et al*. 2011a) but in recent years it has become quite scarce, indicating a 'boom-and-bust' temporal pattern, i.e. substantially declining after a period of very high abundance.

The present study offers a baseline for future assessments of the temporal dynamics of the surveyed species in the region. Biological invasions are very dynamic and thus regular monitoring is needed to assess rates of change and shifts in the composition of biota, as new species arrive and complex biotic interactions and abiotic effects continue to cause changes. Especially climate change is expected to cause substantial modifications in marine communities that need to be carefully monitored to inform conservation efforts and the management of biological resources.

Table 2: Records of the detected target species and estimated occupancy (ψ ± standard error) in the study area (species ranked by occupancy).

Species	Sites	Occupancy
S. rivulatus Forsskål & Niebuhr, 1775	S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30	0.857 ± 0.059
P. gibbesi (H. Milne Edwards, 1853)	S1, S3, S4, S5, S6, S7, S8, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30	0.788 ± 0.073
S. luridus (Rüppell, 1829)	S2, S4, S5, S6, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30	0.774 ± 0.071
G. farinosum (J.V. Lamouroux) K.C. Fan & Yung C. Wang	S5, S7, S8, S10, S11, S12, S13, S14, S16, S18, S20, S21, S23, S24, S25, S26, S27, S28, S29, S30, S33, S34, S35	0.671 ± 0.083
P. imbricata Röding, 1798	S1, S2, S4, S5, S9, S10, S18, S19, S24, S28, S31	0.574 ± 0.248
C. persicus (Swainson, 1821)	S1, S2, S3, S4, S5, S6, S7, S8, S9, S15, S18, S19, S21, S24, S25	0.451 ± 0.089
D. setosum (Leske, 1778)	S12, S14, S19, S22, S23, S24, S26	0.355 ± 0.191
S. schimperi (Kützing) Verlaque & Boudouresque	S1, S2, S3, S6, S8, S9, S35	0.200 ± 0.068
P. miles (Bennett, 1828)	S19, S21, S22, S23, S24	0.150 ± 0.063
O. patagonica de Angelis D'Ossat, 1908	S1, S2, S3, S4	0.114 ± 0.054
A. taxiformis (Delile) Trevisan de Saint-Léon	2, 21, 23	0.090 ± 0.050
S. diaspros Fraser-Brunner, 1940	S1, S3, S5	0.086†
F. commersonii Rüppell, 1838	S24	0.029†
E. anatina (Lowe, 1838)	S18	0.029†
S. reciprocans (Forsskål, 1775)	S8	0.029†

†naïve estimate of occupancy (number of sites with detection divided by the total number of sites)

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References

Dimitriadis Ch., Fournari-Konstantinidou I., Sourbès L., Koutsoubas D., Katsanevakis S. (2021). Long term interactions of native and invasive species in a marine protected area suggest complex cascading effects challenging conservation outcomes. Diversity 13, 71.

Essl F., Dullinger S., Genovesi P, Hulme PE, Jeschke JM, *et al*. (2019). A conceptual framework for range-expanding species that track human-induced environmental change. BioScience 69, 908–919.

Evans J., Arndt E., Schembri P.J. (2020). Atlantic fishes in the Mediterranean: using biological traits to assess the origin of newcomer fishes. Marine Ecology Progress Series 643, 133–143.

Gerovasileiou V., Akel E.H.Kh., Akyol O., Alongi G., Azevedo F., *et al*. (2017). New Mediterranean Biodiversity Records (July 2017). Mediterranean Marine Science 18, 355–384.

Hines, J.E. (2006). PRESENCE2- Software to estimate patch occupancy and related parameters. http://www.mbr-pwrc.usgs.gov/software/presence.html (accessed 1/2/2017).

Issaris Y., Katsanevakis S., Salomidi M., Tsiamis K., Katsiaras N., Verriopoulos G. (2012). Occupancy estimation of marine demersal species: dealing with imperfect detectability. Marine Ecology Progress Series 453, 95–106.

Katsanevakis S., Zenetos A., Mačić V., Beqiraj S., Poursanidis D., Kashta L. (2011a). Invading the Adriatic: spatial patterns of marine alien species across the Ionian-Adriatic boundary. Aquatic Biology 13, 107–118.

Katsanevakis S., Poursanidis D., Yokes B., Mačić V., Beqiraj S., *et al*. (2011b). Twelve years after the first report of the crab *Percnon gibbesi* (H. Milne Edwards, 1853) in the Mediterranean: current distribution and invasion rates. Journal of Biological Research 16, 224–236.

Katsanevakis S., Wallentinus I., Zenetos A., Leppäkoski E., Çinar M.E., *et al*., 2014. Impacts of marine invasive alien species on ecosystem services and biodiversity: a pan-European review. Aquatic Invasions 9(4), 391–423.

Katsanevakis S., Tsirintanis K., Sini M., Gerovasileiou V., Koukourouvli N. (2020). Aliens in the Aegean – a sea under siege (ALAS). Research Ideas and Outcomes 6, e53057.

MacKenzie D.I., Nichols J.D., Royle J.A., Pollock K.H., Bailey L.L., Hines J.E. (2006). Occupancy Estimation and Modeling. Academic Press, San Diego, CA.

Sala E., Kizilkaya Z., Yildirim D., Ballesteros E. (2011). Alien marine fishes deplete algal biomass in the eastern Mediterranean. PLoS ONE 6(2), e17356.

Sini M., Vatikiotis K., Thanopoulou Z., Katsoupis C., Maina I., *et al*. (2019). Small-scale coastal fishing shapes the structure of shallow rocky reef fish in the Aegean Sea. Frontiers in Marine Science 6, 599.

Thessalou-Legaki M., Aydogan Ö., Bekas P., Bilge G., Boyaci Y.Ö., *et al*. (2012). New Mediterranean Biodiversity Records (December 2012). Mediterranean Marine Science 13(2), 312–327.

Verlaque M., Ruitton S., Mineur F., Boudouresque C.F. (2015). Vol 4. Macrophytes. In: "CIESM atlas of exotic species of the Mediterranean", Briand F. (ed), CIESM Publishers, Monaco, pp. 362.

Zenetos A., Arianoutsou M., Bazos I., Balopoulou S., Corsini-Foka M., *et al*. (2015). ELNAIS: A collaborative network on Aquatic Alien Species in Hellas (Greece). Management of Biological Invasions 6(2), 185–196.

Zenetos A., Galanidi M. (2020). Mediterranean non indigenous species at the start of the 2020s: recent changes. Marine Biodiversity Records 13, 10.

THE EFFECTS OF ABIOTIC FACTORS ON THE ECONOMICALLY IMPORTANT BROWN SEAWEED Dictyopteris polypodioides PHYSIOLOGICAL PERFORMANCE

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Abstract

We investigated the relationship of subcellular (chlorophyll *α* fluorescence kinetics for Rapid Light response Curves-RLC, JIP-test, and Quenching analyses, and chlorophyll content) to organismal (relative growth rate-RGR) physiological parameters of the brown seaweed *Dictyopteris polypodioides* with critical seawater abiotic factors. The aim was to understand present and predict future species seasonality in the Mediterranean Sea, from February to August, and gain knowledge on thermal optima for growth and survival to support species' sea or land-based mass cultivation. Statistically significant variation ($p<0.01$) in 19 from 26 parameters tested correlated (RDA) with seawater temperature, salinity and nutrients was shown. Species could photo-acclimate to high temperatures by dissipating the excess energy to heat to regulate the photosynthetic electron transport chain. Species sensitivity to salinity indicated by photoinhibition of PSII (qI) and low Chl *a* and *c* concentrations under fresh-water plumes of terrestrial or Black Sea origin. Soluble Reactive Phosphorus positive correlation with RGR indicated that nutrients might limit species growth even in the mesotrophic North Aegean coastal waters. The RGR rapid decline at 28°C, i.e., near the species' upper thermal limit (30°C), seems to be the reason for species disappearance from the shallow Aegean coasts during late summer as a primary strategy to survive Mediterranean lethal temperatures. Species photo-acclimation potential at low temperatures may indicate a future shift of species appearance and growth towards winter under optimal salinity conditions. Species growth at present and in the future will also be governed by nutrients that change irregularly as influenced by terrestrial activities or lockdowns.

Keywords: *Seaweed, chlorophyll a fluorescence kinetics, relative growth rate, coastal waters*

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1. Introduction

The genus *Dictyopteris*, widely distributed in the oceans of tropical, subtropical, and temperate regions, have been extensively studied for their unique chemical and biological properties and, therefore, their possible usage in biotechnological applications. Among the species, *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux (synonym of *D. membranacea*) is well-known for its anti-inflammatory molecules that may lead to the design of novel anti-inflammatory therapies (Daskalaki *et al.* 2020), and it is, therefore, recommended for commercial cultivation. *Dictyopteris polypodioides* grow in the Mediterranean Sea from the upper to low sublittoral zones, all the year round (Ballesteros *et al.* 1993), with a growing and reproductive season from March to June. During summer, their fronds decline around the central rib or in shallow waters disappear. Therefore, it is facing challenges due to climate change as their photosynthetic and growth performance primarily depends on the external environmental conditions, especially under the current Mediterranean warming trend (Harley *et al.* 2012). Seaweeds have developed mechanisms to acclimate and protect the photosynthetic system from excessive temperature or light stress allowing them to grow successfully in a changing environment and diverse microhabitats (Hurd *et al.* 2014). This study aimed to understand present and predict future species seasonality in the Mediterranean Sea, from February to August, and gain knowledge on thermal optima for growth and survival to support species' sea or land-based mass cultivation.

2. Materials and Methods

2.1 Plant Material and experimental design

Thalli of *Dictyopteris polypodioides* were sampled from Kavala Gulf (40°48'31.41"N, 24°17'1.78"E), North Aegean Sea, Greece, on May 28th, 2019; July 23rd, 2019; February 4th, 2020; May 5th, 2020; and July 14th, 2020, and were transported to the laboratory in plastic bags within 30–40 min. Seawater temperature and salinity were recorded in the field by a salinity/conductivity portable probe (WTW LF330), while 1 l seawater was sampled and transferred to the laboratory for nutrient analyses. PAR data were mean monthly values of three years (2017-2019) measured in a meteorological station ca. 3 km apart from the sampling site. Twelve (12) upper thalli pieces (0.03–0.07 g) were carefully selected free of epiphytes serving as replicates (n=6) of the experiments. All pieces were pre-acclimatized in 2 l aquaria for one night at dark conditions under constant temperature (Haake cryo-thermostats ±0.2°C) and salinity conditions presented in Table 1 before Chlorophyll *α* fluorescence measurements. The growth medium consisted of artificial aerated medium (6 μmol l^{-1} N-NO_3^- and 0.4 μmol l^{-1} P-PO_4^{3-}), produced by sea salt diluted in resin-filtered tap water (<1.5 μS cm^{-1}).

Table 1. Abiotic factors for each sampling effort according to field measurements. PAR=Photosynthetic active radiation. TDIN: Total dissolved inorganic nitrogen; SRP: Soluble reactive phosphorus.

Time	Temperature (°C)	Salinity	Photoperiod (h)	24 h mean PAR (μmol photons m^{-2} s^{-1})	TDIN (μmol l^{-1})	SRP (μmol l^{-1})
May 28, 2019	22	34	15.03	460.65	7.42	0.49
July 23, 2019	27	35.3	14.47	496.08	15.79	0.70
February 4, 2020	10	37.5	10.38	174.35	2.18	0.36
May 5, 2020	16	34.3	15.03	460.65	4.16	0.45
July 14, 2020	28	35.2	14.47	496.08	2.42	0.32

2.2 Chlorophyll-α measurements

2.2.1 RLC parameters

Underwater-Diving PAM (Waltz, GmbH, Effeltrich, Germany) fluorometer Rapid Light Curves (RLC) protocol was used to determine three photosynthetic parameters ($rETR_{max}$, alpha, E_k) described the PSII performance in *D. polypodioides* by illumining the dark-adapted specimens with different levels of actinic light.

2.2.2 JIP-test and quenching parameters

A double-modulation FL3500-F fluorometer (PSI Drasov, Czech Republic) was used for OJIP and Quenching Analysis (QA) measurements. OJIP curves plotted in a logarithmic scale from 10 μs to 1 s, after illumination of dark-adapted specimens with continuous red light (> 2.500 photons m^{-2} s^{-1}) and far-red pre-illumination (for PQH_2 reoxidation *via* PSI activity). F_0 was determined at 20 μs when all RCs are open. The parameters (ABS/RC, TR_0/RC, DI_0/RC, RC/ABS, φ_{P0}, ψ_{E0}, φ_{E0}, φ_{R0}, δ_{R0}, K-band) were calculated from the extracted data, and the basic parameters derived by the JIP-test models. *Quenching analysis* is a protocol based on comparing the maximal fluorescence emission (F_{max}, F_{max}') before and during exposure to actinic light. The fluorescence emission F_{max} (F_{max}') is the correspondence to the reduction of the primary quinone acceptor in all PSII RCs. The calculated parameters (Φ_{PSII}, qP, qL, NPQ, and qI) from the extracted data give information about the quenching and non-quenching mechanisms of photosynthetic machine.

2.3 Photosynthetic pigments

Chlorophyll *α* (Chl *α*), Chlorophyll *c* (Chl *c*), Fucoxanthin (Fx) and Carotenoids (Car) were estimated on the replicates used for JIP-test and QA analyses.

2.4 Relative Growth Rate (RGR)

Six (6) replicates measured for RLC experiments were further cultivated for six (6) days to estimate the relative growth rate (RGR) at different temperatures (see Table 1) in 0.5 l glass vessels submerged in Haake cryo-thermostats (±0.2°C). RGR (day^{-1}) = ($LnWB_{t2} - LnWB_{t1}$)/($t_2 - t_1$), where WB_{t2} was the wet biomass on the final (6th) experimental day, and WB_{t0} was the wet biomass before the start of the experiment. Field material sampled during July 2020 was further cultivated for six (6) days (n=6) to estimate RGR at 15, 20, 25 and 30°C in 0.5 l glass vessels, submerged in Haake cryo-thermostats (±0.2°C).

2.5 Statistical analyses

Data normality was tested by the Shapiro-Wilk's W test, while Levene's test tested the homogeneity. If normality existed, data presented as mean (n = 6) ± standard error (SE), otherwise as median (interquartile range-IQR). JIP-test and QA parameters, photosynthetic pigments, and RGR were analyzed with one-way analysis of variance (one-way ANOVA). Duncan's post hoc test tested the multiple comparisons. In the present study, all samples did not have normal distribution and homogeneity of variance and thus, the significance level (p-value) was defined as 0.01. Redundancy forward analysis (RDA) was used to quantify the correlation of abiotic factors (temperature, salinity, photoperiod, PAR and SRP) with the chlorophyll *a* fluorescence parameters, pigment content, and RGR parameters using Canoco 5.1 (Microcomputer Power, Ithaca, NY, USA). Statistical tests were run using the Monte Carlo permutation procedure. Collinearity of abiotic factors was tested by Spearman rank correlations analysis.

3. Results

3.1 RLC parameters

The factor "time" was significant on the relative maximal electron transport rate ($rETR_{max}$), photosynthetic efficiency (alpha) and saturated irradiance (E_k) ($p<0.01$, Table 2). The maximum median $rETR_{max}$ values were observed in May 19 and the minimum in February 2020. The maximum mean alpha values were observed in February 2020 and the minimum in May 2020. The maximum mean E_k values were observed in May 2019 and the minimum in February 2020.

3.2 JIP-test and QA parameters

The light absorption flux of antenna Chl *a* molecules per RC (ABS/RC), trapped energy flux per RC (TR_0/RC), RCs per PSII antenna Chl *a* (RC/ABS) and activity of oxygen evolving complex (OEC; K-band) were not affected by "time" ($p>0.01$, Table 2). The factor "time" was significant on the dissipated energy flux per RC (DI_0/RC; $p<0.01$). The maximum median DI_0/RC values were observed in February 2020 while the minimum values in July 2019. The factor "time" was significant on the maximum quantum yield for primary photochemistry (φ_{P0}; $p<0.01$). The maximum median φ_{P0} values were observed in July 2019, while the minimum values in February 2020. The efficiency/probability that an electron moves further than Q_A^- (ψ_{E0}) and the quantum yield for electron transport further than Q_A^- (φ_{E0}) were affected by time ($p<0.01$). The maximum ψ_{E0} (median) and φ_{E0} (mean) values were observed in July 2020 and 2019, respectively, while the minimum values in February 2020. The factor "time" was significant on the quantum yield for reduction of end electron acceptors at the PSI acceptor side (φ_{R0}) and efficiency/probability that an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (δ_{R0}) ($p<0.01$). The maximum mean φ_{R0} values were observed in July 2019 while the minimum values in May 2019. The maximum mean δ_{R0} values were observed in July 2020 while the minimum values in May 2019.

Table 2. Parameter values of mean ± standard error or median (IQR), n = 6. Latin Statistical differences from the post hoc test (p < 0.01) are represented using different Latin letters. ABS/RC= $M_0 \times (1/V_J) \times (1/\phi_{P0})$, TR_0/RC= $M_0 \times (1/V_J)$, DI_0/RC= ABS/RC − TR_0/RC, RC/ABS= $(TR_0/ABS) \times (TR_0/RC)^{-1}$, φ_{P0}= $TR_0/ABS = [1 - (F_0/F_M)]$, ψ_{E0}=$ET_0/TR_0 = (1 - V_J)$, φ_{E0}= $ET_0/ABS = [1 - (F_0/F_M)] \times (1 - V_J)$, φ_{R0}= $RE_0/ABS = [1 - (F_0/F_M)] \times (1 - V_I)$, δR_0= $RE_0/ET_0 = (1 - V_I)/(1 - V_J)$, K-band= $V_{300\mu s}$ (variable fluorescence), Φ_{PSII}= $(F_M'-F_S')/F_M'$, qP= $(F_M'-F_S)/(F_M'-F_o')$, qL= qP x (F_0'/F_S), qN= $(F_v-F_v')/F_v$, NPQ= (F_M/F_M') - 1, qI= $(F_M-F_M'')/(F_M-F_0')$.

Parameter	May19	July19	Feb20	May20	July20	p
ABS/RC	2.639 (0.255)^a	2.385 (0.372)^a	2.775 (0.814)^a	2.904 (0.159)^a	2.616 (0.324)^a	0.089
TR0/RC	1.504 ± 0.038^a	1.376 ± 0.021^a	1.366 ± 0.065^a	1.512 ± 0.048^a	1.451 ± 0.041^a	0.081
DI0/RC	1.095 (0.172)^a	0.979 (0.246)^a	1.433 (0.442)^b	1.305 (0.433)^{bc}	1.074 (0.157)^{ac}	**<0.01**
RC/ABS	0.390±0.011^a	0.420±0.014^a	0.352±0.02^a	0.344±0.014^a	0.371±0.025^a	0.039
φ_{P0}	0.582 (0.009)^a	0.590 (0.038)^a	0.477 (0.029)^b	0.551 (0.110)^{ab}	0.565 (0.025)^{ab}	**<0.01**
ψE0	0.601 (0.025)^a	0.615 (0.033)^a	0.536 (0.057)^b	0.599 (0.014)^a	0.619 (0.009)^a	**<0.01**
φE0	0.354 ± 0.004^a	0.358 ± 0.007^a	0.254 ± 0.012^b	0.309 ± 0.012^a	0.329 ± 0.019^a	**<0.01**
φR0	0.080 ± 0.004^a	0.113 ± 0.009^b	0.085 ± 0.010^{ab}	0.106 ± 0.002^{ab}	0.108 ± 0.004^b	**<0.01**
δR0	0.0226 ± 0.010^a	0.315 ± 0.009^{ab}	0.332 ± 0.024^b	0.345 ± 0.012^b	0.479 ± 0.035^c	**<0.01**
K-band	0.172 (0.007)^{ab}	0.149 (0.007)^b	0.181 (0.061)^a	0.177 (0.019)^{ab}	0.163 (0.011)^{ab}	0.033
ΦPSII	0.195 ± 0.183^a	0.187 ± 0.150^a	0.119 ± 0.033^b	0.104 ± 0.077^b	0.191 ± 0.165^a	**<0.01**
qP	0.289 ± 0.005^a	0.284 ± 0.030^a	0.183 ± 0.015^b	0.175 ± 0.018^b	0.290 ± 0.014^a	**<0.01**
qL	0.115 (0.005)^a	0.096 (0.035)^a	0.074 (0.020)^a	0.073 (0.016)^a	0.112 (0.043)^a	0.020
qN	0.171 (0.034)^a	0.137 (0.151)^a	0.130 (0.101)^a	0.239 (0.05)^a	0.179 (0.097)^a	0.732
NPQ	0.122 (0.021)^a	0.100 (0.148)^a	0.063 (0.007)^a	0.119 (0.047)^a	0.147 (0.052)^a	0.234
qI	0.079 (0.026)^a	0.064 (0.174)^a	0.092 (0.041)^a	0.487 (0.057)^b	0.077 (0.036)^a	**<0.01**
RGR	0.055 ± 0.005^a	0.053 ± 0.005^a	0.012 ± 0.003^b	0.057 ± 0.008^a	0.001 ± 0.005^c	**<0.01**
rETRmax	56.426 (23.050)^a	54.958 (28.234)^a	12.745 (4.470)^b	16.695 (1.937)^b	55.351 (8.047)^a	**<0.01**
alpha	0.444 ± 0.034^{ab}	0.525 ± 0.095^{ab}	0.612 ± 0.045^a	0.303 ± 0.044^b	0.549 ± 0.048^a	**<0.01**
Ek	121.807 ± 6.56^a	98.624 ± 13.384^{ac}	22.502 ± 2.323^b	65.151 ± 9.788^c	103.221 ± 9.007^a	**<0.01**
Chl *a*	0.829 ± 0.037^a	1.519 ± 0.094^b	1.842 ± 0.064^c	1.058 ± 0.065^a	1.348 ± 0.084^b	**<0.01**
Chl *c*	0.177 (0.092)^a	1.274 (0.701)^b	0.957 (0.380)^c	0.127 (0.098)^a	0.709 (0.335)^c	**<0.01**
Car	19.051 (6.602)^a	11.588 (4.418)^{ab}	35.918 (0.854)^c	9.515 (4.506)^b	13.226 (2.896)^{ab}	**<0.01**
Fx	0.480 (0.077)^a	0.000 (0.157)^b	0.573 (0.009)^a	0.166 (0.098)^{bc}	0.280 (0.084)^c	**<0.01**
Chl/Car	0.052 (0.011)^a	0.234 (0.090)^b	0.080 (0.020)^a	0.129 (0.033)^a	0.155 (0.089)^{ab}	**<0.01**
Chl/Car+Fx	0.051 (0.010)^a	0.234 (0.092)^b	0.079 (0.020)^a	0.126 (0.030)^a	0.152 (0.088)^{ab}	**<0.01**

The coefficient of photochemical fluorescence quenching assuming interconnected PSII antennae, the coefficient of non-photochemical quenching (qN) and the non-photochemical quenching (NPQ) were not affected affected by "time" ($p>0.01$). The factor "time" was significant on the effective PSII photochemical quantum yield (Φ_{PSII}), photochemical quenching (qP) and photoinhibition of photosynthesis (qI) ($p<0.01$). The maximum mean Φ_{PSII} values were observed in May 2019 while the minimum values in May 2020. The maximum mean qP values were observed in July 2020 while the minimum values in May 2020. The maximum median qI values were observed in May 2020 while the minimum values in July 2019.

3.4 Pigment content

The factor "time" was significant on Chl *α*, Chl *c*, Car, Fx content and on the ratios Chl/Car and Chl/Car+Fx ($p<0.01$, Table 2). The maximum mean Chl *α* values were observed in February 2020 while the minimum values in May 2019. The maximum mean Chl *c* values were observed in July 2019 while the minimum values in May 2020. The maximum mean Car values were observed in February 2020 while the minimum values in May 2020. The maximum mean Fx values were observed in February 2020 while the minimum values in July 2019. The maximum mean ratio Chl/Car and Chl/Car+Fx values were observed in July 2019 while the minimum values in May 2019.

3.5 Relative Growth Rate

The factor "time" was significant on the RGR ($p<0.01$, Table 2). The maximum mean RGR values were observed in July 2020 while the minimum values in February 2020. The temperature experiment of July 20 field material grew optimally between 20 and 25°C and lowest at 15°C. At 30°C the plants did not grew and get injured even after 3 days of cultivation.

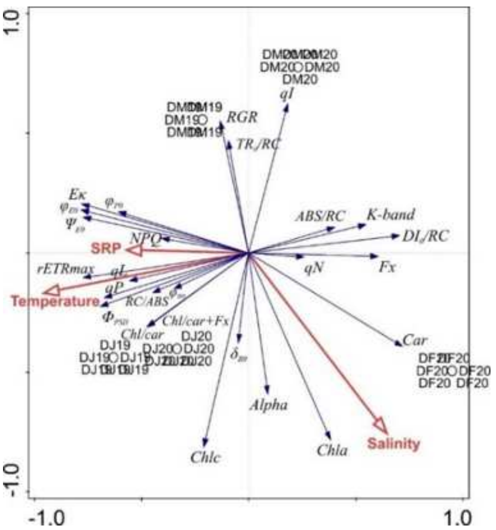

Figure 1. Triplot from the redundancy analysis (RDA) that shows the relationships between the physiological parameters of *Dictyopteris polypodioides* with critical abiotic factors. The length of the arrows indicates the strength of representation and contribution of each parameter to the RDA axes. The first axis accounted for 27.4% of the variation, while the second axis accounted for 12.9%.

3.6 Redundancy forward analysis (RDA)

Analysis of the correlations between the physiological parameters and abiotic factors was performed using an RDA forward selection model (Figure 1). Collinearity between the abiotic factors used in the analysis was weak ($\rho<0.7$). According to the RDA results, temperature (56.3%; Monte Carlo permutation test: pseudo $F = 9.7$, $p = 0.001$), salinity (29.2%; pseudo $F = 5.9$, $p = 0.001$), and SRP (14.5%; pseudo $F = 3.2$, $p = 0.005$) were statistically significant factors that added 100% in total to the explanatory power. The first axis accounted for 27.4% of the variation (Monte Carlo permutation test: $F = 0.918$) and was negatively correlated (-0.877) with the temperature, while the second axis accounted for 12.9% of the variation negatively correlated with the salinity (-0.702) and the third accounted for 5.3% of the variation negatively correlated with the SRP (-0.672). While DI_0/RC and carotenoids were positively correlated with Axis 1, $rETR_{max}$, E_k, ψ_{E0}, φ_{E0}, Φ_{PSII}, and qP were negatively correlated. While qI was positively correlated with Axis 2, Chl *a* and *c* were negatively correlated. RGR was negatively correlated with Axis 3. The experimental points (species) were distributed rather heterogeneously on the RDA1/RDA2 plane, with RDA1 to separate cold from high temperature conditions and with RDA2 high from low salinities.

4. Discussion

Among the three abiotic factors tested, temperature explained the highest variability of *D. polypodioides* physiological parameters (56.3%; $p=0.001$), followed by salinity (29.2%; $p=0.001$) and SRP

(14.5%; p=0.005) (Figure 1). Typical seawater temperature trend in the Mediterranean and the Kavala Gulf, includes lowest values from February to March (lowest mean value 11.45^{o}C, for 2014-15) and highest values during August to September (highest mean value 26.29^{o}C, for 2014-15), that also confirmed in this study (Table 1). Based on the RGR results, such temperature range seems to explain short species seasonality in the shallow Aegean Sea coasts, from February to August. The presence and growth of the species along the year have been observed in other Mediterranean coasts but at low sub-littoral zone, where lower temperatures than the surface exist (Ballesteros *et al.* 1993). On the other hand, photosynthesis performance increased up to 28^{o}C measured under field conditions. Sant & Ballesteros (2020) also reported higher photosynthetic rates at saturation values during summer and lower in winter and spring. The negative correlation between photosynthetic performance ($rETR_{max}$, E_k, ψ_{E0}, φ_{E0}, Φ_{PSII}, and qP) and dissipation of the excess energy to heat (DI_0/RC) indicated the plant's photo-acclimation potential to regulate the photosynthetic electron transport chain rather than photooxidation. High temperatures also increase carotenoid pigments as photo-protectants and antioxidants agents of the photosynthetic apparatus by quenching harmful reactive oxygen species (ROS). This result is also confirmed by TR_0/RC that represents the maximal rate by which an exciton is trapped by the RC, resulting in the reduction of Q_A^-, remained unaffected. The cultivation of the species at 30^{o}C inhibited PSII activity, as indicated by the abrupt reduction of rETRmax and alpha (not shown) even from 3rd day through oxidative damage to PSII proteins (Yamamoto 2016). Regarding high temperatures, it is well known that photosynthesis is affected by thermal stress, which has harmful effects up to death since photosystems and the electron transport system are heat-sensitive.

Seawater salinity fluctuation in the Kavala Gulf differs from the other Mediterranean costs due to Black Sea water influence, being higher during winter and lower during summer. Heavy rains may also affect the salinity of coastal waters locally, as happened during this study affecting species Chl *α,* and Chl *c* contents and photosynthetic efficiency of PSII (alpha). Increased Chl *α* have been reported to enhance the photosynthetic efficiency as a mechanism of the species to enhance PSII cope with stress. Sant & Ballesteros (2020) referred that photosynthetic efficiency (alpha) was higher in spring and summer and lower in winter.

RGR was positively correlated to Soluble Reactive Phosphorus (p=0.005) indicating that species growth may be limited by nutrients even in the mesotrophic North Aegean coastal waters. Species growth at present and in the future will be also governed by nutrients that change irregularly in coastal waters as influenced by terrestrial activities or lockdowns. Exceptionally, max RGR values was measured in May 2020 after the COVID-19 lockdown as laboratory growth medium nutrient pulses may be highly effective in promoting growth.

Acknowledgements

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References

Ballesteros E., Zabala M., Uriz M.J., Garcia-Rubies A., Turon X. (1993). El bentos: les comunitats. In Història Natural de l'Arxipèlag de Cabrera. Monografies de La Societat d'Història Natural de Balears 2. Palma de Mallorca: CSIC-Ed. Moll, 687–730.

Daskalaki M.G., Bafiti P., Kikionis S., Laskou M., Roussis V., Ioannou E., Kampranis S.C., Tsatsanis, C. (2020). Disulfides from the Brown Alga Dictyopteris membranacea Suppress M1 Macrophage Activation by Inducing AKT and Suppressing MAPK/ERK Signaling Pathways. Marine Drugs 18(11), 527.

Harley C.D.G., Anderson K.M., Demes K.W., Jorve J.P., Kordas R.L., Coyle T.A., Graham M.H. (2012). Effects of climate change on global seaweed communities. Journal of Phycology 48(5), 1064–1078.

Hurd C.L., Harrison P.J., Bischof K., Lobban C.S. (2014). Frontmatter. In: Seaweed Ecology and Physiology (2nd ed). Cambridge University Press, p. i–iv.

Sant N., Ballesteros E. (2020). Photosynthetic activity of macroalgae along a bathymetric gradient: Interspecific and seasonal variability. Scientia Marina 84(1), 7–16.

Yamamoto Y. (2016). Quality control of photosystem II: The mechanisms for avoidance and tolerance of light and heat stresses are closely linked to membrane fluidity of the thylakoids. Frontiers in Plant Science 7, 1–13.

LENGTH-WEIGHT RELATIONSHIP AND REPRODUCTION OF THE MARBLED ELECTRIC RAY, *Torpedo marmorata* (RISSO, 1810), FROM THE GREEK SEAS

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Abstract
The present work presents the length-weight relationship, and the reproductive biology of the marbled electric ray, *Torpedo marmorata* (Risso 1810) from the Eastern Ionian and Aegean Seas. The samples were collected from October 2017 to August 2020. A total of 135 females and 109 males were collected, with a total length ranging from 91 to 464 mm for females, and 99 to 313 mm for males. Length at first maturity (L_{50}) was 261 mm for females and 241 mm for males. There was no statistically significant difference in sex ratio. Mature males were recorded throughout the year, whereas ovulating females were present in spring and early autumn. The number of embryos ranged from five to 13 per brood.
Keywords: *Length-weight relationship, length at maturity, sex ratio, gonadosomatic index, torpedo ray*
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1. Introduction

The Mediterranean Sea has the highest percentage of endangered species in the world (Dulvy *et al.* 2016). Extensive overfishing threatens more than 25% of elasmobranch species in the basin, while insufficient data makes it impossible to assess their stocks (Cashion *et al.* 2019). Moreover, the slow recovery of their stocks and the inadequate management measures on national and international level, increase their conservation concern.

Limited information is available on electric rays (Torpedinidae) (Bertrand *et al.* 2000; Damalas & Vassilopoulou 2011; Bellodi *et al.* 2021) due to their low economic value (Relini *et al.* 2000). However, they are caught quite often and discarded by certain types of fishing gear (Tzanatos *et al.* 2007; Morey *et al.* 2006; Gil *et al*. 2018).

The marble electric ray, *Torpedo marmorata* (Risso 1810), is a demersal species with a preference on sandy or muddy bottoms, and shallow depths of the continental shelf, ranging from a few to 300 meters (Tserpes *et al.* 2012). Its distribution extends along the east Atlantic coast and the Mediterranean Sea (Bini 1967; Stehmann & Bürkel 1984). In the IUCN red list it is referred to as "Data Deficient" worldwide (Notarbartolo di Sciara *et al.* 2009), while as "Least Concerned" in the Mediterranean Sea (Notarbartolo di Sciara *et al.* 2016) due to lack of distribution and biological information.

The present study focuses on evaluating the length-weight relationship and several reproductive aspects of *T. marmorata*, such as the length at first maturity, sex ratio, spawning period, parturition and gestation period, from the Ionian (Central Mediterranean) and the Aegean (East Mediterranean) Seas.

2. Materials and Methods

Samples of *T. marmorata* were collected from various research programs and surveys (MEDITS, MEDIAS, DEFISHGEAR, and commercial fishers) in the Ionian and Aegean Seas between October 2017 and August 2020. The depth of their collection ranged from 8 m to 354 m.

Each specimen was sexed, and maturity was determined macroscopically following the MEDITS scale on Sexual Maturity stages of Elasmobranchs (MEDITS Handbook 2017). Length-weight relationships were calculated by applying the formula: $EW = \alpha \times TL^{\beta}$, where EW is the Eviscerated Weight (g), TL is the Total Length (cm), and parameters α(intercept) and β (slope) are the regression coefficients.

The chi-square test was used for the whole sample to determine if the sex ratio (M:F, 1:1) had a statistically significant difference. Size length at first maturity (L_{50}) was calculated for females and males, applying the logistic model: Pm = 1 / (1+ e (- (a + b× TL))) (Ni & Sandeman 1984), where Pm is the probability that a person is mature, while a and b are logistic regression parameters.

The GSI and HSI indices were calculated for males and females separately, according to: GSI = (GW / TW) × 100 and HSI = (LW / TW) × 100 (where LW = liver weight, GW = gonad weight; weighed to the nearest 0.01 g). For both sexes the GSI and HSI relationships were studied and plotted separately per month to examine the reproductive cycle and the spawning period. Embryos collected from gravid females were measured and weighed to the nearest 0.01 mm and 0.01 g, respectively.

3. Results

3.1 Length-weight relationship

From the 244 samples, the 54.35% were females (135) and 45.65% were males (109). No statistically significant difference was detected between the two study areas (p = 0.697), therefore the length-weight relationship was calculated for the whole dataset and each sex. The TL for females ranged from 91 to 461 mm (average ± SD, 257.52 ± 88.32 mm). In males, TL ranged from 99 to 313 mm (average ± SD, 214.22 ± 54.22 mm). Eviscerated weight for females ranged from 26.64 to 1821.00 g (mean ± SD, 475.46 ± 393.10 g) and for males from 24.21 to 512.00 g (230.34 ± 116.05 g). Statistically significant differences were observed between the two sexes in the length-frequency distribution (Kolmogorov-Smirnov test, d = 0.3929, p <0.0001), confirming the large size range and the high mean TL of females. There was a statistically significant difference in weight distribution between the two sexes (Kolmogorov-Smirnov test, d = 0.4205, p <0.0001). The estimated EW - TL relationships (Figure 1) for females (F) and males (M) were:

$EW_f = 0.018 \times TL^{3.006}$, n = 125, $r^2 = 0.98$

$EW_m = 0.027 \times TL^{2.852}$, n = 105, $r^2 = 0.98$

In females, the b value showed an isometric increase (t-test, t (106) = 0.159, p = 0.8743), while in males it showed a negative allometric growth (t-test, t (80) = -2.593, p = 0.0113). A significant difference in b values between males and females was also detected (F (1, 186) = 4.666, p = 0.0320).

3.2 Reproductive biology

The sex ratio (F:M, 1.14:1) was not significantly different (x2 = 1.74, p = 0.187). The length of first sexual maturity (L_{50}) was significantly different between the sexes at the intercepts (Dev = 18.683, p < 0.0001). The L_{50} was estimated at 261 mm for females and 241 mm for males. For females, 70 were immature with a length of 91 to 260 mm, 63 females were mature with a length of 263 to 461 mm, and in two cases no maturity stage was determined. Immature and mature females were found throughout the year. Female ovulation, however, appeared to exist from April to October, while the middle and fully developed embryos were present in May and October, respectively. Regenerated females were observed during three months (October, May, and August). Most males were immature (67) with a length of 99 to 253 mm, while the 42 mature specimens ranged from 230 to 313 mm. All stages were observed throughout the year, while spawning animals were recorded mainly in April and July.

An extended spawning cycle for *T. marmorata* in both the Ionian and Aegean Seas appeared to exist according to the monthly GSI values (Figure 2). This aplacental viviparous species seems to ovulate from April to September (large, fertilized eggs), with a peak in spring and autumn, laying in October and resting in winter. The males appeared to reproduce throughout the year, with the highest GSI values were observed in spring and summer periods. The liver weight followed a similar pattern to the weight of the gonads. The HSI remained stable in males, while for females it was quite high in September and April (Figure 3). The ANOVA test showed differences, in the GSI (F = 28.181, p <0.0001) and HSI (F = 5.9587, p = 0.0156) index, respectively, between sexes. Females also weighed more on the liver and gonads than males. Out of the 63 mature females, only seven were found with embryos, with broods ranging from five and 13 pups. The size of fully developed embryos was from 76 to 103mm (TL) and the TW was from 14.08 to 22.07 g.

4. Discussion

The current study reports no differences in the EW-TL relationships between the two sampling areas (Ionian and Aegean Seas), yet, significant differences were recorded between sexes. *Torpedo marmorata* from the Greek Seas were larger than specimens from the NE Mediterranean (Lamprakis *et al.* 2003; Duman & Basusta 2013), and smaller than those from the Central and Central-western Mediterranean Sea (Consalvo *et al.* 2007; Bellodi *et al.* 2021).

The length–weight relationships showed an isometric and a negative allometric growth in females and males, respectively. Overall, a negative allometric growth trend is detected in the Eastern Mediterranean

(Consalvo *et al.* 2007; Karachle & Stergiou, 2008; Bellodi *et al.* 2021), suggesting the lack of optimum conditions for growth. The marbled electric ray exhibited sexual dimorphism, with females being larger and heavier than males, which could be attributed to large energy expenditure in live-bearing females due to strong selection pressure for large offspring (Sims 2003).

No differences in the sex ratio were detected, indicating that females and males are equally vulnerable to fishing effort and applied sampling methods (Capapé *et al.* 2006). The length at which 50% of the population attained sexual maturity of *T. marmorata* in the present study (261 mm for female and 241 mm in TL for male specimens) was lower in both sexes compared to previous studies (312 mm and 251 mm in TL, Consalvo et al. 2007; 360 mm and 260 mm in TL, Bellodi *et al.* 2021), Egypt (around 355 mm and 255 mm in TL, Abdel-Aziz 1994), Tunisia (390 mm and 290 mm in TL, Capapé 1979), and Senegal (380 mm and 270 mm in TL, Capapé *et al.* 2001), suggesting an early maturation in the study area.

The reproductive cycle of *T. marmorata* is suggested to last from two to three years, based on the GSI and oocyte diameter in female specimens, while males are capable of reproducing throughout the year (Capapé 1979; Abdel-Aziz 1994). Mating takes place between November and January and ovulation for mature females occurs in autumn (Abdel-Aziz 1994; Consalvo *et al.* 2007). Our findings were in agreement with those reported from the Central and S.E. Mediterranean Sea, as maturing eggs were observed all through the year in different sizes, with a peak in spring and autumn. The overall observed litter size was similar to those reported in other areas of the Mediterranean Sea (Abdel-Aziz 1994; Consalvo *et al.* 2007). Fluctuations in torpedinid fecundity have been previously recorded, and were attributed to environmental changes (i.e. salinity, temperature) and/or sampling biases (El Kamel-Moutalibi *et al.* 2013).

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References

Abdel-Aziz S.H. (1994). Observations on the biology of the common torpedo (*Torpedo torpedo*, Linnaeus, 1758) and marbled electric ray (*Torpedo marmorata*, Risso, 1810) from Egyptian Mediterranean waters. Marine and Freshwater Research 45, 693–704.

Bellodi A., Mulas A., Carbonara P., Cau A., Cuccu D., Marongiu MF., Mura V., Pesci P., Zupa W., Porcu C., Follesa MC. (2021). New insights into life–history traits of Mediterranean Electric rays (Torpediniformes: Torpedinidae) as a contribution to their conservation. Zoology 146, 125922.

Bertrand J., Gil-de-Sola-Simarro L., Papakonstantinou C., Relini G., Souplet A. (2000). Contribution on the distribution of elasmobranchs in the Mediterranean (from the MEDITS surveys).

Bini G. (1967). Atlante Dei Pesci Delle Coste Italiane, Vol. 1, Leptocardi, Ciclostomi, Selaci. Mondo Sommerso, Milano.

Capapé C. (1979). La torpillemarbrée, *Torpedo marmorata*Risso, 1810 (Pisces, Rajiformes) des côtestunisiennes: nouvellesdonnées sur l'écologie et la biologie de la reproduction de l'espèce, avec une comparaison entre les populations méditerranéennes et atlantiques. Annales des sciences naturelles. Zoologie et biologieanimale13, 79–97.

Cashion M.S., Bailly N., Pauly D. (2019). Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. Marine Policy 105, 1–9.

Consalvo I., Scacco U., Romanelli M., Vacchi M. (2007). Comparative study on thereproductive biology of *Torpedo torpedo* (Linnaeus, 1758) and *T. marmorata* (Risso, 1810) in the central Mediterranean Sea. Scientia Marina 71, 213–222.

Damalas D., Vassilopoulou V. (2011). Chondrichthyan by-catch and discards in the demersal trawl fishery of the central Aegean Sea (Eastern Mediterranean). Fisheries Research 108, 142–152.

Dulvy N.K., Allen D.J., Ralph G.M., Walls R.H.L. (2016). The conservation status of sharks, rays, and chimaeras in the Mediterranean Sea [Brochure]. IUCN Centre for Mediterranean Cooperation, Malaga, Spain, pp. 14.

Duman Ö., Başusta N. (2013). Age and growth characteristics of marbled electric ray *Torpedo marmorata* (Risso, 1810) inhabiting Iskenderun Bay, North-eastern Mediterranean Sea. Turkish Journal of Fisheries and Aquatic Sciences 13(3), 551–559.

El Kamel-Moutalibi O., Mnasri N., Boumaïza M Ben Amor M.M., Reynaud C., Capapé C. (2013). Maturity, reproductive cycle and fecundity of common torpedo, *Torpedo torpedo* (Chondrichthyes, Torpedinidae) from the Lagoon of Bizerte (Northeastern Tunisia, central Mediterranean). Journal of Ichthyology 53, 758–774.

Gil M.D.M., Catanese G., Palmer M., Hinz H., Pastor E., Mira A., Grau A., Koleva E., Grau A.M., Morales-Nin B. (2018). Commercial catches and discards of a Mediterranean small-scale cuttlefish fishery: implications of the new EU discard policy. Scientia Marina 82, 155–164.

Karachle P.K., Stergiou K.I. (2008). Length-length and length-weight relationships of several fish species from the North Aegean Sea (Greece). Journal of Biological Research 10, 149–157.

Lamprakis M.K., Kallianiotis A.A., Moutopoulos D.K., Stergiou K.I. (2003). Weight-length relationships of fishes discarded by trawlers in the North Aegean Sea. Acta Ichthyologica et Piscatorial 33, 145–152.

Morey G., Moranta J., Riera F., Grau A.M., Morales-Nin B. (2006).Elasmobranchs in trammel net fishery associated to marine reserves in the Balearic Islands (NW Mediterranean). Cybium 30, 125–132.

MEDITS Working Group (2017). MEDITS-Handbook, Version n. 9. MEDITS Working Group.

Ni I-H., Sandeman E.J. (1984). Size at maturity for northwest Atlantic Redfishes (Sebastes). Canadian Journal of Fisheries and Aquatic Sciences 41, 1753–1762.

Notarbartolo di Sciara G., Serena F., Ungaro N., Ferretti F., Pheeha S., Human B. (2009). *Torpedo marmorata*. The IUCN Red List of Threatened Species 2009: e.T161328A5398909. (Accessed 15 May 2021).

Notarbartolo di Sciara G., Serena F., Ungaro N., Ferretti F., Pheeha S., Human B., McCully S., Buscher E. (2016). *Torpedo marmorata*. The IUCN Red List of Threatened Species 2016: e.T161328A16527917. (Accessed 15 May 2021).

Piroddi C., Coll M., Liquete C., Macias D., Greer K., Buszowski J., Steenbeek J., Danovaro R., Christensen V. (2017). Historical changes of the Mediterranean Sea ecosystem: modelling the role and impact of primary productivity and fisheries changes over time. Scientific Reports 7, 44491.

Relini G., Biagi F., Serena F., Belluscio A., Spedicato M., Rinelli P., Follesa M.C., Piccinetti C., Ungaro N., Sion L., Levi D. (2000). I selaci pescati con lo strascico nei mari italiani. Biologia Marina Mediterranea 7, 347–384.

Stehmann M., Bürkel D.L. (1984). Torpedinidae. In: Whitehead PJP., Bauchot M-L., Hureau J C., Nielsen J., Tortonese E. (eds.) Fishes of the north-eastern Atlantic and Mediterranean. UNESCO, Paris. Vol. 1. pp 159–162.

Tserpes G., Maravelias C.D., Pantazi M., Peristeraki P. (2013). Distribution of relatively raredemersal elasmobranchs in the eastern Mediterranean. Estuarine, Coastal and Shelf Science 117, 48–53.

Tzanatos E., Somarakis S., Tserpes G., Koutsikopoulos C. (2007). Discarding practices in a Mediterranean small- scale fishing fleet (Patraikos Gulf, Greece). Fisheries Management and Ecology 14, 277–285.

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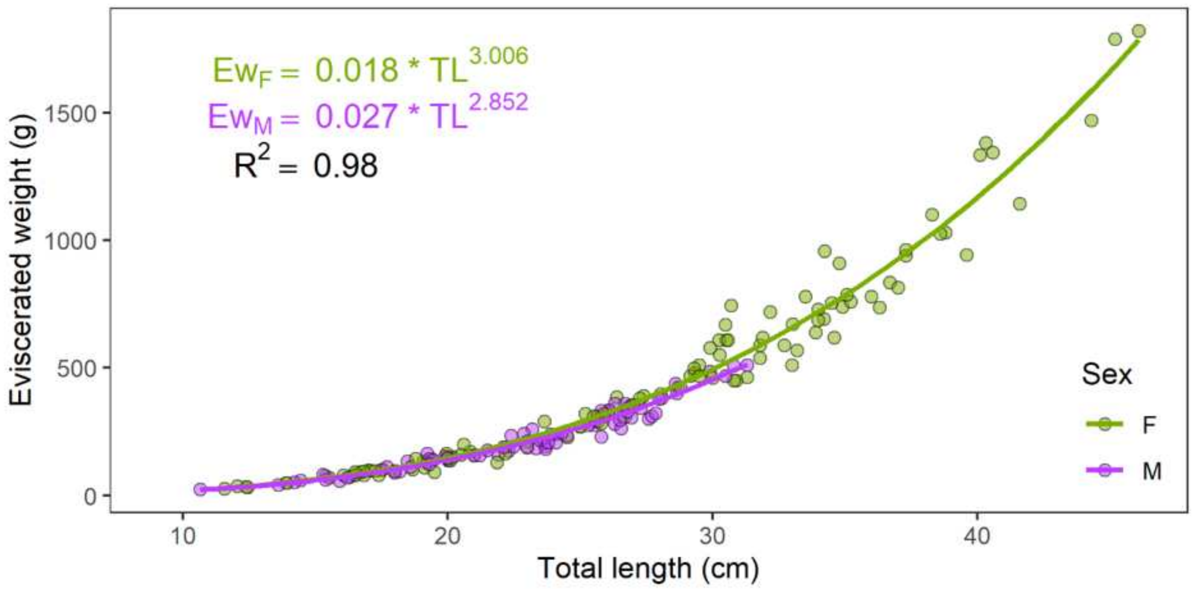

Figure 1. Length–weight relationship for female and male *Torpedo marmorata* from the Ionian and the Aegean Seas.

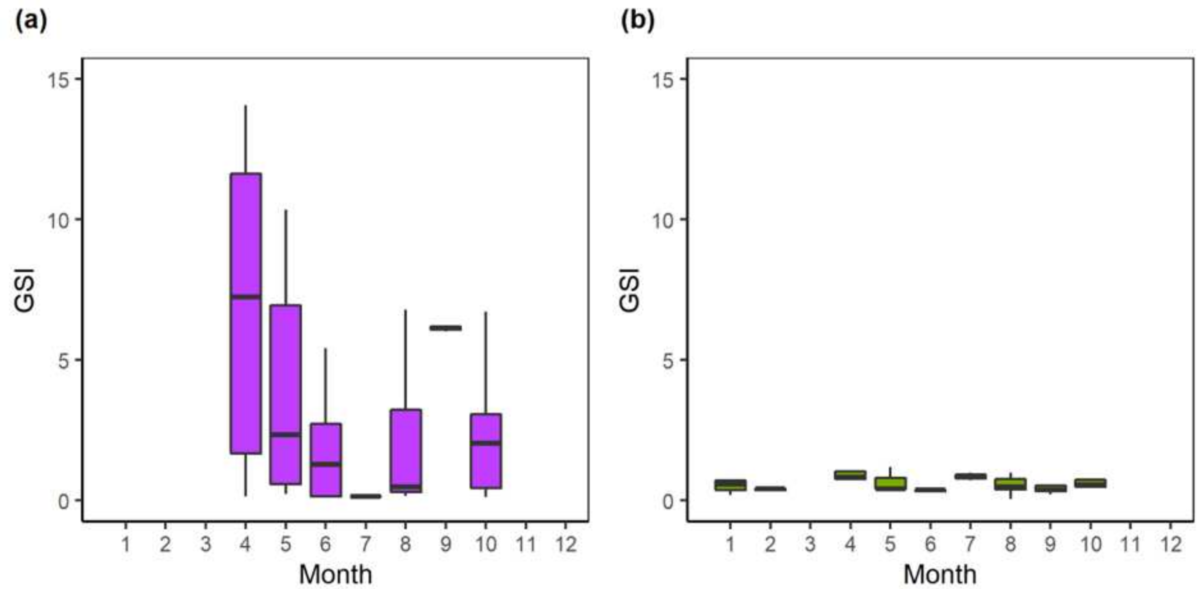

Figure 2. Gonadosomatic index for female (a) and male (b) *Torpedo marmorata* samples from the Greek Seas.

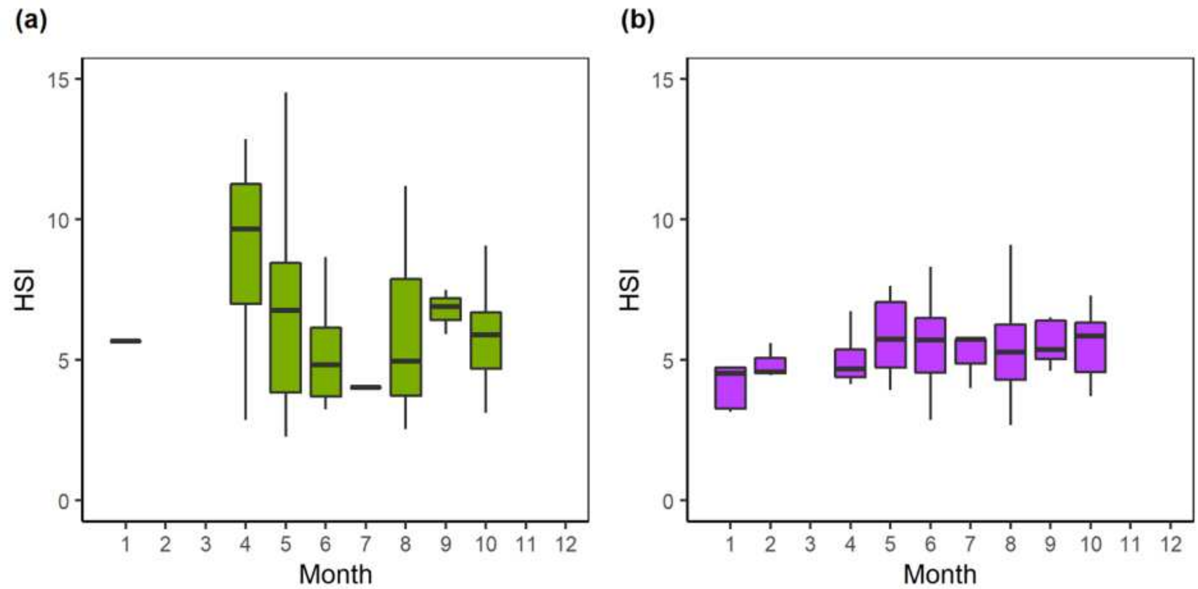

Figure 3. Hepatosomatic index for female (a) and male (b) *Torpedo marmorata* samples from the Greek Seas.

SPATIAL VARIATION OF THE VERTICAL DISTRIBUTION OF THE STATUS OF MACROALGAE COMMUNITIES, SEA URCHINS AND FISH POPULATIONS IN THE EASTERN MEDITERRANEAN

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Abstract
Canopy forming macroalgae (e.g. *Cystoseira sensu lato* forests) increases three-dimensional complexity and spatial heterogeneity of rocky reefs enhancing biodiversity and productivity of coastal areas. In recent years in the eastern Mediterranean two Lessepsian species of the genus *Siganus* (*S. luridus*, *S. rivulatus*) are transforming the *Cystoseira s.l.* forests to barren or turf formations, resulting to the loss of these forests three-dimensional structure and their ecosystem services. At the same time, the populations of the sea urchin *Paracentrotus lividus*, which is one of the main grazers of *Cystoseira s.l.* forests, have collapsed in some areas in the Levantine Sea due to ocean warming. In this study, we examined the differences in fish assemblages and biomass, sea urchins density, and the vertical distribution of the status of macroalgae communities in the north and south Aegean and in the Levantine Sea. Herbivore fish biomass in south Aegean and the Levantine Sea was significantly higher compared to north Aegean. Sea urchins densities estimations revealed a local collapse in the south Aegean and in the Levantine Sea. In almost all sites surveyed, the ecological status of macroalgal communities at depths 0–0.5 m was good or very good. In the south Aegean and in the Levantine Sea, the ecological status was in most sites low or very low at depths ≥2 m. Overall, in the island of Skyros, macroalgal communities were in the best status.
Keywords: *Canopy forming macroalgae, Cystoseira s.l. forests., herbivory, tropicalization*
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1. Introduction

Canopy forming brown macroalgae, such as kelps (Laminariales, Phaeophyceae, Stramenopiles) and fucoids (Fucales, Phaeophyceae, Stramenopiles), are dominant habitat forming species in the intertidal and subtidal zones of most temperate and subpolar regions (Schiel & Foster 2006). These macroalgae are autogenic ecosystem engineers that create structurally complex communities providing shelter and food, thus acting as nursery habitat for many organisms. By increasing three-dimensional complexity and spatial heterogeneity of rocky reefs they enhance biodiversity and productivity of coastal areas (Steneck *et al*. 2002; Cheminée *et al*. 2017).

In the Mediterranean Sea, most canopy forming macroalgae are species of the genus *Cystoseira sensu lato* (including also the recently reinstated genera *Gongolaria* and *Ericaria*) (Fabbrizzi *et al*. 2020). *Cystoseira s.l.* is listed as strictly protected in the Annex I of the Bern Convention. Moreover, *Cystoseira s.l.* are indicators of ecological status in the context of the Water Framework Directive (Directive 2000/60/EEC).

In the last three decades in the Mediterranean, there is a significant number of records of declining *Cystoseira s.l.* forests (Thibaut *et al*. 2015). These declines of *Cystoseira s.l.* forests are the result of direct anthropogenic pressures, such as pollution, urbanisation and human trampling (Thibaut *at al.* 2005; Sales *et al*. 2011), and indirect anthropogenic pressures such as overgrazing (due to trophic cascades caused either by overfishing or invasive species) (Verges *et al*. 2014; Gianni *et al*. 2017).

In the last decades, the Lessepsian species of the genus *Siganus* have been creating and maintaining barren formations in the eastern Mediterranean (Sala *et al*. 2011; Verges *et al*. 2014), while sea urchins populations are declining in that same area due to ocean warming (Yeruham *et al*. 2015). In this study, we examined the vertical distribution of the status of macroalgae communities and the density of sea urchins populations in sites in the Levantine sea (Cyprus) and in the Aegean sea (Lesvos, Skyros, Crete). We also examined the differences in fish assemblages and biomass between sites. All selected sites were dominated by rocky reefs and had at least some coverage of *Cystoseira s.l.*

2. Materials and Methods

The study area included the Aegean Sea (Greece) and the Levantine Sea (Cyprus). In total 12 sites were sampled in four different islands (Lesvos, Skyros, Crete, Cyprus) (Figure 1), between July – October 2020. At each site we assessed the ecological status of macroalgal communities using the Thibaut *et al*. (2017) scoring system (Table 1), for different depth zones using photo-sampling. At the depths of 8 (or 6 if there were no reefs at 8 m), 5, 2 and 1 m the macroalgae communities status was assessed using photo-quadrats (50×50 cm) every 5 m along a 50 m transect line (sampling size: n=10). At the depth zone of 0–0.5 m a smaller photo-quadrat (25×25 cm) was used every 5 metres, to help the diver take photo-samples under the effect of waves; by forming four osculated samples of the smaller photo-quadrat the sampling surface was kept the same.

Table 1. Status scores of the macroalgae communities, sensu Thibaut *et al*. (2017).

Status	4 (very good)	3 (good)	2 (moderate)	1 (low)	0 (very low)
Cover type	Arborescent perennial ≥50%	Arborescent perennial 5 to <50%	Shrubby ≥50%	Shrubby 5 to <50%	Turf encrusting

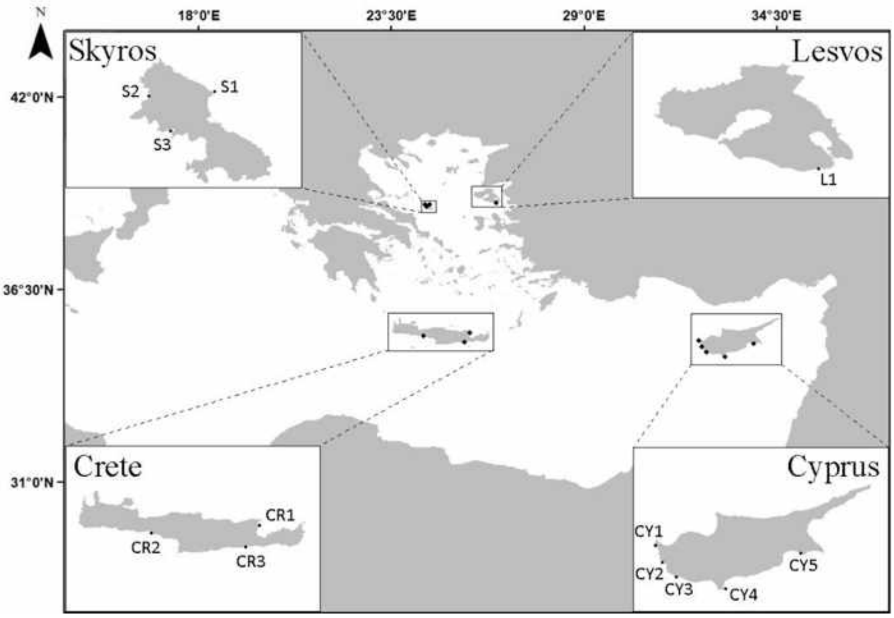

Figure 1. Study area and sampling sites in the Levantine Sea and in the Aegean Sea.

Sea urchin density was estimated at each depth zone under study (8, 5, 2, 1, 0–0.5 m) by counting sea urchins in ten 1×1 m quadrats along a 50 m transect line. The species recorded were: i) *Paracentrotus lividus*, ii) *Arbacia lixula*, iii) *Sphaerechinus granularis*, iv) *Centrostephanus longispinus*, and, v) *Diadema setosum.*

Fish biomass was estimated at each site at 5 m depth using underwater visual surveys. Fish were recorded along six replicated 25 × 5 m^2 strip transects. Moving one-way at constant speed along a transect line, the diver estimated the total length and abundance of the encountered fish. The fish biomass (kg wet mass m^{-2}) was estimated using the length-weight relationship from available literature (Moutopoulos & Stergiou 2002) and FishBase (Froese & Pauly 2021). The fish were assigned to trophic groups based on Thibaut *et al*. (2017). Variability in fish composition among sites was assessed by non-metric multidimensional scaling, based on Bray-Curtis dissimilarity.

3. Results

The fish biomass estimations illustrated a difference in herbivore fish biomass between southern-eastern sites (Crete, Cyprus) and the north Aegean sites (Lesvos, Skyros) (Figure 2). In the southern-eastern sites the herbivorous fish biomass was higher (mean value 3.31 g m^{-2}) than in the north Aegean sites (mean value 0.83 g m^{-2}) and was higher than any other fish trophic group in almost all of the southern-eastern sites. The most abundant herbivorous fish in the north Aegean sites were *Sarpa salpa* and *Sparisoma cretense* and in the southern-eastern were *Siganus rivulatus*, *Siganus luridus* followed by *Sparisoma cretense*. Fish composition in the southern-eastern sites differed in relation to the north Aegean sites (Figure 3).

Sea urchin density estimations revealed the almost collapse of the sea urchin populations in the southern-eastern sites where in most of the sites and depth zones there were no sea urchins, or their density was very low (Figure 4). In contrast, in the north Aegean the sea urchin density was higher in all sites, and the highest densities were recorded in shallow waters (1 and 2 m depths). In most of the islands, *P. lividus* was the most abundant species, except for Crete where *A. lixula* had the highest abundances.

Macroalgal communities scores revealed that in the southern-eastern sites the highest scores (4 or 3) were estimated mainly at depths of 0–0.5 m (87.5% of the south-eastern sites at 0-0.5 m had scores above 2) and the lower scores (2, 1 and 0) were estimated at depths of ≥2 m (100% of the south-eastern sites at 2 m had scores 2 and below, 87.5% at 5 m had scores 2 and below, 87.5% at 8 m had scores 2 and below) (Figure 5). In Skyros, the macroalgal communities scores were 2 and above in almost all depth zones in all sites. In the north Aegean in Lesvos the macroalgal communities scores were high at 0–0.5 m and low (scores 2 and below) in the other depth zones.

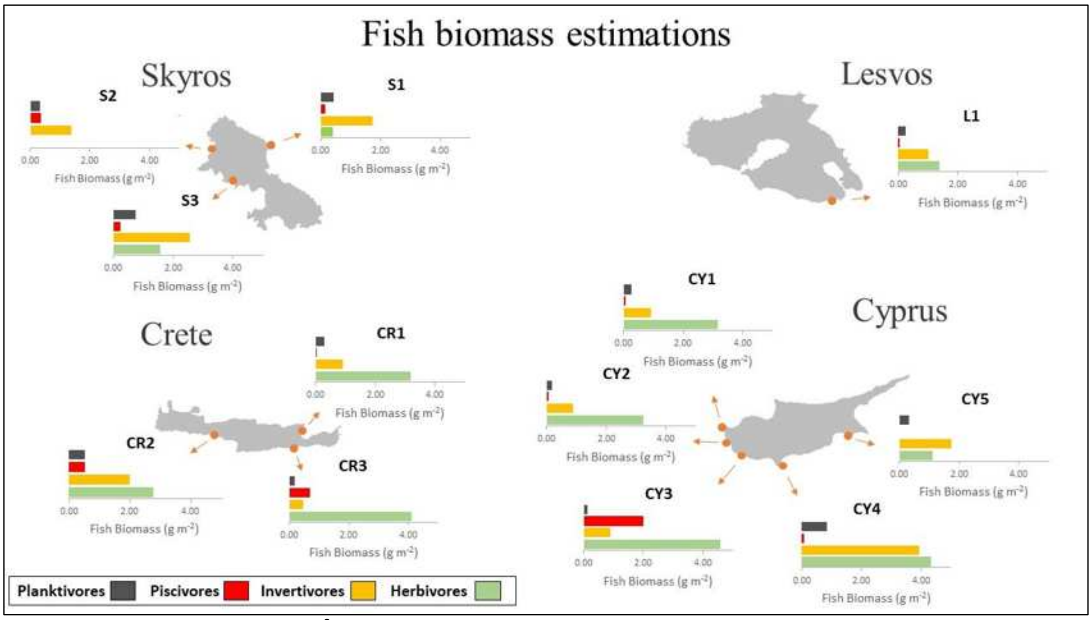

Figure 2: Fish biomass (g m^{-2}) estimations for the different trophic groups for each study site.

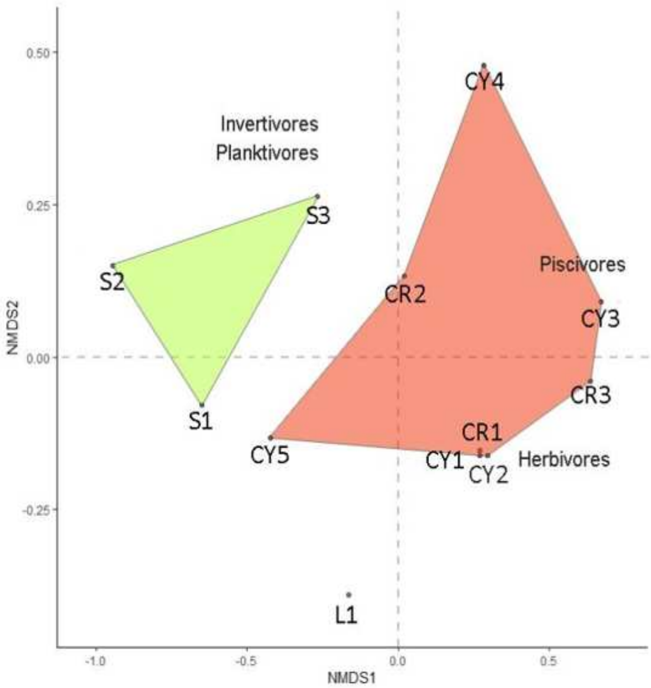

Figure 3: Non-metric multidimensional scaling (NMDS) of fish composition at the surveyed sites, based on the Bray-Curtis dissimilarity (Stress = 0.035).

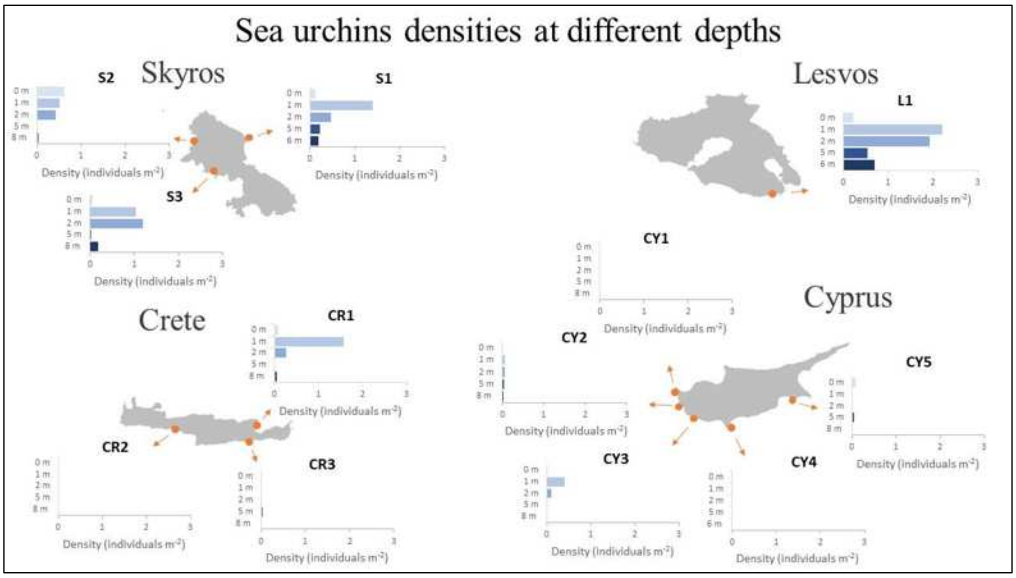

Figure 4: Sea urchins density (individuals m^{-2}) at each study site.

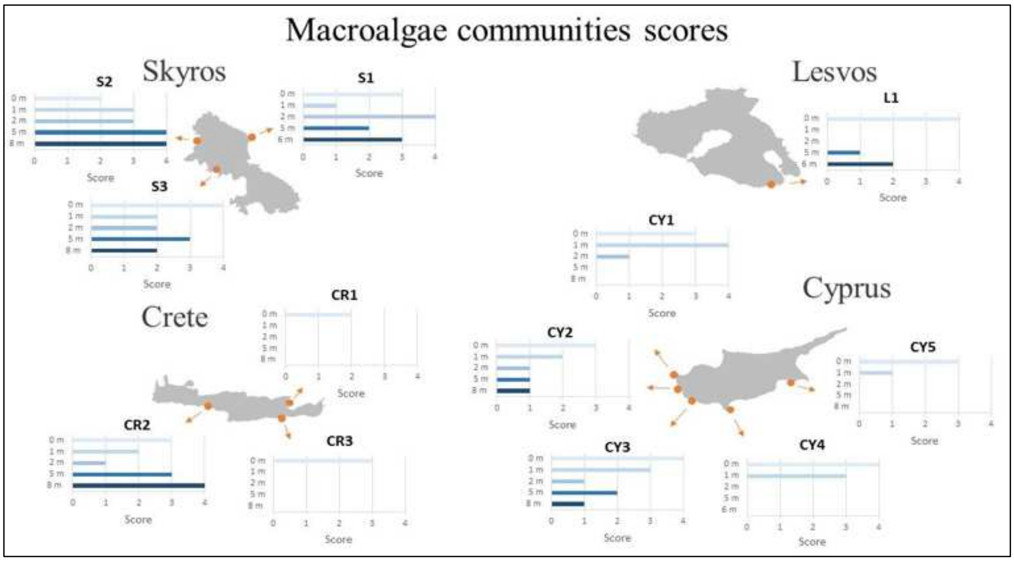

Figure 5: Ecological status of macroalgae communities based on Thibaut *et al*. (2017) (0: very low, 1: low, 2: moderate, 3: good, 4: very good).

4. Discussion

Our results on fish biomass of our study sites, agree with other studies in the eastern Mediterranean which indicate the general lower fish biomass in the eastern Mediterranean compared to the western Mediterranean (Giakoumi *et al*. 2012). It is important, though, to note the significant percentage of the herbivore fish biomass that contributes to the total fish biomass in the southern-eastern sites (Crete, Cyprus), in contrast to the north Aegean sites (Skyros, Lesvos). This great difference between these two geographical areas is caused mainly by the higher abundance of the two Lessepsian *Siganus* species in the warmer southern-eastern waters. The two *Siganus* species have established populations in Cyprus and Crete with great abundances, but in Skyros and Lesvos their populations densities are much lower. That is mainly explained by the typical spatial distribution pattern of thermophilic Lessepsian species, with high richness and abundances in the warmer south-eastern Levantine declining towards the colder north-western Mediterranean (Katsanevakis *et al*. 2014).At the same time, our results indicated an almost-collapse of the sea urchins populations in the southern-eastern sites. Recently in the eastern Mediterranean in the coast of Israel the native sea urchin *P. lividus* populations collapsed due to ocean warming (Yeruham *et al*. 2015). Increasing temperatures might have contributed to the decline of native sea urchins in Cyprus and Crete, and the gradual increase of the thermophilic exotic sea urchin *D. setosum*, as well as competition with the invasive *Siganus* species for the declining macroalgal resources.

We assume that grazing by the two species of *Siganus* is the main reason for the extended observed patterns of the vertical distribution of the status of macroalgal communities in the southern-eastern sites. Pollution, ocean warming or other local pressures can be excluded, as most of the sites have good or very good status at 0 m (i.e. high coverage of canopy forming macroalgae). At very shallow waters, fish herbivores can't easily graze, mainly because this habitat is strongly affected by hydrodynamism and often encounters emersion as a result of wave movement (Verges *et al*. 2009). In addition, these sites aren't affected by the grazing pressure of sea urchins anymore. The north Aegean sites have higher sea urchins densities, but such densities were insufficient to create extensive barrens at depths ≥2 m in contrast to the southern-eastern sites.

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References

Cheminée A., Pastor J., Bianchimani O., Thiriet P., Sala E., Cottalorada., *et al*. (2017). Juvenile fish assemblages in temperate rocky reefs are shaped by the presence of macro-algae canopy and its three-dimensional structure. Scientific Reports 7, 14638.

Moutopoulos D.K., Stergiou K.I. (2002). Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). Journal of Applied Ichthyology 8, 200–203.

Fabbrizzi E., Scardi M., Ballesters E., Benedetti-Cecchi L., Cebrian E., Ceccherelli G., *et al*. (2020). Modeling macroalgal forest distribution at Mediterranean scale: Present status, drivers of changes and insights for conservation and management. Frontiers in Marine Science 7, 20.

Froese R., Pauly D. Editors. (2021). FishBase. World Wide Web electronic publication. www.fishbase.org.

Gianni F., Bartolini F., Pey A., Laurent M., Martins M.G., Airoldi L., *et al*. (2017.) Threats to large brown algal forests in temperate seas: the overlooked role of native herbivorous fish. Scientific Reports 7, 6012.

Katsanevakis S., Coll M., Piroddi C., Steenbeek J., Ben Rais Lasram F., Zenetos A., *et al*. (2014). Invading the Mediterranean Sea: biodiversity patterns shaped by human activities. Frontiers in Marine Science 1, 32.

Sales M., Cebrian E., Tomas F., Ballesteros E. (2011). Pollution impacts and recovery potential in three species of the genus *Cystoseira* (Fucales, Heterokontophyta). Estuarine, Coastal and Shelf Science 92, 347–357.

Schiel D.R., Foster M.S. (2006). The population biology of large brown seaweeds: ecological consequences life histories of multiphase in dynamic coastal environments. Annual Review of Ecology, Evolution and Systematics 37, 343–372.

Steneck R.S., Graham M.H., Bourque B.J., Corbett D., Erlandson J.M., Estes J.A., *et al*. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation 29, 436–459.

Thibaut T., Pinedo S., Torras X., Ballesteros E. (2005). Long-term decline of the population of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albères coast (France, North-western Mediterranean). Marine Pollution Bulletin 50(12), 1472–1489.

Thibaut T., Blanfuné A., Boudouresque C.F., Verlaque M. (2015). Decline and local extinction of Fucales in French Riviera: the harbinger of future extinctions? Mediterranean Marine Science 16(1), 206–224.

Thibaut T., Blanfuné A., Boudouresque C.F., Personnic S., Ruitton S., Ballesteros E., *et al*. (2017). An ecosystem-based approach to assess the status of Mediterranean algae-dominated shallow rocky reefs. Marine Pollution Bulletin 117, 311–329.

Verges A., Alcoverro T., Ballesteros E. (2009). Role of fish herbivory in structuring the vertical distribution of canopy algae (*Cystoseira* spp.) in the Mediterranean Sea. Marine Ecology Progress Series 375, 1–11.

Verges A., Tomas F., Cebrian E., Ballesteros E., Kizilkaya Z., Dendrinos P., *et al*. (2014). Tropical rabbitfish and the deforestation of a warming temperate sea. Journal of Ecology 102(6), 1518–1527.

Yeruham E., Rilov G., Shpigel M., Abelson A. (2015). Collapse of the echinoid *Paracentrotus lividus* populations in the Eastern Mediterranean—result of climate change? Scientific Reports 5, 13479.

Pinna nobilis, IN THE BRINK OF EXTINCTION IN GREEK SEAS

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Abstract

Five years have passed after the first reported mass mortality event of the fan mussel *Pinna nobilis*, and the populations of this emblematic species have mostly collapsed across the Mediterranean basin. Scattered populations are surviving within the basin, in lagoons or enclosed bays with special environmental conditions. Greek fan mussel populations have been severely affected, however the search and protection of surviving healthy populations or individuals, is crucial to increase the chances of recovery. Facing the threat of extinction of *P. nobilis*, this study is an attempt to depict the current status of fan mussel populations in Greece and to identify unaffected or less affected locations, where populations or individuals are resisting the parasitic infection. Currently, the only known populations with surviving individuals are in Kalloni Gulf (Aegean Sea) and Amvrakikos Gulf (Ionian Sea). Further research is needed to investigate anecdotal information about surviving individuals in various locations and depths across the Greek seas, to better understand the causes of the MME, and to examine possible methods to restore *P. nobilis* populations.

Keywords: *Pinna nobilis, Pinnidae, Mass Mortality Event, population assessment, conservation status*

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1. Introduction

The fan mussel, *Pinna nobilis* (Linneus 1758) is Mediterranean's largest endemic bivalve, reaching up to 120 cm in length, with a maximum lifespan of 45 years (Zavodnik *et al*. 1991; Rouanet *et al*. 2015). *Pinna nobilis* can be found with the anterior part buried and anchored by its byssus threads to different types of habitats (such as *Posidonia oceanica* and other seagrass meadows, unvegetated sandy substrates, estuaries, sandy patches among hard substrates), from very shallow waters (0.5 m) down to 60 meters (Zavodnik *et al*. 1991; Basso *et al*. 2015; Zotou *et al*. 2020). The species has a key role in the ecosystem since it is able to reduce turbidity and organic particulate matter, provides a hard substrate for colonizers in soft bottom surroundings, and in large densities can create biogenic reefs, acting as an "ecosystem engineer" (Rabaoui *et al*. 2009; Trigos *et al*. 2014; Katsanevakis 2016).

Habitat degradation (especially of *P. oceanica* meadows due to trawling and uncontrolled anchoring), marine pollution, fishing activities, invasive species and climate change have been listed as major threats for *P. nobilis* survival (Basso *et al.* 2015). Due to these anthropogenic threats, the species has been protected under the European Union Habitats Directive (92/43/EEC, Annex IV), the Protocol for Specially Protected Areas and Biological Diversity in the Mediterranean of the Barcelona Convention (Annex II), and the national legislation of most Mediterranean countries. Despite its protection, poaching has been common in some Mediterranean countries (Katsanevakis *et al*. 2011).

In autumn 2016, the list of the species' threats was enriched with an unknown pathogen that caused mass mortality events (MME) in the Spanish coasts (Vázquez-Luis *et al*. 2017), subsequently spreading in the Mediterranean basin by currents (Cabanellas-Reboredo *et al*. 2019). The MMEs were first observed in the north-western Mediterranean coasts and within short time, high mortality rates were also reported from the eastern coasts of the basin (Kersting *et al*. 2019; Zotou *et al.* 2020; Katsanevakis *et al*. 2021). As a result of the populations decline, *P. nobilis'* was assessed as "Critically Endangered" in the Red List of the International Union for the Conservation of Nature in autumn 2019 (IUCN, 2019), underlining its high risk of extinction.

The pathogen first identified for causing the MME was a cryptogenic species-specific haplosporidian parasite, named *Haplosporidium pinnae* (Catanese *et al*. 2018). However, further studies in other regions linked the disease to an actinobacterium *Mycobacterium* sp. (Carella *et al*. 2019; Lattos *et al*. 2020), while molecular and histological analyses in other surveys confirmed the coexistence of more pathogens (*H. pinnae*, *Mycobacterium* sp., *Vibrio mediterranei* and *Perkinsus* sp.) (Carella *et al*. 2020; Prado *et al*. 2020). A multifactorial disease with multiple pathogens (opportunistic or more specific) could be an explanation for the dramatic decline of fun mussel populations all over the Mediterranean, although the aforementioned pathogens may not be necessarily associated with the MME (Scarpa *et al*., 2020), leading to a conclusion that the causes of the mortality of *P. nobilis* are far from being understood (Katsanevakis *et al*. 2021).

The disease was expressed with mortality rates that commonly exceeded 95%, and such mortality events have occurred so far in all known areas that held fan mussel populations (Katsanevakis *et al*. 2021). Locations scattered across the basin with healthy populations still surviving are considered to be refuges with environmental conditions halting the expansion of the pathogen(s) (Kersting *et al.* 2019). A recent study (Katsanevakis *et al*. 2021) summarized the current status of *P. nobilis* populations in all Mediterranean ecoregions, highlighting the unaffected areas that sustain healthy populations (Figure 1). The Sea of Marmara (Turkey) held healthy populations by September 2020 (Çinar *et al*. 2021), with signs of the disease reported only in its southern gate, i.e. Çanakkale Strait (mortality rate 90%; Özalp & Kersting 2020). However, after a marine mucilage ("sea snot") outbreak in spring 2021, a massive mortality event was observed (M.E. Çinar *et al*. submitted); in the absence of molecular analyses for the detection of pathogen(s), the driver of the observed mortality remains unknown (pathogen or the mucilage outbreak). The situation in the south coasts of Mediterranean is harder to assess, since there were no targeted surveys conducted to evaluate the status of *P. nobilis* these last five years. In general, the status of the species is unknown and understudied in most of the southern coastline of the Mediterranean and the fun mussel populations have never been systematically monitored neither after the 2016 MME nor before (Katsanevakis *et al*. 2021).

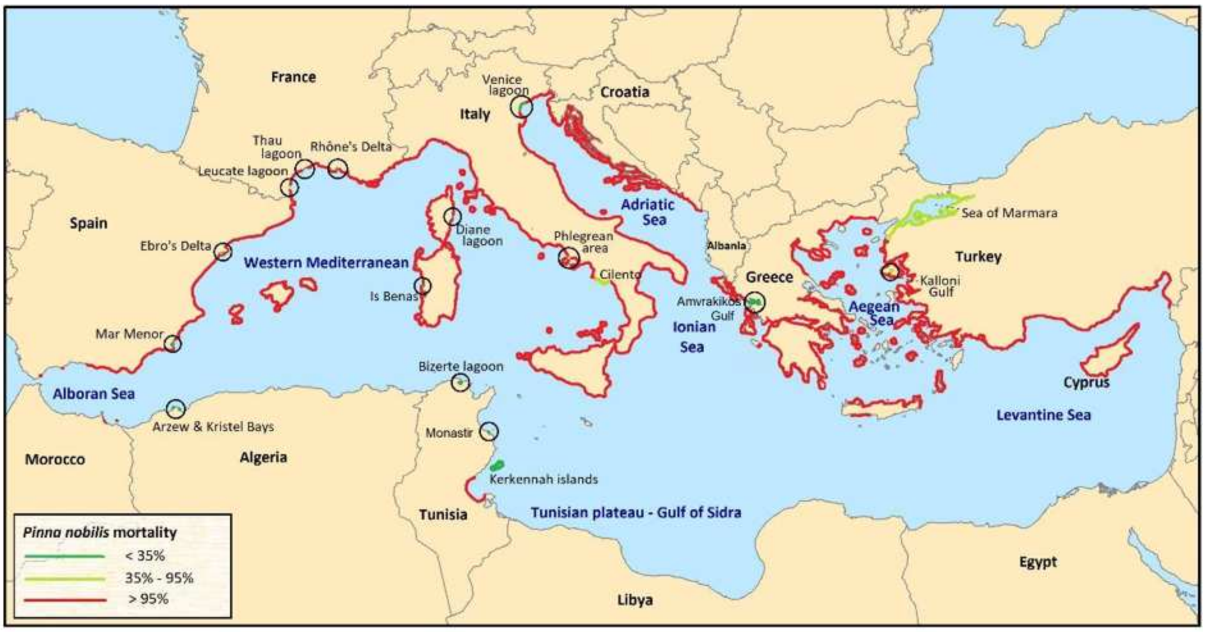

Figure 1. The current (July 2021) status of *Pinna nobilis* populations in the Mediterranean Sea after the parasitic outbreak in 2016. In the parts of the coastline with no color, the situation remains unknown. Even along the coastlines indicated as areas of high mortality, the possibility that yet unidentified healthy populations exist cannot be fully excluded. (Updated from Katsanevakis *et al*. 2021).

The aim of this study was to re-evaluate the status of *P. nobilis* populations in the Greek seas and update the most recent assessment of fan mussel mortality in Zotou *et al*. (2020), by adding more recent data where available. The present study depicts our knowledge on the status of fan mussels in Greece by July 2021.

2. Materials and Methods

This study presents the state of fan mussel populations as a compilation of independent targeted case studies and opportunistic records and observations, collected in the framework of other scientific projects, in various marine areas of Greece. The search for unaffected areas, any sign of recovery in impacted populations (such as recruitment or juvenile individuals), or the expansion of the disease in other locations are crucial information in order to estimate the extent of the MME.

In the targeted case studies, a 45-min visual survey protocol was applied, according to which all *P. nobilis* individuals both alive and dead are counted in order to estimate the mortality rate in the area (Katsanevakis *et al*. 2019). Since the MME has affected Greek seas over the past 3 years, all dead individuals

Table 1. Sampling methodology followed in each marine area for the years 2020-2021 and the estimated mortality rate. "45′ protocol" refers to a 45-min standardized protocol of underwater visual observations, and "Opportunistic" refers to opportunistic records without applying a strict protocol.

Marine Area	Year	# Sites	Mortality Rate	Sampling Methodology
Kalloni Gulf	2020	6	98.4%	45' protocol
	2021	8	97.8%	45' protocol
Gera Gulf	2020	2	100%	45' protocol
	2021	1	100%	45' protocol
N.M.P.A.N.S.	2021	21	100%	45' protocol
Peloponnese	2020	26	100%	45' protocol
	2021	10	100%	Opportunistic
Amvrakikos Gulf	2021	20	33%	45' protocol
Other Areas	2020	19	100%	Opportunistic
	2021	24	99.9%	Opportunistic

were counted, without excluding specimens presumably dead for long periods. From the assessed locations of this study, some areas were visited for the first time after the MME, while some sites had been revisited. Thus, in Kalloni Gulf (Lesvos Island) 6 sites were surveyed between July and September of 2020 and 8 more sites were visited between May and July of 2021. Two sites were assessed in Gera Gulf (Lesvos Island) in the summer (June, July) of 2020 and another site was surveyed in June 2021. In June 2021, 21 sites in the National Marine Park of Alonnisos – Northern Sporades (N.M.P.A.N.S) were visited in order to estimate the mortality rate of the fan mussel in the boundaries of the Marine Park. Around Peloponnese, 26 sites were assessed in August 2020 and another 10 locations were visited in July 2021. In Amvrakikos Gulf, 11 sites were visited in April 2021 and 9 more sites in July 2021. Through surveys for other research projects, opportunistic records for 19 sites were compiled between July and August 2020, and 24 more sites were assessed from April to July 2021 in various localities in Greece (Table 1). All data collected within the framework of this study were combined with those reported by Zotou *et al*. (2020) to depict the status of *P. nobilis* in the Greek seas by July 2021.

3. Results

In total, 137 visual surveys were conducted the past 14 months (June 2020-July 2021) from different research groups along the Greek coastline, at a depth range of 0.3 – 34 m. Overall 4971 *P. nobilis* individuals were recorded, among which 98.6% were dead and only 1.4% were alive, not including those found in Amvrakikos Gulf, where a large and healthy population (a few thousand individuals) with signs of successful recruitment of juveniles has been recently discovered by an ongoing research project that is focusing in the area

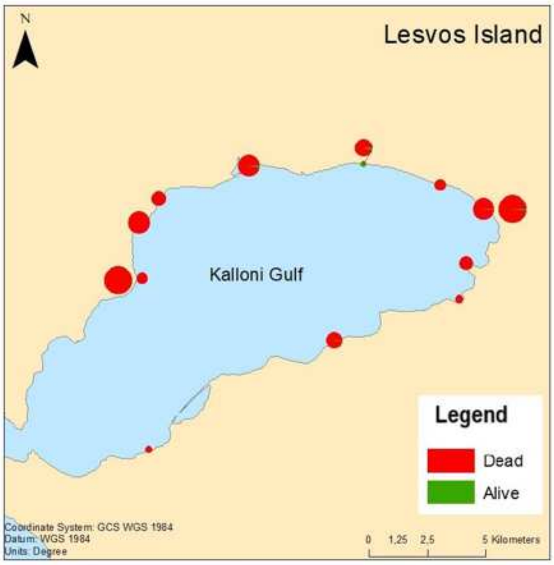

Figure 2. Mortality assessment of *Pinna nobilis* populations in Kalloni Gulf. The size of the pie charts represents the number of recorded individuals. The red and green colour in the pie charts show the proportion of dead and live individuals, respectively.

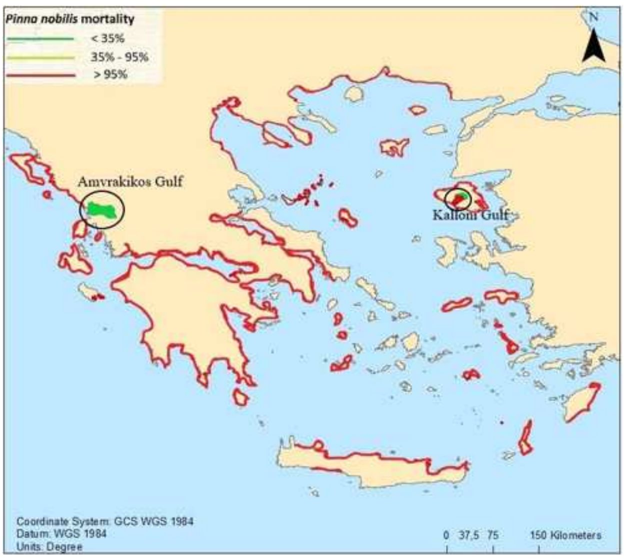

Figure 3. Integrated map of the current status (July 2021) of *P. nobilis* in the Greek seas.

(Y. Issaris, unpublished data). Preliminary data show that the estimated mortality in Amvrakikos Gulf is low (33%) with the fan mussel population seeming to be largely unaffected by the MME. Of the 69 alive individuals found in the rest of Greece, 57.4% were juveniles and all of them were found in Kalloni Gulf (Figure 2), indicating successful recruitment in specific locations, despite the ongoing MME. Apart from Kalloni Gulf (Lesvos Island), only one more alive individual was counted in Thermaikos Gulf as an opportunistic record, among more than 100 dead individuals.

In Kalloni Gulf the estimated mortality rate during the 2020 survey was 98.4%, while during 2021 the mortality rate was 97.8%. In Gera Gulf, N.M.P.A.N.S., and Peloponnese the mortality rate was 100%, since no live individual was found. From the opportunistic records dataset, only 1 individual was found alive, among more than 766 dead fan mussels, leading to a total mortality of approximately 99.9%.

The fan mussel population in Kalloni Gulf has been severely affected, however the few surviving individuals and the juveniles among them indicate some resistance against the pathogen compared to the rest of the Greek coastline (Figure 3). Surviving populations were found near the salt pans in the inner part of the gulf, indicating that increased salinity halted the disease. In Gera Gulf, Peloponnese, N.M.P.A.N.S and all other surveyed areas there were no signs of recovery.

Additional anecdotal information supports that scattered individuals are surviving in various locations and depths in the Greek seas. One specimen was caught on a fisher's net in Thrakiko Pelagos last February (A. Christidis, pers. comm.) and another fan mussel was caught in Samos Island (A. Miliou, pers. comm.). A citizen also reported a small surviving population in the NW coasts of Skyros Island (A. Christidis, pers. comm.), which however could not be confirmed and was thus not included in this study.

4. Discussion

This study documents the large-scale progression of the *P. nobilis* MME throughout the Greek seas during 2020 and 2021 and the collapse of fan mussel populations in most of the marine areas investigated. The spread of the disease and the expansion to previously unaffected populations indicates that the fan mussel MME that started in 2016 is still in progress, and no local population in the Mediterranean may be considered safe. The remaining surviving populations however give hope for the survival of fan mussel. The safety of surviving populations or individuals when found, should be assured, since *P. nobilis* is considered a delicacy in many countries and is being poached, despite its protection status (Katsanevakis *et al*. 2011). Additionally, accidental killing of *P. nobilis* by illegal fishing methods, anchoring or extreme weather conditions should be limited.

Continuous monitoring of the fan mussel and especially the search for juveniles and recruits is needed in order to observe a possible "natural recovery" of the species. Larvae collectors are a useful tool for early detection of new recruits (Kersting *et al*. 2020) and collected juveniles could be cultivated ex situ or be transplanted in parasite- free locations (Katsanevakis 2016).

Interestingly, in Spain where *P. nobilis* coexists with the congeneric species *Pinna rudis*, that wasn't affected by the disease, hybrid individuals have been detected (Katsanevakis *et al*. 2021) and seem to be resistant to the parasite (Vázquez-Luis *et al*. 2021). Further research is needed to understand the reasons for the survival of both *P. rudis* and the hybrids and the pathogeny of fan mussels in general. As the species is near the brink of extinction, improving our knowledge base on the mass mortality event and adequately protecting the remaining individuals both in situ and ex situ are vital for securing the global survival of the species. In Greece, there are still areas that are unassessed (Figure 3), whereas even in assessed localities surveys are mostly restricted in shallow waters. Intensification of efforts to identify the remaining populations is needed. Characteristic example is the case of Amvrakikos Gulf, where a healthy important population was recently discovered, being previously unknown (Zotou *et al*. 2020).

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References

Basso L., Vázquez-Luis M., García-March J., *et al*. (2015) The pen shell, *Pinna nobilis*: A review of population status and recommended research priorities in the Mediterranean Sea. Advances in Marine Biology 71, 109–160.

Cabanellas-Reboredo M., Vázquez -Luis M., Mourre B., *et al*. (2019) Tracking a mass mortality outbreak of pen shell *Pinna nobilis* populations: A collaborative effort of scientists and citizens. Nature Scientific Reports 9, 13355.

Carella F., Aceto S., Pollaro F., *et al*. (2019) A mycobacterial disease is associated with the silent mass mortality of the pen shell *Pinna nobilis* along the Tyrrhenian coastline of Italy. Scientific Reports 9, 2725.

Carella F., Elisabetta A., Simone F., *et al*. (2020) In the wake of the ongoing mass mortality events: Co-occurrence of *Mycobacterium*, *Haplosporidium* and other pathogens in *Pinna nobilis* collected in Italy and Spain (Mediterranean Sea). Frontiers in Marine Science 7, 48.

Catanese G., Grau A., Valencia J.M., *et al*. (2018) *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with massive mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea. Journal of Invertebrate Pathology 157, 9–24.

Çinar M.E., Bilecenoglu M., Yokes M.B., Güçlüsoy H. (2021) *Pinna nobilis* in the south Marmara Islands (Sea of Marmara); still remains uninfected by the epidemic and acts as egg laying substratum for an alien invader. Mediterranean Marine Science 22, 161–168.

IUCN (2019). Mediterranean Noble Pen Shell Crisis (*Pinna nobilis*) – January 2020 Update. https://www.iucn.org/news/mediterranean/202001/mediterranean-noble-pen-shell-crisis-pinna-nobilis-january-2020-update (Assessed: 15/1/2020).

Katsanevakis S. (2016) Transplantation as a conservation action to protect the Mediterranean fan mussel *Pinna nobilis*. Marine Ecology Progress Series 546, 113–122.

Katsanevakis S., Carella F., Çinar M.E., *et al*. (2021). The Fan Mussel *Pinna nobilis* on the Brink of Extinction in the Mediterranean. Imperiled: The Encyclopedia of Conservation, 9p, https://doi.org/10.1016/B978-0-12-821139-7.00070-2

Katsanevakis S., Poursanidis D., Issaris Y., et al. (2011) “Protected” marine shelled molluscs: Thriving in Greek seafood restaurants. Mediterranean Marine Science 11, 429–438.

Katsanevakis S., Tsirintanis K., Tsaparis D., *et al*., (2019). The cryptogenic parasite *Haplosporidium pinnae* invades the Aegean Sea and causes the collapse of *Pinna nobilis* populations. Aquatic Invasions 14.

Kersting D., Benabdi M., Cižmek H. *et al*. (2019) *Pinna nobilis*. The IUCN Red List of Threatened Species 2019, e.T160075998A160081499, pp. 24.

Kersting D.K., Vázquez-Luis M., Mourre B., *et al*. (2020) Recruitment disruption and the role of unaffected populations for potential recovery after the *Pinna nobilis* mass mortality event. Frontiers in Marine Science 7, 594378.

Lattos A., Giantsis I.A., Karagiannis D., Michaelidis B. (2020). First detection of the invasive Haplosporidian and Mycobacteria parasites hosting the endangered bivalve *Pinna nobilis* in Thermaikos Gulf, North Greece. Marine Environmental Research 155 (104889).

Özalp H.B. and Kersting D.K. (2020). A pan-Mediterranean extinction? *Pinna nobilis* mass mortality has reached the Turkish Straits System. Marine Biodiversity 50, 81.

Prado P., Carrasco N., Catanese G., *et al*. (2020). Presence of *Vibrio mediterranei* associated to major mortality in stabled individuals of *Pinna nobilis* L. Aquaculture 519, 734899.

Rabaoui L., Tlig-Zouari S., Cosentino A., Ben Hassine O.K. (2009). Associated fauna of the fan shell *Pinna nobilis* (Mollusca: Bivalvia) in the northern and eastern Tunisian coasts. Scientia Marina 73, 129–141.

Rouanet E., Trigos S., Vicente N. (2015). From youth to death of old age: the 50-year story of a *Pinna nobilis* fan mussel population at Port – Cros Island (Port – Cros National Park, Provence, Mediterranean Sea). Scientific Reports of Port – Cros National Park (29), 209–222.

Scarpa F., Sanna D., Azzena I., *et al*. (2020) Multiple non-species-specific pathogens possibly triggered the mass mortality in *Pinna nobilis*. Lifestyles 10, 238.

Trigos S., Garcıa-March J.R., Vicente N., Tena J., Torres J. (2014). Utilization of muddy detritus as organic matter source by the fan mussel *Pinna nobilis*. Mediterranean Marine Science 13(3), 667–674.

Vázquez-Luis M., Alvarez E., Barrajon A., *et al*. (2017) S.O.S. *Pinna nobilis*: A mass mortality event in western Mediterranean Sea. Frontiers in Marine Science 4, 109.

Vázquez-Luis M., Nebot-Colomer E., Deudero S., Planes S., Boissin E. (2021). Natural hybridization between pen shell species: *Pinna rudis* and the critically endangered *Pinna nobilis* may explain parasite resistance in *P. nobilis*. Molecular Biology Report 48, 97–104.

Zavodnik D., Hrs-Brenko M., Legac M. (1991). Synopsis on the fan shell *Pinna nobilis* L. In the eastern Adriatic Sea. Les Espècies Marines à protéger en Méditerranée (eds Boudouresque, C. F., Avon, M. & Gravez, V.): 169–178.

Zotou M., Gkrantounis P., Karadimou E., *et al*. (2020). *Pinna nobilis* in the Greek seas (NE Mediterranean): On the brink of extinction? Mediterranean Marine Science 21, 575–591.

FUNCTIONAL TRAITS OF POLYCHAETES CHANGE BETWEEN DIFFERENT HABITAT TYPES OF *Posidonia oceanica*

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Abstract
Meadows of *Posidonia oceanica* harbor high diversity and are major providers of ecosystem services in the Mediterranean Sea, yet Biological Traits Analyses of the resident benthic communities, that can relate diversity and ecosystem functions, are lacking. In this study, the functional diversity of polychaetes is investigated between different habitat types of *P. oceanica*, namely unmodified plain meadow, strips/patchy formations, and dead matte. The study aims to provide insight on the functional reference conditions, expected ranges and pilot indicators that can be used in future applications of functional ecology or impact studies. The results showed that unmodified meadow and strips/patchy formations were different in functional compositions, diversity, and thus, the ecosystem processes involved. Although functional diversity was found to be different between unmodified meadow and dead matte, an overlap was observed in functional compositions, highlighting the importance of the latter on benthic functioning. The similarities were likely attributed to the remaining below-ground structure, which, however, can be highly vulnerable to burial or uprooting and therefore, the current exclusion of dead mattes from conservational legislation may have serious consequences on benthic functioning. In terms of assessing *P. oceanica* habitat modification, the classification of species to ecosystem engineering types distinguished the habitat types in similar patterns with the other analyses and showed interesting potential for wider use as indicator.
Keywords: *Patches, dead matte, fragmentation, BTA, ecosystem engineers*
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1. Introduction

Understanding how biodiversity relates to environmental change has been one of the hot topics of marine ecology over the past 50 years, where myriad of studies on taxonomic diversity have been conducted. However, the emerging need to persuade managers that decreases in biodiversity have ramifications beyond simply loss of species, has led to the consideration of functional diversity (i.e., study what organisms do through their morphological, behavioral, and life history traits), for relating biodiversity with important ecosystem processes (Hewitt *et al*. 2008). Biological Traits Analysis (BTA) is a modern formulation of this approach and since its introduction in marine benthic macroinvertebrates, there has been a constant increase of publications on the topic (Beauchard *et al.* 2017). In addition, a growing number of habitat-based legislative agreements and environmental policies (e.g., Convention on Biological Diversity, European Marine Strategy Directive, Habitats Directive) have started to consider human impacts in ecosystem functioning and require management schemes to address their assessment (Frid *et al.* 2008).

Despite the increasing interest by both ecologists and managers, there is a lack of even basic knowledge about functional composition in many marine habitats, and this type of information is crucial for all stages of assessment and management (Bremner 2008). For instance, meadows of *Posidonia oceanica* harbor a unique and remarkably diverse macroinvertebrate community (Gambi *et al.* 1995; Somaschini *et al.* 1994) and are major providers of ecosystem services in the Mediterranean Sea, yet Biological Traits Analyses of the resident benthic communities, that can relate diversity and ecosystem functions, are lacking. The observed high diversity can be associated with the complex habitat structure that encompasses several microhabitats, organized into three ecosystem compartments: the leaf, rhizomes, and matte layers (Gambi *et al.* 1995). However, under either natural or anthropogenic pressure, the structure of the plain meadows can be modified into different types, such as strips/patchy formations, and dead mattes, strongly affecting the associated macroinvertebrate communities (e.g., Borg *et al.* 2006; Harmelin 1964; Somaschini *et al.* 1994).

So far, assessing impacts with indices of taxonomic diversity has repeatedly showed poor detectability in fragmentation events and ecotones (e.g., Sânchez-Jerez & Ramos Esplâ 1996). The interaction of the adjacent communities, which follows fragmentation, may often facilate the number of species and mask the course towards serious impacts on biodiversity, if fragmentation progresses to loss of habitat (Fahrig 2003). On the other hand, BTA can possibly provide early insight on the underlying impacts, by considering the functional traits that are particularly related to the structural complexity of the meadow and can be linearly affected by the modification of it. Among resident macroinvertebrate taxa, polychaetes are considered herein as the most suitable group to study benthic functional response, not only because they are dominant in *P. oceanica* rhizomes and matte layers (Gambi *et al.* 1995; Somaschini *et al.* 1994), but because they also exhibit vast variability in their functional traits (Hutchings 1998) and may be relevant with the study aims.

For instance, their functional traits related to habitat creation can interact with the structural complexity of seagrasses in a predictable pattern, since the different forms of the 'ecosystem engineering' species show an antagonistic relationship for space (hypothesis of Bouma *et al*. 2009). To elaborate further on this, Jones *et al*. (1994) divided the habitat-forming species to 'autogenic engineers' (i.e., create habitats via their own living and non-living material) and 'allogenic engineers' (i.e., create habitats via performing an action). According to the hypothesis of Bouma *et al*. (2009), whenever autogenic engineers create physical structures that cover most of the substrate space (e.g., canopy, rhizomes, and matte of seagrasses), allogenic engineers (e.g., bioturbators and galley-forming polychaetes) are excluded. This pattern shows interesting potential for use in assessing impacts caused by modification of *P. oceanica* structure on macroinvertebrate communities and benthic ecosystem functioning.

In the present study, the functional diversity of polychaetes is investigated between different habitat types of *P. oceanica*, namely unmodified plain meadow, strips/patchy formations, and dead matte. The study aims to provide insight on the functional reference conditions and expected ranges that can be used in future applications of functional ecology and impact studies. To this end, the study also pilots the use of classifying benthic species to ecosystem engineering types as a tool in successful assessments of *P. oceanica* habitat modification.

2. Materials and Methods

Geras Gulf in Lesvos Island (Greece, Mediterranean Sea) is a shallow semi-enclosed water body, with a restricted connection to the Aegean Sea, via a long narrow channel. Extensive *P. oceanica* meadows can be found around the channel mouth, but the distribution of *P. oceanica* is limited to a few kilometers inside the gulf (pers. obs.), due to the unfavorable water regime, characterized by high turbidity and sedimentation. Two sampling stations were selected southwest of the channel mouth, on naturally occurring dead matte (herewith referred as DM) and on unmodified plain meadow (PPM), both presenting mattes rising from the sea bottom. The third station was 3 km inside Geras Gulf, at the boundaries of *P. oceanica* distribution, on strips and patches formations (PSP). In contrast to the previous types, the mattes here were not raised from the nearby substrate and high sedimentation was observed on the surface of the leaves and rhizomes.

Samples of the associated macroinvertebrates were collected by scuba diving, using a hand-held cylindrical corer made of transparent PVC (0.018 m^2 surface), sieved in a mesh of 1 mm and fixed with 10% formalin, stained with Rose Bengal. In total, three replicated samples were taken per station and per season (winter, summer, autumn). At the laboratory, the samples were washed and the macrofauna was sorted from the residuals, taking care to take apart all the plant tissues. Then all polychaete species were identified to the lowest possible taxonomic level and their abundance was counted. A total of 20 traits (associated to habitat structure and important benthic processes), consisting of 69 modalities were assigned to the species, using fuzzy coding, and after weighting with abundances, a traits-sites matrix was created.

The traits-sites matrix was transformed with square root and NMDS with Bray-Curtis similarity was plotted. Significance of difference between habitat types and season was tested, by applying a two-way crossed ANOSIM. Then, the major aspects of functional diversity, Functional Richness (FRic) and Functional Divergence (FDiv) were calculated (Villéger *et al.* 2008). Their variation was tested for normality (Shapiro-Wilk test) and homogeneity (Barlett test), transformed accordingly and statistically significant differences were tested between habitat types and season (ANOVA and Tukey HSD post-hoc tests). Significance level was set to 0.05. Finally, all species were classified into one of three ecosystem engineering types (i.e., allogenic, autogenic, non-engineering). The proportion of each type was calculated for each sample and their variation was tested for statistical significance between habitat types, as above. All analyses were made in R.

3. Results

Communities showed over 60% similarity across all samples. All samples of PPM were associated with DM, whilst most samples from PSP showed widespread distribution, implying high spatial variation to the other types and within (Figure 1). Pairwise comparisons of two-way crossed ANOSIM suggested community overlap between PPM and DM ($p = 0.085$, $R = 0.36$). In contrast, PPM and PSP types showed significant difference with no overlap ($p = 0.03$, $R = 0.661$), while DM and PSP showed significant difference, but with some community overlap ($p = 0.03$, $R = 0.374$). Although Functional Richness mean and maximum values (172.59 ±77.49 and

265 respectively) were higher in PPM, no statistical significance was found by the two-way ANOVA between habitat types or seasonally (p = 0.310 and 0.869 respectively). In contrast, Functional Divergence showed significant different ranges between PPM and both PSP, DM (p = 0.00011 and p = 0.0057, respectively).

Non-engineering species had clear dominance in PPM and DM (66.46 ± 12% and 62.83 ± 6% respectively), compared to PSP whereas there were almost never dominant (28.60 ± 14%). The differences were statistically significant between the latter and both PPM (p = 0.00001) and DM (p = 0.0001), but not between PPM and DM. In contrast, allogenic engineering species were dominant in PSP (av. 58.72 ± 12.43%), with significant differences towards both PPM (p = 0.00001) and DM (p = 0.00195). Autogenic engineering species had similar percentages in habitat types having live rhizomes and canopy, namely PPM and PSP (av. 5 ± 6% and 12.67 ± 14% respectively), while were scarce in DM (0.62 ± 1%) and significantly different than PSP (p = 0.02087). Their proportions across habitat types are depicted in Figure 2.

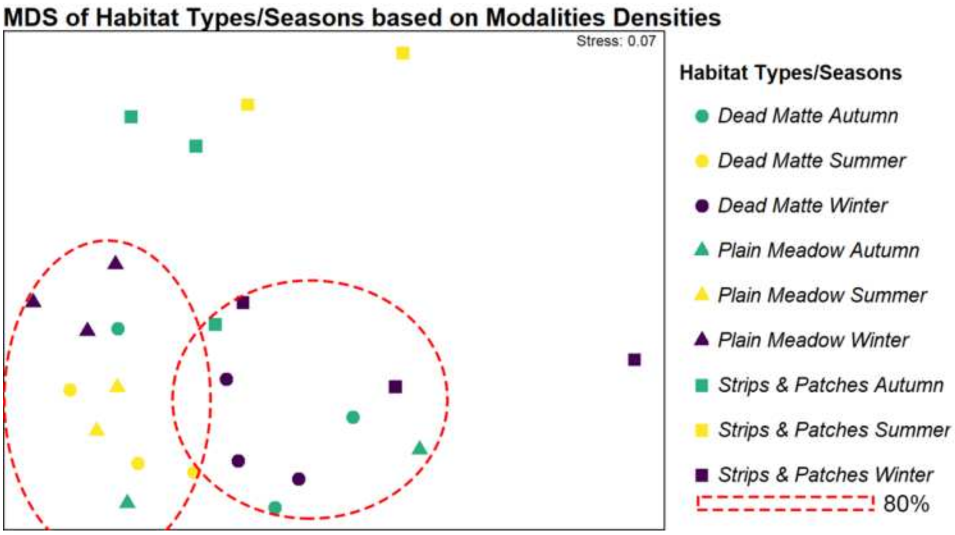

Figure 1. MDS of modalities distributions between different habitat types and seasons (highlighted with different color and shape). Similarity circles of >80% are marked.

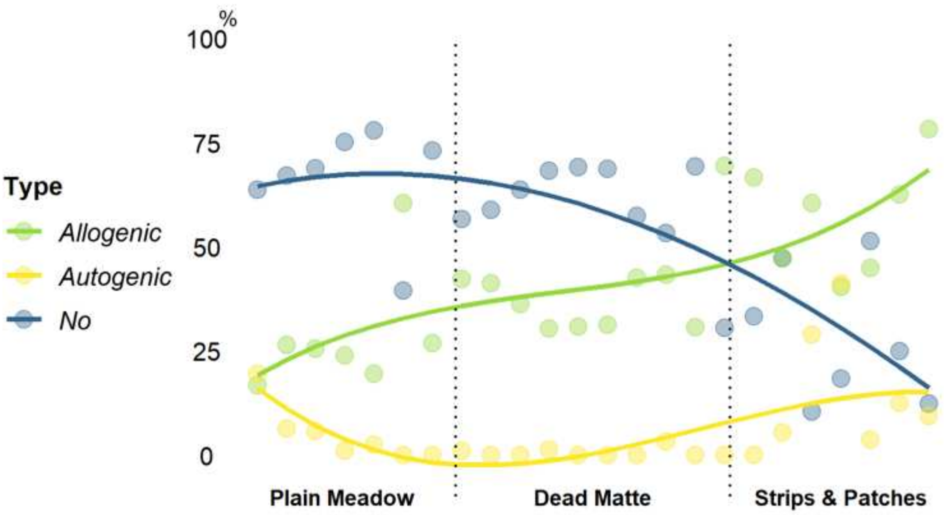

Figure 2. Proportions of each type of ecosystem engineering, with overlayed smooth line (polynomial interpolation), along the habitat types.

4. Discussion

A summary of our results shows an overlap between PPM and DM, whilst differences were apparent between PPM and PSP. Patterns in polychaetes communities seemed to be influenced by the different degrees of complexity between the habitat types, due to relation of the selected traits to *P. oceanica* structure. Reduced complexity and the presence of the adjacent sedimentary habitats were the governing factors in PSP, where modalities fit for sedimentary habitats (tube/galley-dwellers, detritus feeders, low sensory) had dominant or comparatively higher densities, than in the functional composition of PPM (vagile species, carnivores, high sensory). On the other hand, the community overlap between PPM and DM indicated that the below-ground structures were the main factor affecting functional compositions herein, in agreement with past studies that found rhizome and matte layers to be more important than leaf substratum in polychaetes species assemblages (Gambi *et al.* 1995; Somaschini *et al.* 1994)

Dead matte is usually composed of spongy parts and less compact structures that permit more species to form galleys, but still retains high structural complexity of a rhizomes-roots network (Borg *et al.* 2006). Of course, present results could never equate the ecological value of the dead matte with living *P. oceanica* meadows, for numerous reasons, such as the fact that the latter offers a high variety of ecosystem services not examined herein and that polychaetes constitute only one compartment of the associated fauna. But it does highlight the importance of dead matte in benthic functioning, as an independent habitat type, in agreement with past studies finding higher taxonomic diversity than living mattes (Borg *et al.* 2006; Harmelin 1964; Somaschini *et al.* 1994). Despite having available evidence of the diversity and ecological importance of dead mattes since the 60s (Harmelin 1964), this habitat type has been left out from large-scale habitat mapping and conservation legislation so far, also leaving its below-ground structure that sustains the functional composition found herein, vulnerable to anthropogenic damage and erosion.

In contrast to modalities distributions, Functional Richness (FRic) was not found to be significantly different between the habitat types. This index is based on the presence of the species that have the most extreme modalities combination in a community, which set the borders of functional space, and is not accounting how this space is filled (Villéger *et al.* 2008). Therefore, species exclusion or turnover could be overlooked (e.g., modalities fit for both seagrass and adjacent habitats in PSP), if there are species with similar extreme modalities combinations present in the community. Similar responses of the FRic index in ecotones or intermediate disturbances have been already documented (e.g., D'Alessandro *et al.* 2020) and it is regarded relatively weak in detecting early changes (Legras *et al.* 2018). On the other hand, Functional Divergence (FDiv), which is weighted with abundance, was found to be significantly higher in PPM, compared both to PSP and DM, constituting the only functional metric clearly distinguishing PPM and DM. This indicates that specialist species were more abundant in PPM than the other habitat types. Communities with high Functional Divergence, such as in PPM, are linked with high degree of niche partitioning and lower resource competition (Villéger *et al.* 2008). It is noteworthy that the popularly used Functional Diversity indices are quite recently developed and it will require time to comprehend their advantages and drawbacks over many ecological scenarios, a constrain that must be considered when interpreting results (Legras *et al.* 2018).

Finally, it could be useful for future applications that ecosystem engineering types were found remarkably relevant to *P. oceanica* structure, probably reflecting wider aspects of species life strategies. In the present study, they distinguished the habitat types in similar patterns to the analyses using in-depth functional compositions, confirming the hypothesis of Bouma *et al.* (2009). The mattes of PPM and DM facilitated the dominance of non-engineering species and excluded allogenic engineers, which in turn were dominant in PSP, where more space was available in the sediment matrix. However, proportional abundances of autogenic engineers were not different between PPM and PSP, despite the less available hard substrate for tube attachment in the latter. This can be explained by the fact that most autogenic engineers among polychaetes are suspension feeders and can be facilitated by eutrophic water column conditions, such as the presence of high turbidity and agricultural run-offs in Geras Gulf. Therefore, *P. oceanica* structure alone does not determine their densities, as in the other engineering types. The classification to types of ecosystem engineering shows interesting potential in assessing impacts of *P. oceanica* habitat damage or loss and its application could be investigated for cases of various human activities causing burial or uprooting and affecting matte structure, such as e.g., coastal constructions, trawling and mooring. Firstly, it may have linear response to increasing habitat fragmentation, to the contrary of taxonomic diversity that often increased with interaction of adjacent communities, and secondly, it does not require the time and effort of applying a thorough functional composition analysis.

References

Beauchard O., Veríssimo H., Queirós A.M., Herman P.M.J. (2017). The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. Ecological Indicators 76, 81–96.

Borg J.A., Rowden A.A., Attrill M.J., Schembri P.J., Jones M.B. (2006). Wanted dead or alive: High diversity of macroinvertebrates associated with living and "dead" *Posidonia oceanica* matte. Marine Biology 149(3), 667–677.

Bouma T.J., Olenin S., Reise K., Ysebaert T. (2009). Ecosystem engineering and biodiversity in coastal sediments: Posing hypotheses . Helgoland Marine Research 63(1), 95–106.

Bremner J. (2008). Species' traits and ecological functioning in marine conservation and management. Journal of Experimental Marine Biology and Ecology 366(1–2), 37–47.

D'Alessandro M., Porporato E.M.D., Esposito V., Giacobbe S., Deidun A., Nasi F., Ferrante L., *et al*. (2020). Common patterns of functional and biotic indices in response to multiple stressors in marine harbours ecosystems. Environmental Pollution 259, 113959.

Fahrig L. (2003). Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34, 487–515.

Frid C.L.J., Paramor A.L., Brockington S., Bremner J. (2008). Incorporating ecological functioning into the designation and management of marine protected areas. Hydrobiologia 606, 69–79.

Gambi M., Giangrande A., Martinelli M., Chessa L. (1995). Polychaetes of a *Posidonia oceanica* bed off Sardinia (Italy): Spatio-temporal distribution and feeding guild analysis. Scientia Marina 59(2), 129–141.

Harmelin J.G. (1964). Etude de l'endofaune des "mattes" d'herbiers de Posidonia oceanica Delile. Rec. Trav. St. Mar. End. 35(January 1964), 43–105.

Hewitt J.E., Thrush S.F., Dayton P.D. (2008). Habitat variation, species diversity and ecological functioning in a marine system. Journal of Experimental Marine Biology and Ecology 366(1–2), 116–122.

Hutchings P. (1998). Biodiversity and functioning of polychaetes in benthic sediments. Biodiversity and Conservation 7(2), 1133–1145.

Jones C.G., Lawton J.H., Shachak M. (1994). Organisms as ecosystem engineers. Oikos 69(3), 373–386.

Legras G., Loiseau N., Gaertner J.C. (2018). Functional richness: Overview of indices and underlying concepts. Acta Oecologica 87, 34–44.

Sânchez-Jerez P., Ramos Esplâ A.A. (1996). Detection of environmental impacts by bottom trawling on *Posidonia oceanica* (L.) Delile meadows: Sensitivity of fish and macroinvertebrate communities. Journal of Aquatic Ecosystem Stress and Recovery 5(4), 239–253.

Somaschini A., Gravina M.F., Ardizzone G.D. (1994). Polychaete depth distribution in a *Posidonia oceanica* bed (rhizome and matte strata) and neighbouring soft and hard bottoms. Marine Ecology 15(2), 133–151.

Villéger S., Mason N.W.H., Mouillot D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89(8), 2290–2301.

PRELIMINARY RESULTS ON THE EFFECT OF GROWTH IN THE SKELETAL ARCHITECTURE OF THE ECHINOID *Diadema setosum*

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Abstract

Biomineralization plays an important role in the growth and regeneration mechanics of echinoids. The present study presents preliminary results regarding the changes in the architecture of the stereom of the plates and spines of *Diadema setosum*. Measurements of pore diameter and trabeculae width for different skeletal regions and specimen size classes were taken from electron micrographs in order to determine the formation patterns of the porous meshwork. It was evident that small animals tend to possess plates with narrow pores surrounded by thick meshwork, which then slims down possibly serving the enlargement of the plate, followed by further increase in the diameter of the pores in large individuals. The spines followed a similar trend with small animals exhibiting thick trabeculae and narrow pores, which become wider while the trabecular width decreases in medium-sized specimens, to finally thicken in large individuals. The region also played a significant role in the skeletal architecture of the plates, with oral plates exhibiting both lowest trabecular width and pore diameter, which increases towards the periproct. Spines showed a tendency to form narrower pores with slimmer trabeculae near the base, while an inverse trend was observed towards the tip.

Key words: *Skeletal architecture, biomineralization, growth, regeneration, invertebrates*

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1. Introduction

Echinoids are equipped with a biomineralized endoskeleton made up of a porous meshwork of $CaCO_3$ arranged in rows of interlocked plates, which are bound together by collagenous fibers. These plates contain protuberances, where the spines are connected via a ball-socket joint. Most echinoids have a biphasic live style, shifting from a larval stage to settling as juvenile through a process called metamorphosis. The first formed plates are found in the apical disc, which have partially derived from the larval stage (Emlet 1989). After settlement, sea urchins grow in size by enlarging their plates, while also adding new ambulacral and interambulacral plates (Smith 2005). Addition and enlargement of the plates vary among species. Generally, the evolutionary trend has been a shift from addition of new plates to enlargement of new ones. These biomineralization processes take place through molecular and cellular pathways, changing the morphology of the skeleton (Thompson *et al*. 2021).

Diadema setosum belongs to the subclass Euechinoidea, which utilize both the accretion of plates and the addition of new ones, in contrast to most Paleozoic echinoids, whose plates remained undifferentiated, while moving from aboral to oral orientation. It is, however, not known if and to what extent the aboral and oral plates are differentiated from each other in terms of architecture. *D. setosum* recently invaded the eastern Mediterranean through the Suez Canal (Yokes & Galil 2006). Its abundance in the Aegean Sea and data about its size has also been reported (Vafidis *et al*. 2020). This species is one of the few to possess venomous spines. These are slender and extremely long, while also being hollow, deprived of inner stereom possibly to further enhance the utilization of toxins. The mechanical properties of the test and spine in regard to the skeletal architecture of this species have also been discussed elsewhere (Voulgaris *et al*. In press). However, the effect of size as well as the relative sections of the skeleton on its architecture remains for the most part unknown. The present study determines variations in the plates and spines of *D. setosum* quantitatively, focusing on the porous stereom and the micromorphology of these skeletal elements.

2. Materials and Methods

2.1 Collection and preparation of samples

Individuals of *D. setosum* (n=60) were collected in July 2020 from the southern Dodecanese complex. Measurements of the test diameter (TD) of each individual were taken using a Vernier caliper. Three size classes were determined each with a 30 mm width interval, namely small (TD <40 mm), medium (TD <70 mm) and large (TD >70 mm) and the test and spines of five specimens from each size class were examined. Three plates and three spines per individual were taken from three distinctive regions of the test (oral, ambital, aboral) and each spine was further divided into three sections (base, shaft, tip). The samples were then bleached to remove the soft tissues, following the method of Collard *et al*. (2016): immersion in NaOCl 2.5% for 1.5 h, then NaOCl 5.25% for a further 2.5 h and subsequently air dried for 24–48 h. Finally, the specimens were mounted on metal stabs with carbon-based tape and coated with carbon for SEM observations.

2.2 Scanning Electron Microscopy

The longitudinal section of the spines and the cross section of the plates were observed, and micrographs were taken, using a JEOL JSM 6510 electron microscope. The specimens were examined, using a 10 kV acceleration voltage with a 26–31 mm working distance. A region of roughly 200×200 μm of each micrograph was selected to measure the trabeculae width and pore diameter and a minimum of 30 measurements per image were performed using ImageJ software (Figure 1).

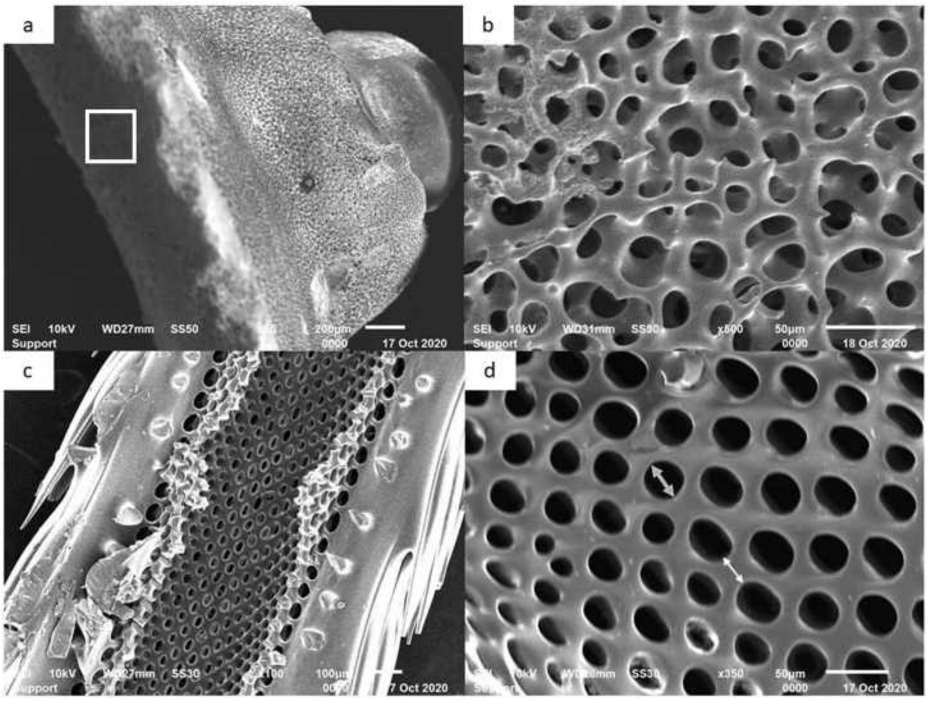

Figure 1. Electron micrographs of the plates (a, b) and the spines (c, d) of *D. setosum*. a) Cross section of a plate; the white box indicates the area of higher magnification. b) The trabecular meshwork of the plate. c) Longitudinal section of the spine. d) Higher magnification of the stereom surrounding the lumen; Yellow arrow indicates the pore diameter, while white arrow shows the measurement taken for the width of the trabeculae.

2.3 Statistical Analysis

Samples were first tested for homogeneity and normality and then a comparison of the width of the skeletal meshwork and the diameter of the pores for the plates and spines of the three size classes of *D. setosum* as well as to determine differences in the size of pore and trabeculae among different regions of the spines and test, was carried out using one-way ANOVA. Tukey's post-hoc test was used to determine statistically significant variations between groups.

3. Results

One-way ANOVA and post-hoc tests showed that both the trabeculae width and the pore diameter vary with size class both in the plates and the spines, except for the diameter of the pores of the spines in medium and large individuals. Small individuals appeared to have smaller pores (12.41 ± 4.28 μm), medium-sized individuals exhibited intermediate values (14.329 ± 6.01 μm), while in large specimens the pore was the widest (20.31 ± 9.29 μm). Regarding the width of the trabeculae, small animals exhibited highest values (11.29 ± 3.42 μm). Lowest values were observed in the medium class (7.78 ± 1.99 μm), while large individuals appeared to have intermediate trabeculae width (9.89 ± 3.42 μm) (Table 1; Figure 2).

Table 1. Results for the analysis of variance determining statistical differences for the diameter of pores and the width of the trabeculae of the plates and spines among different size classes, different positions of the plates in the test, as well as different areas of the spine. (F-variance of the group means for ANOVA; *p*-probability value*).*

		Pore diameter		Trabeqular width	
		F	*p*	F	*p*
Plates	size	54.85	*<0.0001*	54.58	*<0.0001*
	section	9.92	*<0.0001*	4.38	*0.013*
Spine	size	72.82	*<0.0001*	76.28	*<0.0001*
	area	122.44	*<0.0001*	15.67	*<0.0001*

The spines of *D. setosum* exhibited lowest values for the diameter of the pores in small animals (18.94 ± 6.94 µm), while large and medium individuals showed no statistical differences (23.91 ± 6.24 µm; 24.1 ± 6.39 µm respectively). Observing the width of the trabecular meshwork, the same pattern as in the plates was followed, namely widest trabeculae in the small class (17.97 ± 3.33 µm), while the medium-sized specimens exhibited lowest values (14.93 ± 2.91 µm). Finally, the largest individuals showed intermediate values (16.56 ± 3.92 µm), (Table 1; Figure 2).

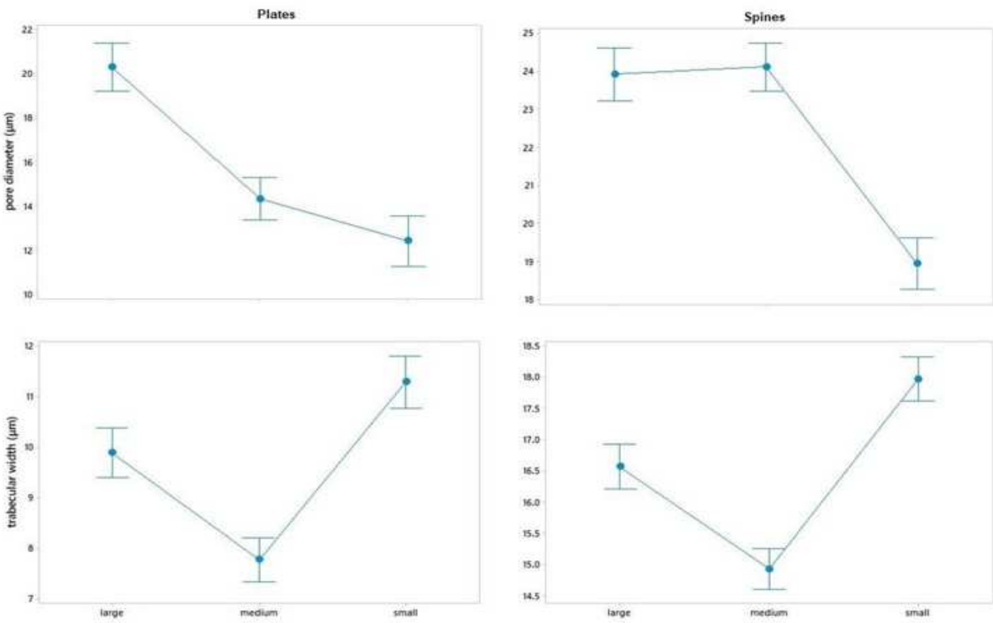

Figure 2. Interval plots of the pore diameter and trabecular width with size; error bars indicate the 95% confidence intervals.

Regarding differences in the size of pores and trabecular meshwork among plates of different sections of the test, it was shown that plates from the aboral region had highest values for pore diameter (17.62 ± 8.98 µm), while the oral region exhibited lowest values (13.96 ± 6.29 µm). The ambital region (15.8 ± 7.06 µm) did not show any statistical differences compared with the other two sections of the test. Examining the spine, different areas exhibited different pore diameters, with the base showing lowest (18.77 ± 5.9 µm), the shaft intermediate (22.86 ± 5.95 μm) and the tip highest values (25.88 ± 6.89). Finally, regarding the width of the trabeculae lowest values were observed in the base (15.66 ± 3.5 μm), while highest in the shaft and tip (17.1 ± 3.7 μm; 16.59 ± 3.5 μm respectively), (Table 1; Figure 3).

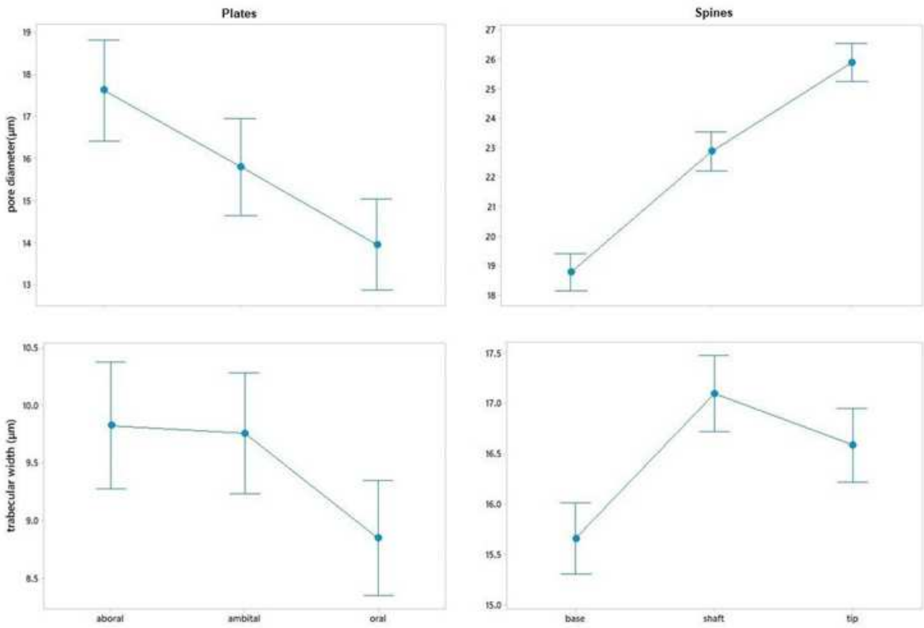

Figure 3. Interval plots of the pore diameter and trabecular width with the position of the plates in the test and the different sections of the spine; error bars indicate the 95% confidence intervals.

4. Discussion

The results of this study reveal a pattern both in the test and spines of *D. setosum*, where small individuals present narrow pores surrounded by a thick trabecular meshwork, which tends to slim down so that the pores can be enlarged. In large animals the meshwork seems to be then further thickened possibly to enhance the tenacity of the skeleton (Figure 2). These results are preliminary towards a better understanding of the specific pathways of biomineralization of sea urchins from the perspectives of development, growth and regeneration. The same pattern that is described in this study was also observed in studies regarding regeneration of spines in other species, where the porous stereom is initially built and will be subsequently thickened when regeneration is complete (Gorzelak *et al*. 2017).

Growth of the test of the animal is dictated by two procedures, specifically the enlargement of existing plates and the addition of new ones from a growth zone. As a consequence of this addition, earlier plates are shifted away from the apical disc with the first ones found closest to the oral region (Smith 2005). After the formation of the new plates the process of enlargement takes place, where stereom is accreted both peripherally and in the center of the plate creating a new growth line (Smith 1980). The results of the present study further explain the process of plate enlargement in that they show a trend of the stereom of the plate to become denser. Oral plates have the smallest pores and the slimmest trabeculae, which indicates that the stereom becomes denser in that it stretches during enlargement. In contrast, newly formed plates appear to have thick trabeculae with a more open stereom, since the pore diameter was observed to be higher in the plates of the aboral region (Figure 3).

Regarding the spines, regeneration plays a key role in their function. During the formation of the spines, either initially or during regeneration, the stereom is first created in the form of microspines orientated from the base to the tip and it is then thickened for the septa to be created (Heartfield 1971). The width of the trabeculae and the diameter of the pores both present the lowest values near the base of the spine, which is also the oldest part of the spine, while highest values are presented in the tip of the spine. Thus, it becomes evident that the architecture of the spine shifts from a dense stereom in the base to a progressively more open one near the tip, which might enable the spine to partially fracture. This way regeneration becomes a highly energy-efficient process.

The present study deals with growth-related changes in the skeletal architecture of *D. setosum*, a highly mobile sea urchin with hollow, toxin-bearing spines. The plates and the spines present differences in the size of the pores and the trabeculae not only regarding the size of different individuals but also different regions of the skeletal elements. Certain traits of the skeletal architecture in *D. setosum* may dictate the specific position, where the spine tends to fracture. In addition, variations in the morphometric features of the meshwork of the plates could possibly make the test of this species lighter and consequently more mobile. Changes in architecture are most likely related with processes of biomineralization both during the development and the regeneration of echinoids. These processes interrelate the chemical composition of the skeleton with each morphology and may be affected by various factors. Seasonal changes of the chemical composition of the skeleton of three Mediterranean echinoid species have been reported (Varkoulis *et al*. 2020). However, more research has to be conducted towards a better understanding of how changes of the skeletal properties are dictated both by environmental and interspecific factors.

References

Collard M., Rastrick, S.P.S., Calosi P., Demolder Y., Dille J., Findlay H.S., Hall-Spencer J.M., Milazzo M., Moulin L., Widdicombe S., Dehairs F., Dubois P. (2016). The impact of ocean acidification and warming on the skeletal mechanical properties of the sea urchin *Paracentrotus lividus* from laboratory and field observations. ICES Journal of Marine Science 73, 727–738.

Emlet R.B. (1989). Apical skeletons of sea urchins (Echinodermata: Echinoidea): two methods for inferring mode of larval development. Paleobiology 15, 223–254.

Gorzelak P., Dery A., Dubois P., Stolarski J. (2017). Sea urchin growth dynamics at microstructural length scale revealed by Mn-labeling and cathodoluminescence imaging. Frontiers in Zoology 14, 42.

Heatfield B.M. (1971). Growth of the calcareous skeleton during regeneration of spines of the sea urchin *Strongylocentrotus purpuratus* (stimpson): A light and scanning electron microscope study. Journal of Morphology 134, 57–89.

Smith A.B. (1980). Stereom microstructure of the echinoid test. Special Papers in Palaeontology 25, 1–83.

Smith A.B. (2005). The pre-radial history of echinoderms. Geological Journal 40, 255–280.

Thompson J.R., Paganos P., Benvenuto G., Arnone M.I., Oliveri P. (2021). Post-metamorphic skeletal growth in the sea urchin *Paracentrotus lividus* and implications for body plan evolution. EvoDevo 12, 3.

Vafidis D., Antoniadou C., Voulgaris K., Varkoulis A., Apostologamvrou C. (2021). Abundance and population characteristics of the invasive sea urchin *Diadema setosum* (Leske, 1778) in the south Aegean Sea (eastern Mediterranean). Journal of Biological Research-Thessaloniki 28, 11.

Varkoulis A., Voulgaris K., Zaoutsos S., Stratakis A., Vafidis, D. (2020). Chemical composition and microstructural morphology of spines and tests of three common sea urchins species of the sublittoral zone of the Mediterranean Sea. Animals 10, 1351.

Voulgaris K., Varkoulis A., Zaoutsos S., Stratakis A., Vafidis D. (In press). Mechanical defensive adaptations of three Mediterranean sea urchin species. Ecology and Evolution.

Yokes B., Galil B.S. (2006). The first record of the needle-spined urchin *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) from the Mediterranean Sea. Aquatic Invasions 3, 188–190.

FRIENDLY AQUACULTURE; ARE FISH FARMS UNFAIRLY HELD RESPONSIBLE FOR DEATHS OF MEDITERRANEAN MONK SEAL?

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Abstract
Among the marine mammals observed or living in the Mediterranean, *Monachus monachus* is the Europe's most endangered marine mammal. Since the population numbers declined dramatically in the past century, the species is currently considered "critically endangered, CR". The interaction between monk seals and marine aquaculture is a well-known incident and *M. monachus* is one of the major problems for fish farms. In this study, the interaction between fish farms and Mediterranean monk seals is discussed with a special emphasis to the seal observation on the fish farm cage in Güllük Bay. We observed a Mediterranean monk seal resting on the floats of the offshore farm's cages in Göltürkbükü, Muğla. The estimated length and weight was 280–290 cm and 300–350 kg, respectively.
Keywords: *Mediterranean monk seal, Monachus monachus, aquaculture, Turkey*
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1. Introduction

As the awareness of the integral importance of marine mammals in healthy aquatic ecosystems has increased in recent years, so has the interest in these animals' lives, both by the public and the scientific and management communities. A number of human activities, fisheries included, have started threat their survival and their habitats. Numerous studies have tried to evaluate the extent of marine mammal and fisheries interactions. These studies have mostly focused on the operational interactions, such as the accidental entanglement or mortality of marine mammals in fishing operations and the damage caused by marine mammals to fishing gear. In the Mediterranean Sea a characteristic and a widely publicized example of such an interaction is the case of the Mediterranean monk seal with coastal fisheries.

Monachus monachus, also known as the Mediterranean monk seal, is one of the 33 extant species of Pinnipeds and belongs to a genus that inhabits exclusively subtropical and temperate waters. The Mediterranean monk seal is found throughout almost the entire Mediterranean basin, mostly in coastal areas. The species is now mainly confined to two surviving populations, one occupying the Atlantic coast of northwest Africa, and the other, the northeastern Mediterranean (Dendrinos *et al*. 2020). Although it is known that the largest population is found in Greece and Turkey, small number specimens of the species have also been seen in Morocco, Algeria, Libya, in some Portuguese Islands, Croatia, and Cyprus.

Population numbers of *M. monachus* declined dramatically in the past century and the species is currently considered "critically endangered" by the International Union for Conservation of Nature. The shy and reclusive nature of the species and the inaccessibility of its habitat have precluded accurate population estimations. Conventional wisdom, however, suggested that fewer than 600 individuals survive (Reijnders *et al*. 1997). According to Güçlüsoy *et al*. (2004), there were about 100 individuals in the Turkish coasts and the number of identified monk seals in the northeastern Mediterranean Sea was given as 42 by Gücü *et al*. (2009). Pollution, overfishing, bacterial, fungal and viral infectious diseases effect to decline the population (Öztürk 1992; Androukaki *et al*. 1999). Dendrinos *et al*. (2007) gave the factors that threaten the species on a global scale and in Hellas seas under six main headings. According to the list given by the researchers, these are as follows: (1) Habitat loss and deterioration, (2) Deliberate killing (mostly by fishermen), (3) Accidental death due to entanglement in fishing gear, (4) Lack of food and depressed physical condition as a result of overfishing, (5) Lack of international coordination and funding of conservation and management actions, (6) Stochastic events (i.e. disease epidemics, cave collapses, oil spills, toxic algae blooms or other).

On the other hand, world demand for fish, both as a source of food for human consumption and for reduction to fishmeal, has grown at a steady pace since the end of Second World War. Until recently, demands were met by the expansion of capture fisheries. Exploiting wild stocks that reach the upper limits has motivated developing in aquaculture and using aquaculture technology to support fisheries such as sea ranching. Due to the ecological limits capture fisheries face, a further expansion has taken place through intensive marine aquaculture production, which leads to new ways of producing seafood in encircled spaces in marine areas (Saguin 2016; Ertör & Ortega-Cerdà 2019). Total production from aquaculture was raised from 19 million tons in 1994 to as much as 39 million tones by the year 2010. This development generates profit and income, but it also bears risks of negative environmental impact, such as pollution, landscape modification, or biodiversity change. The environmental impact is primarily a function of feed composition and feed conversion, fecal waste generation, organic and inorganic fertilizers, liming materials, algicides and herbicides, disinfectants, antibiotics, inducing agents, osmoregulators, piscicides, probiotics etc. (Tacon & Metian 2008). The marine aquaculture entered Turkey's agenda in the early 1980s. The main farmed marine species were gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) that produced from 1985 onwards. While production levels were initially quite low in the late 1980s, the sector began to witness growth in the 1990s, although Turkey's total production volume was still very limited compared with its competitors. In 2019, marine aquaculture production reached more than twice the level of inland aquaculture production.

The interaction between monk seals and marine aquaculture is a well-known occurrence and *M. monachus* sometimes becomes one of the major problems for fish farms due to attacks on fishes in cages (Güçlusoy & Savaş 2003). In this study, the interaction between fish farms and Mediterranean monk seals is discussed with a special emphasis to the seal observation on the fish farm cage in Güllük Bay.

2. Materials and Methods

A Mediterranean monk seal of an estimated 280–290 cm length and 300–350 kg weight was observed resting on the floats of the offshore farm's cages owned by Hatko Technical Equipment Representation Trade Inc., which produces sea bream and sea bass, near the small rabbit island located 10 km northwest of Göltürkbükü sub-district in Bodrum district of Muğla (Figure 1-A). *M. monachus* was recorded with videos and photographs by the employees and they tried to interact by swimming next to the animal. In this way, it was determined that the animal was a male by observing the white patch under its abdomen, and its length and weight were estimated by measuring the distance between the struts (brackets) of the floats on which he was lying (Figure 2-A & B). When trying to obtain information about the monk seal by interviewing the fish farm workers, we were informed that the seal was seen resting or basking on the floats of the fish cages many times. No mention was made of any attacks on either the employees or the cages. Due to throwing feed to the fish in the cages, the feed and feed residues mixed with the seawater cause to assemble and settle under and around the cages free-ranging fish schools such as mullet, sardines, coupes, sarpa and others. Therefore, it is estimated that this ichthyofauna under the cages also allure predators such as seals, tuna and dolphins for feeding purposes.

Figure 1. (A) The male Mediterranean monk seal resting on the floats of the offshore farm's cages located 10 km northwest of Göltürkbükü in Bodrum district, Muğla, (B) *Monachus monachus* on the sea-cages in the Kazikli Cove of Güllük Bay (taken from Akyol & Ceyhan 2020).

3. Results and Discussion

Coastal aquaculture along the Eastern Aegean coasts has been going through a period of unprecedented growth over the past ten years (Yucel-Gier 2013). Turkish aquaculture production has increased from 34,000 tons in 2000 to around 75,000 tons in 2009 (Turkstat 2010). The actual production for Gulluk Bay was 67,000 tons in 2011, making it the premier production area in Turkey. Furthermore this growth has led to the drafting and implementation of new planning and management policies by the Turkish government (Yucel-Gier 2013). Especially Gulluk Bay, which is one of the seven largest gulfs and bays on the west coast of Turkey and contributes about 70% of countries' aquaculture production, has attracted special attention recently since rapid and uncontrolled urban development activities, as well as mariculture, have created considerable threats and problems on its valuable resources. This bay is the location where two monk seals observed basking or resting on fish cages.

Akyol & Ceyhan (2020) observed *M. monachus* various times, especially when swimming around sea-cages in Güllük Bay and occasionally, basking on the cages. It was closely photographed (Figure 1-B) by an aquaculture technician on 12 February 2017 in Kazıklı Cove, Güllük Bay. Gerovasileiou *et al*. (2017) reported several sightings (n=7) around four sea-cage fish farms, 3 in Güllük, and 1 in Gerence Bays between March 2016 and February 2017 along the Turkish Aegean Sea coasts. When we consider the entire Güllük Bay, angling is carried out intensively in the region with boats and vessels smaller than 20 meters. In addition, the presence of many large and small rocky shores and islands near the coastal region of the Hatko Fish Farm strengthens the estimation that they may breed in the region.

Figure 2. (A) The curious farm worker, who is eager to understand the reaction of the Mediterranean monk seal by swimming close to the animal that is resting or basking on the farm cage, (B) While diving in the water, a large white patch on the abdomen of the male *Monachus monachus* is observed; the animal is uncomfortable when the employee approaches it.

According to Dendrinos *et al*. (2007), based on the results of more than 90 necropsies and on information received through the Rescue and Information Network, deliberate killing is the most frequent cause of non-natural death for the species in Hellas. Especially adult individuals are affected, as they are considered a threat to the livelihood of fishermen and aqua culturists. Approximately 50% of adult monk seals found dead in Hellas were deliberately killed. The Turkish monk seal population is also under pressure of deliberate killing. Güçlüsoy *et al*. (2004) reported that five out of 22 dead seals had been deliberately killed. Öztürk (2007) also reported that 12 out of 24 mortalities observed during 1986-1996 were due to deliberate killing. During 2012-2014, two more deliberate killing cases were recorded. A juvenile, female Mediterranean monk seal was found dead by local people on the coast of Meydan Village, Hatay Province on 5 December 2012. In the gross necropsy, two sharp force injuries were observed as the evidence of deliberate killing (Ergün and Altuğ 2012). Danyer *et al*. (2013b) reported a deliberate killing of a monk seal by rifle on Antalya coast of Turkey in April 2013. It was an adult male monk seal, well-recognized and even nicknamed “Duman” by local people.

An adult, female monk seal was found stranded on Antalya coast in August 2013. In the gross necropsy, seal was emaciated and nematodes, cestodes, and trematodes were found in the gastrointestinal tract (Danyer *et al*. 2013a). Similarly, Danyer *et al*. (2014) published their opinions and predictions about necropsy findings performed on a male Mediterranean monk seal of 3–3.5 months old stranded near Yasilovacık Harbour, Mersin on 28 February 2014. According to findings of necropsy, emaciation and severe pneumonia might have led to the monk seal’s death. They predicted and even claimed that perhaps due to the noise stress the seal could not exit from the cave to feed, then his nutritive condition decreased, consequently that was why this stranded seal was in a poor health condition. In opposition to this claim, Yiğit *et al*. (2018), who had worked in the same locality, stated that major threat on the monk seal were by catch in gill nets, bottom trawl nets, overfishing and deliberate killing rather than the pier constructions caused noise, turbidity and over-lighting. According to them, the observing frequencies were being increased in the harbor area and the reason for this increase was though due to the alternative employment for local people, which mitigated the conflicts between monk seals and fishermen.

More recently, fish farm operators have also come into conflict with monk seals that raid their facilities, particularly where adequate protective netting has not been installed (Güçlüsoy & Savas 2003). In their study, Güçlusoy & Savaş (2003) recorded that *M. monachus* were attacking the wild fish that gather around the pens as a result of feed contamination and accumulation rather than attacking the captive fish in the cage complexes.

Güçlüsoy and Savaş (2003) indicated that fishermen and fish farmers had used lights, pesticide-injected fish, noise generation and warning and/or direct shots with rifles to keep them away from fish cages.

Fish farmers in the Muğla region reported the increasing incidences of fish-farm attacks by the Mediterranean monk seal in recent years (Akyol *et al*. 2019). Akyol *et al*. (2019) determined potential conflicts between small-scale fisheries and sea-cage fish farms in the Aegean Sea and stated that the main problems of coastal fishing activities, as highlighted by small-scale fisheries, were the pollution caused by fish farms, the space limitation for fishing, recreational fishers, and net damage caused particularly by dolphins and monk seals. Moreover, in addition to conflicts between fish farmers and small-scale fisheries, a significant increase of dolphins (i.e., *Delphinus delphis*, *Tursiops truncatus*, and *Stenella coeruleoalba*) had been reported by both groups (Akyol *et al*. 2019). They believe that Mediterranean monk seal and sea turtle populations have been increasing in Muğla region (Akyol *et al*. 2019).

Because of the attacks on the fish in cages, the monk seal could be a big problem for the aquaculture. Güçlüsoy and Savaş (2003) reported 40 attacks on 11 fish farms in the Turkish Aegean Sea, which resulted in damage to cage nets and the escape of reared fishes. In terms of the predators, 84% of Muğla and 75% of Izmir fishermen mentioned predator attacks to the sea-cage farms. The common predators were dolphins, monk seals, bluefin tuna, bluefish, sharks, and sea turtles (Akyol *et al*. 2019). Additionally, 46% of Muğla and 60% of Izmir fish farmers mentioned fish escapes from sea-cages. The fish escapes occurred due to damages to cage nets, storms, fish fall from dip nets during the harvesting, and predator attacks. Furthermore, damages to cage nets, caused particularly by monk seal and bluefish depredation, were also reported by 18% of Muğla and 40% of Izmir fish farmers. These events were one of the sources of significant economic losses. Indeed, the losses of fish in 2018 were approximately 5,000 (more than 4 t) seabass in Muğla region, and 1,000 gilthead seabream and 20,000 seabass in the Izmir region.

If you are one of the components of the same ecosystem, and if you share the same ecosystem, it is inevitable that these components will come together in some way and a competition will occur between them. Just as the big fish eat the small fish, the stronger one will win in this competition as well. As Mahatma Gandhi once said, "the earth provides every human being's need, but not every human being's greed!". We well know that human beings are often the winner in that competition between species in the ecosystem, that is why many species were vanished already and that is why many of them are in danger of extinction today as well. The debate on the effects of fish farming on the marine environment has been ongoing from the beginning. It is likely that this debate will continue until resolved by a true coastal zone management plan. Coastal zone management has to protect sensitive habitats, to ensure the sustainability of water resources and natural ecosystems, and finally to prevent potential conflicts among coastal users. Some case reports, such as this one, will contribute to the understanding of the impacts of fish farms on coastal environment, and to help to establish to this kind of planning and management tools.

Over the last 20 years, due to the increase in the coast area utilization around Gulluk Bay by fish farming enterprises there has been extra conflict with other coastal users especially with tourism, fisheries and marine transportation. The development of Turkish marine aquaculture site selection and zoning should address all the issues through a participatory process involving stakeholders, scientists and government (Yucel-Gier *et al*. 2013). The debate on the effects of fish farming on the marine environment has been ongoing from the beginning. When the rapid deterioration of water quality became obvious in parallel to the increasing number of fish cages in the coastal zones, naturally, local communities blame the farming companies without a scientific evidence base. It is likely that this debate will continue until resolved by a true coastal zone management plan. Coastal zone management has to protect sensitive habitats, to ensure the sustainability of water resources and natural ecosystems, and finally to prevent potential conflicts among coastal users. Consequently, research studies, observations and case reports may contribute to better understanding of the impacts of fish farms on coastal environment as well as the Mediterranean monk seals, and may help us to establish better planning and management tools. The more friendly fish farm in the Mediterranean, the more healthy and happy monk seal population.

References

Akyol O., Ceyhan T. (2020). Wild fish communities associated with sea-cage fish farms in the Aegean Sea. Marine Aquaculture in Turkey: Advancements and Management, Deniz Çoban,Menekşe Didem Demircan, Deniz D. Tosun, Editör, Turkish Marine Research Foundation (TUDAV), İstanbul, pp. 401-415.

Akyol O., Özgül A., Şen H., Düzbastilar F.O., Ceyhan T. (2019). Determining potential conflicts between small-scale fisheries and sea-cage fish farms in the Aegean Sea. Acta Ichthyologica et Piscatoria 49(4), 365–372.

Androukaki E., Adamantopoulou S., Dendrinos P., Tounta E., Kotomatas S. (1999). Causes of mortality in the Mediterranean monk seal (*Monachus monachus*) in Greece. Contributions to the Zoology and Ecology of the Eastern Mediterranean Region 1, 405–411.

Danyer E., Aytemiz I., Gücü A.C., Tonay M.A. (2014). Preliminary study on a stranding case of Mediterranean monk seal *Monachus monachus* (Hermann, 1779) on the Eastern Mediterranean coast of Turkey, Journal of the Black Sea/Mediterranean Environment 20(2), 152–157.

Danyer E., Aytemiz I., Özbek E.Ö., Tonay A.M. (2013a). Preliminary study on a stranding case of Mediterranean monk seal *Monachus monachus* (Hermann, 1779) on Antalya coast, Turkey, August 2013. Journal of the Black Sea/Mediterranean Environment 19(3), 359–364.

Danyer E., Özbek E.Ö., Aytemiz I., Tonay A.M. (2013b). Preliminary report of a stranding case of Mediterranean monk seal *Monachus monachus* (Hermann, 1779) on Antalya coast, Turkey, April 2013. Journal of the Black Sea/Mediterranean Environment 19(2), 278–282.

Dendrinos D, Karamanlidis A.A., Adamantopoulou S., Koemtzopoulos K., Komninou A., Tounta E. (2020). LIFE-IP 4 NATURA: Integrated actions for the conservation and management of Natura 2000 sites, species, habitats and ecosystems in Greece. Deliverable Action A.1: Action Plan for the Mediterranean monk seal (*Monachus monachus*). Hellenic Ministry of Environment and Energy, Athens.

Dendrinos S., Adamantopoulou E., Androukaki A., Chatzispyrou A.A., Karamanlidis V., Paravas E., Tounta S., Kotomatas S. (2007). Mediterranean monk seal *Monachus monachus* and fisheries: Conserving biodiversity and mitigating a conflict in Hellenic seas. State of Hellenic Fisheries - Chapter 6, p. 23–43.

Ergün Y., Altuğ E.M. (2012). Necropsy Report. Veterinary Faculty of Mustafa Kemal University (No:96174695/045/1340).

Ertör I., Ortega-Cerdà M. (2019). The expansion of intensive marine aquaculture in Turkey: The next-to-last commodity frontier? Journal of Agrar Change19, 337–360.

Güçlüsoy H., Savas Y. (2003). Interaction between monk seals *Monachus monachus* (Hermann, 1779) and marine fish farms in the Turkish Aegean and management of the problem. Aquaculture Research 34, 777–783.

Güçlüsoy H., Kiraç C.O., Veryeri N.O., Savas Y. (2004). Status of the Mediterranean monk seal, *Monachus monachus* (Hermann, 1779) in the coastal waters of Turkey. E.U. Journal of Fisheries & Aquatic Sciences 21, 201–210.

Gücü A.C., Sakinan S., Ok M. (2009). Occurrence of the critically endangered Mediterranean monk seal, *Monachus monachus*, at Olympos-Beydaglari National Park, Turkey. Zoology in the Middle East 46, 3–8.

Öztürk B. (1992). Mediterranean Monk Seal. Anahtar Kitaplar, Istanbul, 215 pp. (in Turkish).

Öztürk B. (2007). Mediterranean Monk Seal and Its Protection. Yalıkavak Çevre ve Fok Araştırmaları Derneği Yayın No:1, Muğla Turkey, 132 pp. (in Turkish).

Reijnder P.J.H., Verriopoulos G. Brasseur, S.M.J.M. (1997). Status of Pinnipeds Relevant to the European Union. Wageningen, The Netherlands, Institute for Forestry and Nature Research (IBN-DLO). 1-195.

Saguin K. (2016). Blue revolution in a commodity frontier: Ecologies of aquaculture and agrarian change in Laguna Lake, Philippines. Journal of Agrarian Change 16(4), 571–593.

Tacon A.G.J., Metian M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285(1–4), 146–158.

TURKSTAT (2010). Fishery Statistics Prime Ministry Republic of Turkey Turkish Statistical Institute Fishery Statistics in 2009. ISSN 1013-6177 (In Turkish) (http://www.turkstat.gov.tr)

Yiğit N., Ayas D., Çolak E. (2018). Occurrence of the Mediterranean Monk seal (*Monachus monachus*) in Yeşilovacık bay (Mersin, Turkey); a case report for anthropogenic impact assessments. Biological Diversity and Conservation 11, 97–105.

Yucel-Gier G., Pazi I., Kucuksezgin F. (2013). Spatial Analysis of Fish Farming in the Gulluk Bay (Eastern Aegean). Turkish Journal of Fisheries and Aquatic Sciences 13, 737–744.

THE USE OF THE mtDNA AS A MOLECULAR TOOL TOWARDS AUTHENTICITY OF THE RED PORGY *Pagrus pagrus*

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Abstract
The red porgy (*Pagrus pagrus*) is a species of high commercial value, widely traded in the Greek market. Its wild populations are located in the Mediterranean Sea, but also in the Atlantic coasts of America, South Europe and Africa. *P. pagrus* shows similar morphological features with the closely-related species of Sparidae, as *Pagellus erythrinus*, and *Dentex gibbosus*. *Pagrus* fish farming products sold in the Greek market are found under various names, with the majority reported as *Pagrus major*. Fish samples can be identified and classified by their morphological characteristics however, the strong similarities between the representatives of the genus *Pagrus*, or with other closely related species, make the identification very difficult, especially when the products are frozen, cooked or processed. The molecular techniques and phylogenetic analyses based on the identification of genetic material (DNA) and the possibility of cross-referencing data with bioinformatics, are wildly used in Fisheries and Aquaculture. Determining the molecular identity of each species results in a number of effective tools for assessing the genetic authenticity and traceability of catches. The mitochondrial DNA (mtDNA) is a common molecule used in the analysis of molecular mechanisms and evolutionary relations between different organisms and especially, part of the cytochrome oxidase subunit I gene (COI) is considered as a barcode for species identification. Other regions of mtDNA (*cytb*, 16RNA, D-loop) can also be used in populational genetics. The aim of this study is the identification of specific molecular markers, which can be used to effectively identify *Pagrus pagrus*, caught in the Greek seas, compared to other species sold in the Greek market. In this study, the complete mtDNA of *Pagrus pagrus*, captured in the central Aegean Sea, was sequenced. Moreover, the COI barcode was determined for *Pagrus* samples (a) collected from Greek seas, (b) Greek fish farms, and (c) imported sold at super markets. Data inferred from the complete mtDNA sequence along with sequences mined from databases (GenBank, Boldsystems) were used in different molecular techniques (sequencing, PCR-RFLP, multiplex PCR, HRM), in order to establish a fast and reliable method for species identification within *Pagrus* genus. By fish species authentication, fish fraud could be controlled with exceptional precision from their catch to the consumer. Moreover, it aims to improve the competitiveness of the Greek Fisheries sector, safeguard biodiversity in Greek seas and protect the consumers.
Keywords: *Mitochondrial DNA, barcoding, PCR, Pagrus pagrus, authenticity*
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1. Introduction

The red porgy is a benthopelagic species widely traded in the Greek market. Its wild populations are located the Mediterranean Sea, but also in the Atlantic coasts of America, South Europe and Africa (Fishbase). According to the "Red Book of Endangered Animals of Greece" has been described as an "endangered" species for International / Mediterranean waters, while the populations in the Greek seas have not been assessed (Legakis & Maragos 2009). Its high commercial value raises as its size increases. European regulations set the minimum size (total length) of its catch in the Mediterranean at 18 cm. Wild *Pagrus pagrus* from fisheries in Greece shows similar morphological features (pink color with silver reflections, lighter in the abdomen, while its head may appear darker, as well as the axes of the pectoral fins) with the corresponding imported, and closely-related species of Sparidae, as the common pandora *Pagellus erythrinus*, and the pink dentex *Dentex gibbosus*. During the previous years, due to increased consumer demand, many fish farms have included the genus *Pagrus* in their production. Specifically, the red porgy covers 50% of the production of "new species", which in total constitute 3% of fish production in Greece, with an expected increase in production in the coming years (FGM 2019). In the Greek market the genus *Pagrus* is sold either as frozen or as fresh, therefore it can be Greek or imported. Usually, the fresh products come either from domestic catch or from the Greek fish farms. In the same genus, worldwide, there are other species with similar morphological characteristics, such as the Japanese porgy (*Pagrus major*), the blue spotted seabream (*Pagrus caeruleostictus*), the red banded seabream (*Pagrus auriga*) and the silver seabream (*Pagrus auratus*). *Pagrus* fish farming products sold in the Greek market are found under various names, with the majority reported as *Pagrus major*. The trading of wild, reared, or imported fish in the market, raises the question of authenticity and proper labeling of *Pagrus* products. The higher commercial value of wild *Pagrus pagrus*, compared to imported or cultured counterparts, but also the need for consumer awareness, raises the need for correct and accurate identification of the species. The strong similarities within the representatives of this family, mainly the color and the shape, result in the mixing of the fish and their sale under the same name (Cocolin *et al*. 2000). For example, in the German market it has been observed that the *Pagrus pagrus, Pagrus caeruleostictus* and *Pagrus auratus* are commonly sold as "Dorade Rose" (Schiefenhöve & Rehbein 2013). Although fish can be classified by their morphological characteristics or by protein-based techniques, if a sample has been processed, damaged, or cooked, specialists may not be able to identify the sample and proteins have been denatured. Molecular techniques based on genetic material (DNA) are currently being used for accurate and robust species identification, to detect possible fraud in fishery products of any kind. Having the ability to cross-check the sequences with databanks, the molecular identity of each species is determined, therefore the genetic authenticity and origin of each sample can be assessed (Friedheim 2016). The advantage of these methods is that they can identify samples of any form: raw, cooked, processed, frozen. The mitochondrial DNA (mtDNA) is used regularly for investigating molecular mechanisms and evolutionary relations between different fish species (Ceruso *et al*. 2019). A very common approach is DNA barcoding, where the sequence of part of mitochondrial cytochrome c oxidase I (COI) gene can is used to uniquely identify the species (Ratnasingham & Hebert 2007), in the same way that a supermarket scanner uses the familiar black stripes of the UPC barcode to identify an item against its reference database. However, for the discrimination between closely related species or populations within species, data could also be drawn from other mtDNA regions (*cytb*, 16sRNA, D-loop etc.) or even the total mt genome analysis. Up to date, the complete mitochondrial genome of *P. major*, *P. auriga* and *P. caeruleostictus* have been determined. In this study, the complete mtDNA of *Pagrus pagrus*, captured in the central Aegean Sea, was sequenced. Data inferred from this sequence along with barcoding of samples collected in the Greek market (locally fished, reared, imported) were used in different molecular techniques (DNA barcoding, FINS, PCR-RFLP), in order to establish a fast and reliable method for species identification within the *Pagrus* genus and for estimation of population differentiation within wild *Pagrus pagrus*.

2. Materials and Methods

The specimens tested included wild *P. pagrus* (80 samples) caught in the Greek seas, FAO subareas 37.2 (Ionian Sea division 37.2.2) and 37.3 (Aegean and Cretan Seas), farmed *Pagrus* (15 samples from various Greek fish farms), *Pagrus* caught in lagoon fish traps (3 samples) and imported (labelled as *P. pagrus*), purchased from fish markets and supermarkets (10 samples). Fish species were identified according to their morphological characteristics and categorized in groups by their origin. The difference between *Pagrus pagrus* and *Pagrus major* is the absence of white line at the end of the ureter fin in the latter (Merentitis, Organic Life). Regarding species of the same family but of a different genus, the main characteristic of *Dentex gibbosus* is that the first two thorns are low, but the third and fourth are very tall, a feature that sets it apart from other species. Between *Pagrus* spp. and *Pagellus erythrinus* the differences are found in body shape, colour and lateral fins (Figure 1).

Figure 1. Morphological characteristics of *Pagrus* spp., *Dentex gibbosus*, and *Pagellus erythrinus*.

Total DNA was extracted from the skeletal muscle or liver using NucleoSpin® Tissue Macherey-Nagel, according to the manufacturer's instructions. For the determination of total mtDNA sequence, a specimen caught at Central Aegean FAO division 37.3.1 was selected. For the amplification mtDNA, primers were either designed using EUROFINS Oligo Analysis Tool or selected from Miya & Nishida (2000). Total mtDNA was amplified in 42 overlapping fragments ranging from 300-4.500 bp and sequenced in both strands. For barcoding, a fragment of 648 bp of COI was amplified using universal primers (Ward *et al*. 2005). In order to distinguish *P. pagrus* populations, Forensically Informative Nucleotide Sequencing (FINS) was carried out for cytochrome b gene. A 583 bp fragment was amplified using primers designed in this study. Sequences were identified with

Blast search tool, compared and aligned with sequences from BOLD database and aligned with clustalW2 (Sievers *et al*. 2011). After in silico analysis of complete mitochondrial genomes, COI and *cytb* genes, 10 fragments were chosen for Restriction Fragment Length Polymorphism analysis (PCR-PCR-RFLP) using a set of 10 restriction enzymes. Smaller fragments of COI (100-300 bp) were used in multiplex PCR experiments (qPCR) and High-Resolution Melting (HRM) analysis.

3. Results

The complete mtDNA of *Pagrus pagrus* has a size of approximately 17 kb and revealed the same structure as all mtDNAs of teleosts containing: 13 protein, 22 tRNA, 2 rRNA genes and two non-coding regions D-loop and L-origin of replication (Figure 2). Gene arrangement was typical of Sparidae species, with most of the genes encoded by the heavy strand, except for the NADH dehydrogenase subunit 6 (ND6) and eight tRNAs that are encoded by the light strand.

Figure 2. Gene organization of *Pagrus pagrus* mtDNA.

The degree of similarity of closely related species was estimated by complete mitochondrial genomes alignment with CLUSTALW. Sequence homology is reflected in the phylogenetic tree of Figure 3, where the highest degree of similarity of *Pagrus pagrus* with *Pagellus erythrinus* is evident.

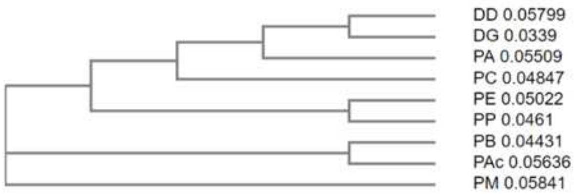


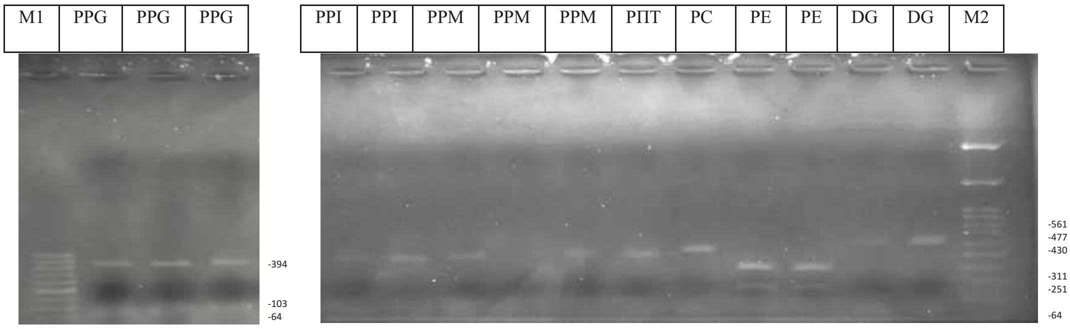
Figure 3. Neighbor-Joining tree without distance correction for species comparisons. PA: *Pagrus auriga* (AB124801.1, PC*: Pagrus caeruleostictus* (MN319701.1, PP: *Pagrus pagrus*, PE: *Pagellus erythrinus* (NC_037732.1, PM: *Pagrus major* (AP002949.1) , DG: *Dentex gibbosus* (NC_037731.1,) , DD: *Dentex dentex* (NC_037755.1) PG: *Pagellus bogaraveao* (NC_009502.1), PAc: *Pagellus acarne* (NC_037505.1).

DNA barcoding method was used to characterize *Pagrus pagrus* caught in Greek seas with a genetic ID. The results showed that there is a clear distinction between wild specimens, imported and reared ones. According to homology in FishBol, all specimens from fisheries were identified as *P. pagrus* presenting the same sequence regardless their geographical origin. All specimens from Greek fish farms as well as *Pagrus* caught in lagoon fish traps (the so-called "divaria") were identified *as Pagrus major*. Specimens of imported *Pagrus* were identified as *Pagrus pagrus* with characteristic homologies with sequences in Genbank, from the Atlantic Ocean with the exception of one sample that was identified as *Pagrus caeruleostictus.* In 6 cases where morphological identification was not possible, DNA barcoding identified 3 of them as *Pagrus pagrus* while 2 as *Dentex gibbosus* and 1 as *Pagrus major*. In addition, for 10 samples of juvenile fish there were doubts about the classification, based on their morphological characteristics, as they appeared like *Pagellus erythrinus* while they were sold as *Pagrus pagrus.* Barcoding confirmed that they were *Pagellus erythrinus*. Sequences (561 bp), of the same samples that were analyzed for *cytb* (FINS), where in accordance with findings of COI, and revealed a smaller region which was more indicative, excibiting many polymorphisms, being a candidate for distinguishing between *Pagrus pagrus* populations. More samples need to be examined, in order to confirm this finding.

Results from PCR-RFLP analysis for COI barcode digested with HindIII and Sau3AI, showed a pattern that could distinguish *P. pagrus* from *P. major, P. caeruleostictus, Pagellus erythrinus, D. gibbosus* but not between Greek and imported *P. pagrus*. When part of *cyt b* (561 bp) digested with Sau3AI (Figure 4A) and a longer fragment L10 (1243 bp) containing D-loop digested with XbaI, were used, a unique band pattern for *Pagrus pagrus* caught in the Greek seas was obtained (Figure 4B). Preliminary results of multiplex PCR and HRM analyses from regions of COI, and *cytb* (data not shown), are indicative and promising for fish species and population identification. Preliminary results of multiplex PCR using three pairs of primers designed for COI, showed different band patterns between Pagrus species and other Sparidae species as shown in Figure 5.

Moreover, a COI fragment of 120 bp was used in HRM experiments (data not shown) could distinguish between *Pagrus pagrus* and other species.

A.



B.

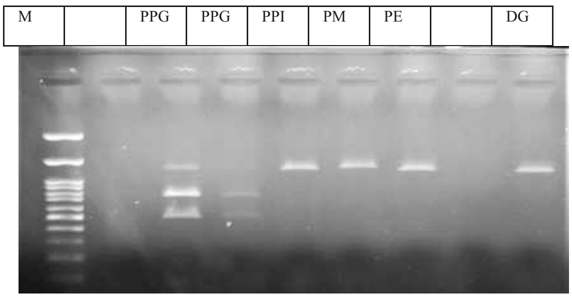


Figure 4. PCR-RFLP analysis of mtDNA regions. A: partial *cytb* (561 bp) with Sau3AI, B: fragment L10 (12430 bp) digested with Xba. PC*: Pagrus caeruleostictus* PP: *Pagrus pagrus*, PM: *Pagrus major,* PE: *Pagellus erythrinus,* DG: *Dentex gibbosus*, PPG: fish caught in Greek seas, PPI: imported *Pagrus pagrus,* PPT: *Pagrus* caught in fish traps of lagoons M1: marker 50 bp ladder, M2: marker 100 bp ladder.

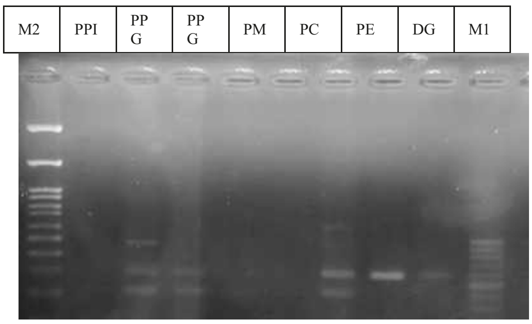


Figure 5. Multiplex PCR for COI. PC*: Pagrus caeruleostictus* PP: *Pagrus pagrus*, PM: *Pagrus major,* PE: *Pagellus erythrinus,* DG: *Dentex gibbosus*, PPG: fish caught in Greek seas, PPI: imported *Pagrus pagrus,* PPT: *Pagrus* caught in fish traps of lagoons M1: marker 50 bp ladder, M2: marker 100 bp ladder.

4. Discussion

The results of the phylogenetic analysis of the complete mtDNA proves the close relationship between the species *Pagrus pagrus* and *Pagellus erythrinus*, despite their systematic classification. On the contrary the distance between *Pagrus pagrus* and *Pagrus major*, is remarkable. The *Pagrus pagrus* DNA barcodes collected from fish caught in Greek seas show a predominant sequence, but also a polymorphism that is extremely similar to sequences from fished individuals in Turkey. This result is expected, due to the proximity of fishing areas and the lack of borders at sea, which allows the movement and mixing of populations. Moreover, there is a high level of similarity (98–99%) with barcodes of fish caught across the Mediterranean, a result that is expected taking into consideration that it is a sea with boundaries and the populations are in proximity compared to the Atlantic Ocean. On the other hand, comparison of sequences from Atlantic populations, either on the American or African side, gives us notable differences. The species *Pagrus pagrus* is not found in Asia and the Pacific, which is why it differs so strongly from other species of the genus *Pagrus*. DNA sequencing techniques provide the highest accuracy and species can be directly identified (Landi *et al.* 2014). However, they are time consuming, specially trained scientific staff is required and they have a relatively high cost per sample. It is therefore difficult to apply them to systematic market controls, where a large number of samples will need to be collected and rapidly analyzed. PCR techniques (PCR-RFLPs, multiplex PCR, HRM) shown in this study aim to a simpler, and more rapid technique, which will enable the detection of fraud with high resolution and low cost.

Fisheries plays an important socioeconomic role in Greece, providing the market with products of high nutritional value. Due the high price of red porgies, it is important to conserve and validate the value of these products. The detection of *P. major* as a common species in Greek porgy aquaculture today raises the questions about the environmental impact from the rearing of an alien species (escapes, mixes with wild populations) in the Mediterranean and authenticity issues as described in the Regulation (EU) No 1379/2013. In order to enable consumers to make informed choices, it is necessary to provide clear and comprehensive information on, inter alia, the origin and the method of production of the products. *P. pagrus*, fished in Greek seas show similar morphological features with the corresponding imported, farmed fish of the same genus and closely-related species of Sparidae. Furthermore, in case the fish has been processed (exfoliated, fillet or cooked), even classification experts are unable to identify the sample. Data of this study, will ensure the identity, originality and authenticity of Greek fishery products. Moreover, extended sequence data are already being used for the development of a methodology (qPCR, HRM) that will provide a rapid tool against fish mislabeling and phenomena of fish fraud.

Acknowledgements

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References

Ceruso M., Mascolo C., Anastasio A., Pepe T. Sordino P. (2019). Frauds and fish species authentication: Study of the complete mitochondrial genome of some Sparidae to provide specific barcode markers. Food Control 103, 36–47.

Cocolin, L., D'Agaro, E., Manzano, M., Lanari, D., Comi, G. (2000). Rapid PCR-RFLP method for the identification of marine fish fillets (seabass, seabream, umbrine, and dentex), Journal of Food Science 65, 1315–1317.

Eurofins Genomics (no date) Oligo Analysis Tool. https://eurofinsgenomics.com/en/resources/design-tools/oligo-analysis/.

Federation of Greek Maricultures (2019). Greek Aquaculture. https://www.fgm.com.gr/uploads/file/FGM_19_GR_WEB _Spreads(4).pdf

Friedheim S. (2016). Comparison of Species Identification Methods: DNA Barcoding versus Morphological Taxonomy Comparison of Species Identification Methods, Mānoa Horizons 1, 74–86.

Landi M., Dimech M., Arculeo M., Biondo G., Martins R., Carneiro M., Carvalho G.R., Lo Brutto S., Costa F.O. (2014). DNA barcoding for species assignment: The case of Mediterranean marine fishes. PLoS ONE 9(9), e106135.

Miya M. Nishida M. (2000). Use of Mitogenomic Information in Teleostean molecular Phylogenetics: A Tree-Based Exploration under the Maximum-Parsimony Optimality Criterion. Molecular Phylogenetics and Evolution 17, 437–455.

Organic Life. https://www.organiclife.gr/el/diatrofi/7790-i-megali-apati-poulane-gia-fagri-iaponiki-tsipoura-ixthiotrofeiou-me-xrostikes.html

Ratnasingham S. Hebert P.D.N. (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7, 355–364.

Schiefenhövel K. Rehbein H. (2013). Differentiation of Sparidae species by DNA sequence analysis, PCR-SSCP and IEF of sarcoplasmic proteins. Food Chemistry 138, 154–160.

Sievers F., *et al*. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology 7, 539.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 360, 1847–1857.

Legakis A. Maragos P. (2009). The Red Book of Endangered Animals of Greece. Athens (Greece): Greek Zoological Society, p. 528.

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NUMERICAL SIMULATION OF HYDRODYNAMIC PARAMETERS OVER RIVER CONTROL STRUCTURES

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Abstract
Fish habitat suitability in natural rivers is a very complex phenomenon related to ecological, biological, hydrodynamic and topographic conditions that are necessary to be accurately simulated. Estimation of basic hydraulic parameters, such as velocity, water depth and energy attributes of river channels is essential for an accurate modeling of biological processes. In rivers different hydraulic structures are widely used to control water depth, to manage flow discharge and to retain specified flow conditions. The objective of the current research work is to apply the Flow-3D finite-volume model in order to numerically investigate the effects of a broad-crested weir construction in river hydrodynamic conditions. The three-dimensional Reynolds-averaged Navier-Stokes equations were solved in order to simulate water depth and velocity variation along an experimental channel, over a flat-crested structure. Numerical results of water depth variation were compared with available experimental measurements to evaluate the ability of the simulation procedure in estimating hydrodynamic parameters distribution that affect inland watersheds and natural river ecosystems. In Addition, useful flow energy distribution results were derived and the proposed numerical procedure results to a substantial methodology for river ecosystem management.
Key words: *River, Hydrodynamic Conditions, CFD model*
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1. Introduction

River hydraulic control structures and especially overflowed structures like weirs are commonly used in natural rivers for raising upstream water depth, controlling flow discharge and creating sufficient conditions in order to retain ecological consistency. Broad-crested weirs can be a foundation for an upstream ramp development area, protected against erosion due to lower flow velocities, so that fish and other aquatic species can live. Free-surface variation along those constructions is an important issue for an environmental management of river ecosystems, related to upstream hydrodynamic conditions, stream inflow discharges and to several downstream factors such as water level associated with downstream river slope and river roughness conditions.

In the past years, different methods have been applied to investigate hydraulic characteristics for free-surface flows, over weirs in rivers, including analytical, field and laboratory experiments. Gonzalez & Chanson (2007) performed experiments and studied pressure and velocity variation along broad-crested weirs. Farsirotou *et al*. (2004) studied water surface variation along a triangular bottom sill based on laboratory measurements, Salmasi *et al*. (2011) investigated the values of discharge and velocity coefficients in different weir geometries and Farsirotou & Kotsopoulos (2015) conducted laboratory experiments on free-surface flow variation over a broad-crested weir construction. The impact of bed sills geometry on the river bed and bank scouring process was also investigated with laboratory measurements by Sohrabi *et al*. (2019). Moreover, numerical simulation procedures of free-surface variation in natural rivers were developed using different computational methods as a result of computers' processing ability improvement. Numerical models to simulate free-surface flow over different hydraulic constructions were developed by Morales *et al*. (2012), Daneshfaraz & Ghaderi (2017), Darvishi et al. (2017), Xu & Jin (2017), Říha et al. (2019) and Farsirotou et al. (2020). Computational Fluid Dynamics (CFD) three-dimensional models were also applied due to their ability to simulate fluid flow problems, water surface and any other hydraulic flow characteristics in complex geometries, for example by Kim & Park (2005), Shen & Diplas (2008), Moradinejad *et al*. (2015) and Velísková *et al*. (2018).

In the current research work the Flow-3D CFD model was used for free-surface simulation along an open channel with a weir construction using the Volume of Fluid method. Reynold's Averaged Navier-Stokes solver based on a structured orthogonal computational mesh was applied for the numerical simulation procedure. Calibration and verification of the computational methodology was performed using available laboratory experiments, through a broad-crested weir, conducted by Farsirotou & Kotsopoulos (2015). The validated three-dimensional numerical model can be highly applicable in investigating hydrodynamic properties in inland aquafers and can assess flow behavior in physical models

2. Materials and Methods

Numerical Implementation

Firstly, a model of the laboratory channel with a broad-crested weir was conducted in Autocad 3D and entered in the Flow 3D software. The computational fluid dynamics program Flow-3D was used in order to simulate numerical solution of the Reynolds-averaged Navier-Stokes equations which are discretized and solved for each computational cell. Finite volume approximations were used to discrete the computational domain (Flow Science, 2018).

2.1 Governing hydrodynamic equations in Flow-3D model

The general mass continuity equation at three-dimensional Cartesian coordinates is:

$$V_F\frac{\partial\rho}{\partial t}+\frac{\partial}{\partial x}(\rho u A_x)+\frac{\partial}{\partial y}(\rho v A_y)+\frac{\partial}{\partial z}(\rho w A_z)= R_{DIF}+R_{SOR} \quad (1)$$

where ρ is the fluid density, u, v, w are velocity components in the coordinate directions x, y, z, respectively, A_x, A_y, A_z are fractional areas open to flow in the x, y, z directions, respectively and V_F is the fractional volume open to flow, related to the volume of fluid, defined as:

$$\frac{\partial F}{\partial t}+\frac{1}{V_F}\left[\frac{\partial}{\partial x}(FA_x u)+\frac{\partial}{\partial y}(FA_y v)+\frac{\partial}{\partial z}(FA_z w)\right]=0 \quad (2)$$

where F (x, y, z, t) is the volume of fluid function which represents the volume of fluid per unit volume.

The volume of fraction V_F represents the amount of fluid in each computational grid point and takes values between 0, if there is no fluid, and 1, if it is totally occupied by fluid. The Volume of Fluid (VOF) method based on the volume of fraction of the computational cells is used for the numerical simulation of free surface profile and consists of three main components, the definition of the volume of fluid fraction, the numerical solution of the VOF transport equation (Equation 2) and the boundary conditions at the free surface. R_{SOR} is a density source term to model mass injection, for example through porous obstacle surfaces and R_{DIF} is the turbulent diffusion term, for turbulent mixing processes in fluids having a non-uniform density given as:

$$R_{DIF}=\frac{\partial}{\partial x}\left(u_p A_x\frac{\partial\rho}{\partial x}\right)+\frac{\partial}{\partial y}\left(u_p A_y\frac{\partial\rho}{\partial y}\right)+\frac{\partial}{\partial z}\left(u_p A_z\frac{\partial\rho}{\partial z}\right) \quad (3)$$

where the diffusion coefficient $u_p = c_p\mu/\rho$, with μ the coefficient momentum diffusion and c_p is a constant called turbulent Schmidt number. This term of mass diffusion makes sense only for turbulent mixing processes in fluids having non-uniform density. For incompressible fluids, where fluid density is stable, the mass continuity equation results as:

$$\frac{\partial}{\partial x}(uA_x)+\frac{\partial}{\partial y}(vA_y)+\frac{\partial}{\partial z}(wA_z)= 0 \quad (4)$$

The three-dimensional Navier-Stokes equations of motion for fluid velocity components (u, v, w) are:

$$\frac{\partial u}{\partial t}+\frac{1}{V_F}\left(uA_x\frac{\partial u}{\partial x}+vA_y\frac{\partial u}{\partial y}+wA_z\frac{\partial u}{\partial z}\right)=-\frac{1}{\rho}\frac{\partial p}{\partial x}+G_x+f_x-b_x-\frac{R_{SOR}}{\rho V_F}(u-u_w-u_s) \quad (5)$$

$$\frac{\partial v}{\partial t}+\frac{1}{V_F}\left(uA_x\frac{\partial v}{\partial x}+vA_y\frac{\partial v}{\partial y}+wA_z\frac{\partial v}{\partial z}\right)=-\frac{1}{\rho}\frac{\partial p}{\partial y}+G_y+f_y-b_y-\frac{R_{SOR}}{\rho V_F}(v-v_w-v_s) \quad (6)$$

$$\frac{\partial w}{\partial t}+\frac{1}{V_F}\left(uA_x\frac{\partial w}{\partial x}+vA_y\frac{\partial w}{\partial y}+wA_z\frac{\partial w}{\partial z}\right)=-\frac{1}{\rho}\frac{\partial p}{\partial z}+G_z+f_z-b_z-\frac{R_{SOR}}{\rho V_F}(w-w_w-w_s) \quad (7)$$

where p is the fluid pressure, G_x, G_y, G_z is the acceleration created by body fluids, f_x, f_y, f_z is viscosity acceleration in the three dimensions, b_x, b_y, b_z are flow losses in porous media or across porous baffle plates, U_w= (u_w, v_w, w_w) is the velocity of a source component and U_s = (u_s, v_s, w_s) is the velocity of the fluid at the source relative to the source itself and this term is zero when the source is of the stagnation pressure type and not of the static pressure type.

2.2 Numerical Approximations

FLOW-3D Computational Fluid Dynamics model numerically solves Navier Stokes equations (1), (5), (6) and (7) using finite-difference or finite-volume approximations. Fixed rectangular cells compose the computational mesh into the simulated flow region area. Local average values of all hydraulic dependent variables are associated with each computational cell. All variables are located at the centers of the cells except for velocities which are located at cell-faces (staggered grid arrangement). A typical computational cell is presented in Figure 1 where u, v, w velocities and fractional areas A_x, A_y, A_z are located at the centers of cell-faces normal to x, y, z directions, respectively, while pressures (p), volume of fractions (V_F), densities (ρ), internal energy (I), turbulence quantities for energy (q), dissipation (D) and viscosity (μ) are located at computational cell centers.

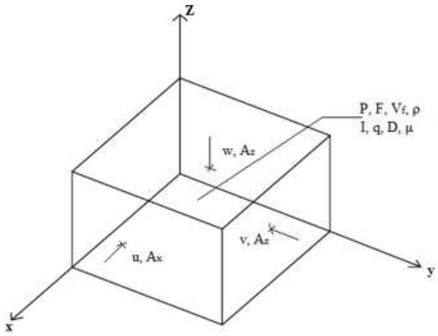


Figure 1: Location of variables in a typical mesh cell.

2.3 Steps of numerical simulation

The basic steps in order to perform an accurate numerical simulation with the Flow-3D model include: geometry preparation through Autocad 3D software, defining the problem and the equations to be solved, defining fluid characteristics, boundary and initial conditions in the flow field, computational mesh, output parameters, convergence criteria, calculation method, simulation performance, display results, results validation according to available data.

3. Results

To verify the credibility and the accuracy of the applied numerical simulation procedure, available experimental measurements of water depth variation through a broad-crested weir along a laboratory open channel, under equilibrium flow conditions, were used. A broad-crested weir is a flat crested structure with a length of crest large enough compares to the flow thickness over the crest of the weir and pressure distribution above the crest is hydrostatic with the critical flow depth observed on the weir crest. A schematic view of the rectangular broad-crested weir is presented in Figure 2 and the hydrodynamic conditions, upstream and downstream of the weir were analytically presented by Farsirotou & Kotsopoulos (2015). Laminar flow conditions were developed for all laboratory experimental simulations. A three-dimensional numerical simulation of water surface variation over the flow control structure was performed, for different inflow conditions, inflow discharges, under steady flow conditions. Numerical predictions of water surface profiles along the flow direction were adequately compared with experimental measurements and comparisons for three different inflow discharges, Q, are given in Figure 3. A preliminary assessment of k-ε and k-ε RNG turbulence models (Flow Science, 2018) was applied and simulated results provide the ability of the CFD to simulate both laminar or turbulence flow conditions. Flow depth, h(m) and water velocity, V(m/s) distribution along the flow channel and over the broad-crested weir are presented in Figures 4(a) and (b), respectively, for the maximum experimental inflow discharge equal to 0.0009(m^3/s). Taking into account the results from a CFD simulation, such control structures should be carefully designed to minimize their impact on the aquatic environment. Considering the inherent flow depth and velocity distribution, low velocity regions, produced upstream from the weir, may be significant enough to sustain healthy populations. Moreover, Froude number, Fr=V/(gh)^0.5, distribution is presented in Figure 4(c) providing useful results of flow type variation. Lower energy environments, where flow depth is higher and flow regime is subcritical are developed upstream from the broad-crested weir and produce natural hydraulic conditions. At the downstream area supercritical flow conditions are observed with higher energy conditions.

Figure 2: Laboratory equipment of the broad-crested weir and free-surface flow presentation.

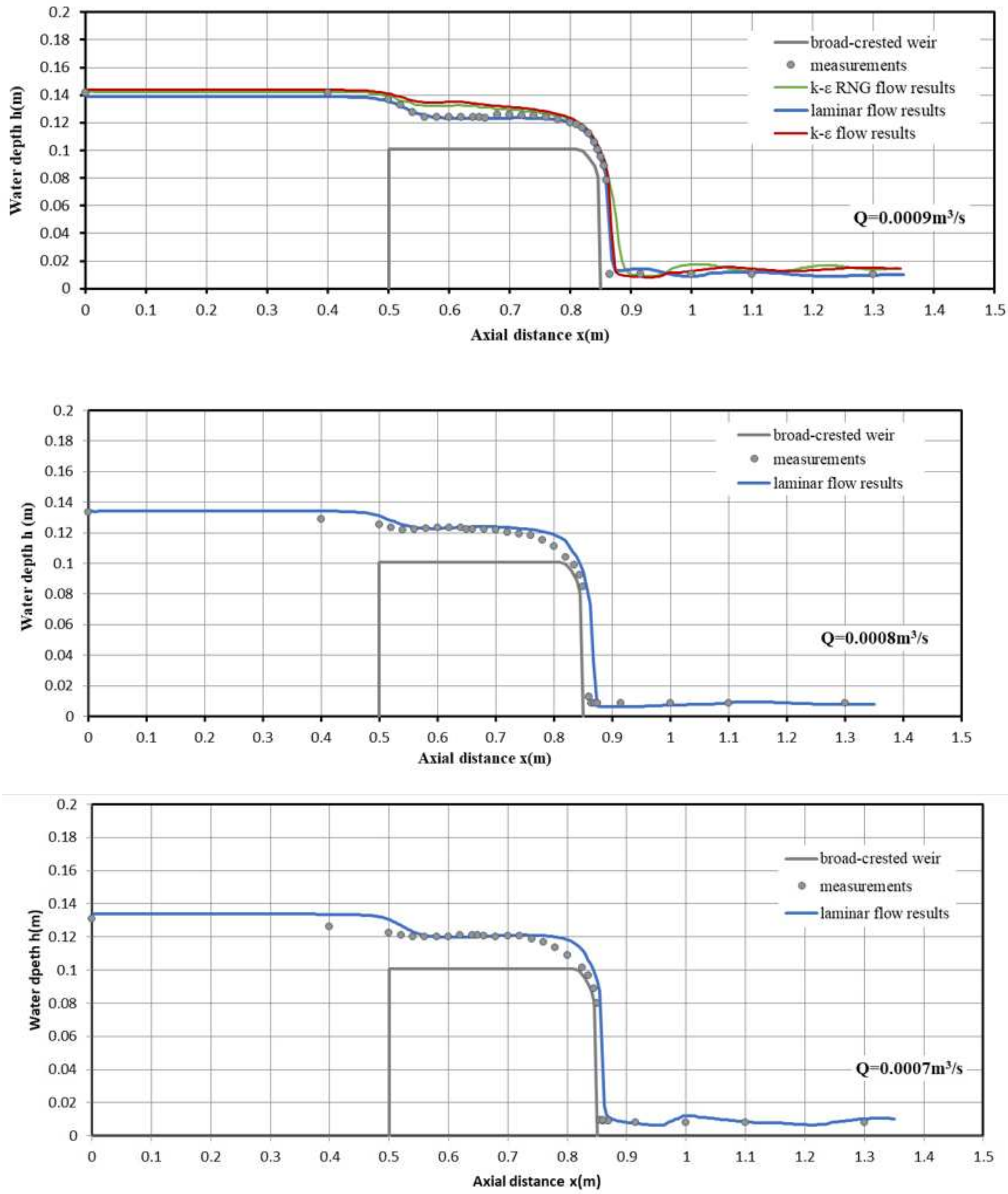
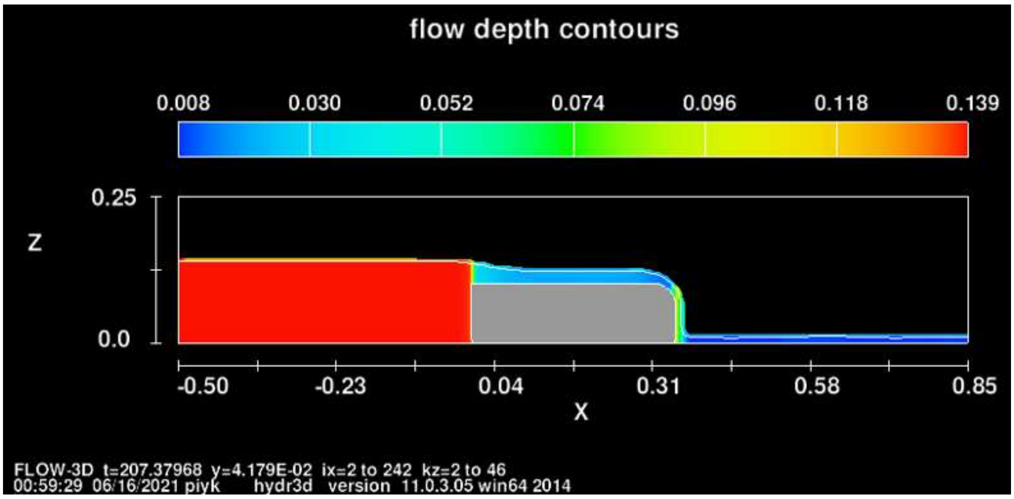
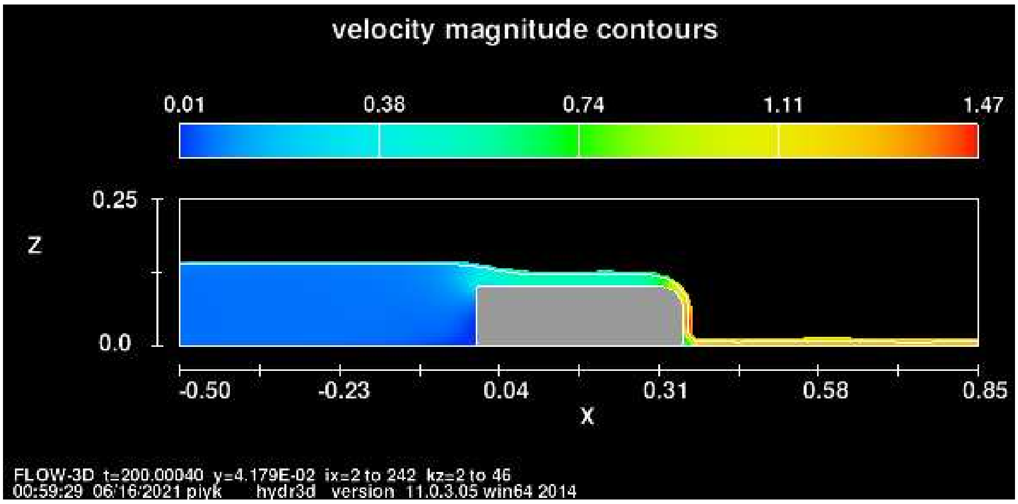


Figure 3: Comparison between numerical and experimental water depth variation over the weir.



(a)



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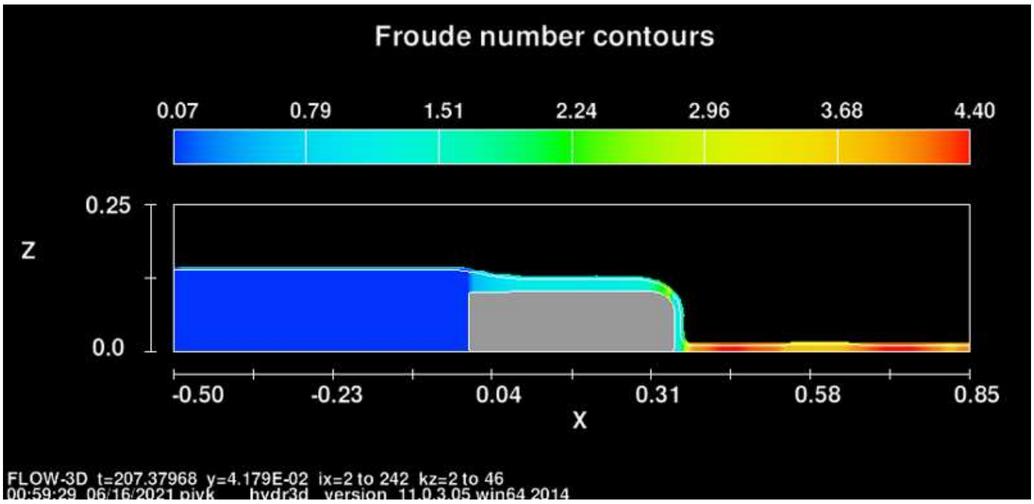

(c)

Figure 4: Numerical results of flow depth, velocity and Froude number distribution along the channel.

Conclusions

In this research, numerical analysis of the effect of a broad-crested weir on the hydraulic characteristics of an open channel flow, such as water surface profile, using the FLOW3D model was investigated. Numerical results were validated with available experimental measurements and the three-dimensional model can be used to predict water depth variation and provide useful estimation of river flow patterns including essential hydrodynamic parameters such as velocity, water depth, Froude number and total hydraulic head. Certain laboratory measurements and visualizations can be adequately predicted with numerical procedures. The FLOW-3D CFD software was able to accurately simulate variations of different target hydrodynamic parameters that affect inland watersheds and natural river ecosystems. Flow energy distribution results were also derived and the proposed numerical procedure results to a substantial methodology for environmental management of rivers that can be extended to turbulent flow conditions with sediment transport and river bed level variation.

References

Daneshfaraz R., Ghaderi A. (2017). Numerical investigation of inverse curvature ogee spillway, Civil Engineering Journal, 3(11), 1146-1156.
Darvishi, E., Fenton, D., Kouchakzadeh, S. (2017). Boussinesq equations for flows over steep slopes and structures. Journal of Hydraulic Research, 55 (3), 324–337.
Farsirotou E., Kotsopoulos S., Xafoulis N., Sanatsios G. (2004). Experimental investigation of non-uniform flow in rivers. Proceeding of the 12th International Conference on Protection and Restoration of the Environment, 873-880.
Farsirotou E., Kotsopoulos S. (2015). Free-surface flow over river bottom sill: experimental and numerical study. Journal of Environmental Process, 2, S133-S139.
Farsirotou E., Panagiotopoulos A., Soulis J. (2020). Explicit and implicit finite-volume methods for depth averaged free-surface flows. Journal of Applied and Computational Mechanics, 6 (SI), 1270-1282.
Flow Science Inc, (2018). FLOW-3D® Version 12.0 User's Manual (2018). Santa Fe, NM: https://www.flow3d.com
Gonzalez C., Chanson H. (2007). Experimental measurements of velocity and pressure distributions on large broad-crested weirs. Flow Measurement and Instrumentation, 18, 107-113.
Kim D., Park J., (2005). Analysis of flow structure over ogee-spillway in consideration of scale and roughness effects by using CFD model. Ksce Journal of Civil Engineering, 9 (2), 161-169.
Moradinejad A., Parssai A., Noriemamzade M. (2015). Numerical modeling of flow pattern in Kamal Saleh dam spillway approach channel. Applied Science Reports 10 (2), 82-89.
Morales V., Talia T., Garcia M. (2012). Numerical modeling of ogee crest spillway and tainter gate structure of a diversion dam on canar river. Ecuador, XIX International Conference on Water Resources.
Říha J., Duchan D., Zachoval Z., Erpicum S., Archambeau P., Pirotton M., Dewals B. (2019). Performance of a shallow-water model for simulating flow over trapezoidal broad-crested weirs. Journal of Hydrology and Hydromechanics, 67, 322–328.
Salmasi F., Poorescandar S., Dalir A., Zadeh D. (2011). Discharge relations for rectangular broad-crested weirs. Journal of Agricultural Sciences 17, 324-336.
Shen Y., Diplas P. (2008). Application of two- and three-dimensional computational fluid dynamics models to complex ecological stream flows. Journal of Hydrology, 348, 195–214.
Sohrabi M., Keshavarzi A., Javan M. (2019). Impact of bed sill shapes on scour protection in river bed and banks. International Journal of River Basin Management, 17 (3), 277-287.
Velísková Y., Chára Z., Schügerl R., Dulovičová R. (2018). Velocity profile deformation by flooded obstacle in free surface flow. Journal of Hydrology and Hydromechanics, 66, 448–456.
Xu T., Jin Y. (2017). Numerical study of the flow over broad-crested weirs by a mesh-free method. Journal of Irrigation and Drainage Engineering, 143 (9): 04017034.

RIVER INTEGRITY ASSESSMENT BASED ON HABITAT QUALITY AND THE MACROINVERTEBRATE COMMUNITY

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Abstract
High anthropogenic impact on the aquatic ecosystem of the Zapadna Morava River (Serbia) gave rise not only to a deterioration of water quality, but also to the abundance of the allochthonous fauna representatives. A brief field survey using the Index of Habitat Integrity methodology (IHI) was conducted along three river sections in order to identify the level of habitat modification and degradation. How established river fauna reacts to present changes? This study showed that habitat conditions along the investigated sector of the Zapadna Morava River were moderately modified without significant impact on aquatic macroinvertebrates diversity and functionality.
Keywords*: habitat modification, impact, aquatic macroinvertebrate, large river*
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1. Introduction

The Zapadna Morava River is the left tributary of the Velika Morava River. It arises at the confluence of Golijska Moravica and Đetinja rivers near Požega. The river bed in its upper and middle course is rocky and pebbly, while muddy substrate dominates in the reservoirs and downstream course (Markovic & Simovic 1994). The catchment area of the Zapadna Morava river basin is 15,754 km^2 with river length of 308 km. Hydroelectric power plants "Ovčar Banja" and "Međuvršje" were constructed on the river, whose reservoirs are almost completely covered with fine sediment. The ecological quality of the Zapadna Morava River is assessed as moderate (III class) (Novaković 2013).

Zapadna Morava belongs to Type 2 watercourses, according to the valid regulation (Official Gazette of RS 74/2011), large rivers with medium grain-size mineral substrates. The Zapadna Morava River basin is exposed to pollution of different origin: organic (biodegradable waste waters) and inorganic (suspended sediment, tailings leaching, and chemicals), and very often there is a combination of organic and inorganic waste with industrial or communal pollution (Marković *et al.* 2000). Illegal landfills and communal wastewaters are also becoming more common. By releasing water from agricultural land polluted with fertilizers and pesticides, as well as by discharging organic waste from livestock farms, the process of eutrophication (algal bloom) of aquatic ecosystems is intensified. Also, the exploitation of gravel in the coastal part of the Zapadna Morava is a major problem, affecting the degradation of the ecosystem. Hydro-morphological changes, such as regulations of the watercourse, have made this sector suitable for bio-invasions (Djikanović *et al.* 2013).

The Index of Habitat Integrity methodology, described by Kleynhans (1996), was undertaken to verify the habitat classification of the Zapadna Morava River. The IHI methodology rates the riverine habitat by assessing the change of instream and riparian habitat from natural conditions.

The aim of this work was, using the Index of Habitat Integrity methodology, to examine how river habitat modification influenced the biological quality elements, i.e. river fauna composition.

2. Material and methods

A brief field survey of the Zapadna Morava River using the Index of Habitat Integrity methodology (IHI) (Kleynhans 1996) was conducted in October 2020 (Σφάλμα! Λανθασμένη αναφορά σελιδοδείκτη στον εαυτό του.). The IHI methodology was applied along three river sections (Figure 1) in order to investigate the level of habitat modification and degradation. The IHI provides a tool for assessing instream (9 instream channel criteria) and riparian habitat (8 riparian zone criteria) by scoring impacts on the river system under present conditions. The severity of impact of the modifications is based on six categories which comprise of ratings ranging from 0 to 25: where 0 (no impact), 1 to 5 (small impact), 6 to 10 (moderate impact), 11 to 15 (large impact), 16 to 20 (serious impact) and 21 to 25 (critical impact).

A total of 19 sites were investigated. Five sites were sited in Section 1 (Pojate - Kruševac), seven sites in Section 2 (Kruševac -Adrani), and seven sites in Section 3 (Adrani – Preljina) **(**Figure 1**)**. For each sampling site GPS co-ordinates (latitude and longitude) were measured and photographs have been collected.

Figure 1. Map of sampling sites in three investigated sections of the Zapadna Morava River

The samples of benthic macroinvertebrates were collected with standard hand net (mesh size 500 µm) using semi-quantitative Kick and Sweep technique in bank regions (up to a depth of 1.5 m). Samples were collected from all available habitats according to EN 27828 standard, at the same time with brief habitat survey. Specimens were observed in laboratory using the Carl Zeiss, Stemi 2000-C binocular magnifier. The collected individuals were identified using the appropriate identification keys.

The main ecological features of the recorded community (the characterization of the species with regard to saprobic preference, current preference and substrate type) were taken from ASTERICS software Version 4.0.4 (AQEM 2002) and used to estimate the functional groups within the community. Status assessment was carried out according to the national legislation (Official Gazette of the Republic of Serbia 74/2011), based on the class boundaries for rivers Type 2 (large rivers with medium grain-size mineral substrates) using several biotic parameters calculated from AQEM (2002) (saprobic index, BMWP and ASPT score, Diversity index Shannon-Weaner, Total number of taxa, Tubificide and EPT indices).

3. Results and discussion

The field survey results of IHI of the Zapadna Morava River pointed that the three investigated sections were moderately modified habitats belonging to a Category C. Section 3 (Adrani – Preljina) showed the highest integrity (76%) due to the functionality and state of the river, but still remained in a Category C and moderately modified state. The reasons for the better status in this part, compared to sections 2 and 1, were the widening of the riverbed, the quality of the gravel substratum and the meandering nature of the Zapadna Morava River. The riverbanks also retained pockets of dense vegetation, which buffered the run-off from agriculture lands (Table 1). Riverbanks of the Zapadna Morava were heavily modified by agriculture which likely led to increased sedimentation and deposition of the river bed. In addition, gravel mining was also noted instream along the course with a great impact to the river bed.

Macroinvertebrate community sampled in the Section 3 of the Zapadna Morava River stood out by number of identified bottom fauna taxa (47) and by high diversity of Oligochaeta, while in the other two sections the fauna diversity was lower, 27 (Section 2) and 22 (Section 1) (Table 2). Percentage participation of identified macroinvertebrate taxa groups per investigated riverine sections is presented in Figure 2.

According to the ecological classification of the taxa (AQEM 2002), with regard to saprobic conditions (saprobic valence of the species) and taking into consideration all recorded taxa within the investigated Sections, the majority could be considered as tolerant to moderate organic load (in total of 71.58% alpha- and beta-mesosaprobic species in Section 3, 70.07% in Section 2 and 61.97% in Section 1). The larger number of species adapted to high organic load (poly-saprobic) were found in Section 1 (16%), while the highest number of taxa sensitive to organic pollution (xeno-saprobic and oligo-saprobic taxa) were observed in Section 2 (24.72%) (Table 2).

Table 1. IHI evaluated habitats along investigated riverine sections

River Section	Ecological category for river section based on field results (IHI- Kleynhans, 1996)
Section-1: Pojate - Kruševac	C category (68% integrity). The river in this section was moderately modified according to the IHI, based on riverbank land use, rubbish dumping and encroachment of towns and agricultural activities on banks. Instream sand mining was noted. A few allochthonous and invasive macroinvertebrate species were founded in this section. The water level inundation was increased. The riparian habitat was more impacted than the instream habitat which retained in a relatively natural sequence capable of supporting some element of the natural freshwater biotic community.
Section-2: Kruševac -Adrani	C category (69% integrity). The river in this section was moderately modified and in a similar state to river section 1. Industrial activities were noted on the banks in this catchment (associated with a decreased water quality) and some instream activities such as gravel excavation. A large portion of the riparian and wetland areas along the riverbanks have been cleared for agriculture and urban use. The water inundation level was increased. The instream habitat sequence remained largely intact and capable of supporting a natural freshwater biotic community.
Section-3: Adrani-Preljina	C category (76% integrity). The river in this section was moderately modified tending to be predominantly natural, with high self-purification functionality, as consequence of widening of the riverbed, gravel substratum and slowing of the river flow. Along the riverbanks was a zone of dense vegetation, buffering the run-off from agriculture lands. In a few localities instream sand mining activities were noted. Overall, the instream habitat sequence remained largely intact and capable of supporting a largely natural freshwater biotic community.

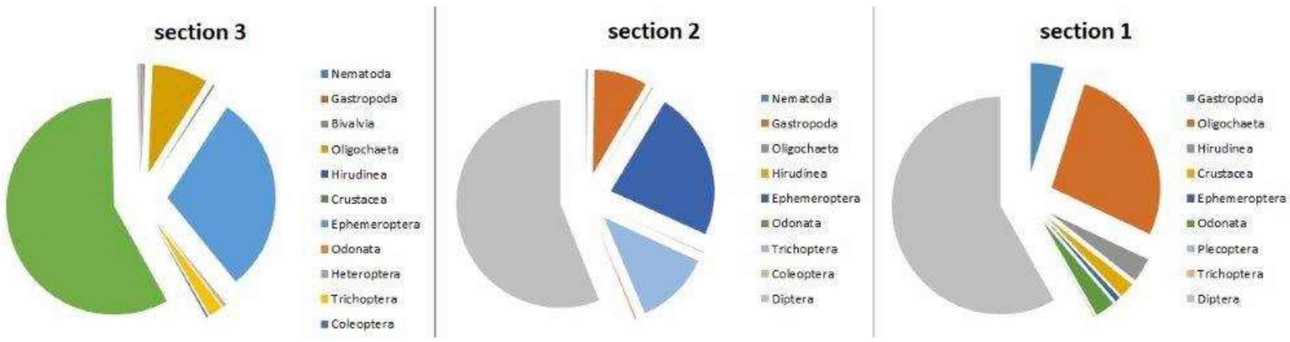

Figure 2. Share of different taxa group in three sections

Regarding current preference, species characterized as limnophilous (together LB, LP and LR type of species, preferring standing waters) had the smallest participation in the macroinvertebrate communities in all three Sections (from 0.42% - Section 2 to 4.78% - Section 1). The recorded communities were characterized by a domination of rheophilous taxa (together RL, RP and RB type of species) from 31.28% - section 3 to 25.42% - Section 2). This type of species prefers fast-flowing streams and lotic zones (Table 2).

Most of the identified species was adapted to bottom substrate types typical of large lowland rivers (substrate types pelal, psammal and argylal) (Table 2).

Based on macroinvertebrate community composition, the ecological quality of sectors 2 and 3 of the Zapadna Morava was assessed as good (II class of Water quality) according to all indices analyzed (saprobic index Zelinka & Marvan, BMWP and ASPT score, Diversity index Shannon-Weaner, Total number of taxa, Tubificide and EPT indices). Section 1 stood out by moderate ecological quality (III water quality class) according to Shannon-Weaner and Tubificid indices and by bad ecological quality (V water quality class) according to presence of EPT taxa (Table 2).

Table 2. AQEM parameters using macroinvertebrate community as biological parameter along riverine sectors

Metrics	section 3	section 2	section 1
Number of Taxa	47	27	22
Saprobic Index (Zelinka & Marvan)	2.082	1.972	2.498
Saprobic Valence [%]			
- xenosaprobic species	0.253	0.527	0.026
- oligosaprobic species	21.51	24.197	18.605
- beta-mesosaprobic species	50.727	49.038	37.557
- alpha-mesosaprobic species	20.849	21.028	24.414
- polysaprobicspecies	2.699	0.025	15.992
- no data available	3.962	5.184	3.405
BMWP Score	132	109	89
Average score per Taxon	5.739	5.737	5.235
Diversity (Shannon-Wiever-Index)	1.877	1.861	1.415
Current preference [%]			
- limnobionts (LB)	0.06	0.084	0
- limnophilous (LP)	0	0	0.065
- limnorheophilous (LR)	2.681	0.334	4.715
- rheolimnophilous (RL)	5.958	4.515	26.13
- Type rheophilous (RP)	22.639	14.716	1.506
- rheobionts (RB)	2.681	6.187	0.262
- Type indifferent (IN)	4.26	11.371	6.483
- no data available	61.722	62.793	60.838
Microhabitat preference [%]			
- pellophilous	5.946	2.726	16.536
- Argyllophilous	0.867	0.602	0.013
- Type Psammophilous	5.034	2.074	10.327
- Type Akalophilous	3.664	3.06	0.635
- Type Litophilous	11.558	16.798	3.268
- Type Phytophilous	7.846	8.445	4.669
- Type Pom detritophilous	3.622	3.135	3.477
- Type Other	0.217	0.033	0.236
- No data available	61.245	63.127	60.838
EPT-Taxa [%]	31.933	34.783	0.917
Tubificidae %	1.39	0.08	27.11

Based on river bank land use and encroachment of towns, agricultural practices and industry a decrease in quality from run off (increased salts, nutrients and pesticides and potentially decreased oxygen concentration) is possible.

Hydro-morphological changes have also caused greater presence of a non-native fish species in the studied area (Djikanovic *et al.* 2013). In particular this had been attributed to deteriorated water quality, regulated river bed, negligence in river bottom exploitation, non-periodical work of the Međuvršje HE plant and turbulent water flow release (Markovic & Veljovic 2005; Markovic & Simic 1994).

Aquatic habitats are, due to their unique features, among the most vulnerable ecosystems. This study showed that habitat conditions along the investigated sector of the Zapadna Morava River were moderately modified without significant impact on aquatic macroinvertebrate community, i.e. diversity and functionality. Hydro-morphological modification caused changes in habitat characteristics and can potentially form suitable habitat for non-native species. Considering that river regulation and habitat modification in the investigated sector of Zapadna Morava River may become more intensive, the impacts may become more severe. All that activities may also cause a disappearance of habitats suitable for some protected aquatic species, like *Astacus astacus* and *Unio crassus.*

Acknowledgments

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References

AQEM (2002). Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. (Contract No: EVK1-CT1999-00027).

ASTERICS Software (2013). ASTERICS - einschließlich Perlodes - (deutsches Bewertungssystem auf Grundlage des Makrozoobenthos) - Software-Handbuch für die deutsche Version. Wageningen.

Djikanović V., Marković G., Skorić S. (2013). New record of *Neogobius fluviatilis* (Pallas, 1814) (Gobiidae) in the Danube River Basin (Serbia). Archive for Biological Science 65 (4), 1469-1472.

EN 27828 (1994). Water quality - Methods for biological sampling – Guidance on hand-net sampling of benthic macro-invertebrates.

Kleynhans C.J. (1996). A qualitative procedure for the assessment of the habitat integrity status of the Luvuvhu River (Limpopo system, South Africa). Journal of Aquatic Ecosystem Health 5, 41-54.

Markovic G., Simovic S. (1994). Ichthyofauna of the middle course of Zapadna Morava as a saprobic indicator. Archives of Biological Science Belgrade 46 (1-2), 39-44.

Markovic G., Veljovic P. (2005). Biotic indices to be used for assessment of ichthyofauna structure of the Zapadna Morava River (West Serbia, the Danube basin). Proc Nat Sci Matica Srpska Novi Sad 109, 29-37.

Marković G., Milovanović Z., Simović S., Vukićević D., Nikolić, D. (2000). Contribution to the development of the cadastre of water pollutants in the Moravica district. [Prilog izradi katastra zagađivača voda Moravičkog okruga]. In. Water resources of the Velika Morava basin and their use. II conference. Andrejević K. (ed.). Kruševac, Andrejević Foundation, Belgrade, 236-238

Novaković B. (2013). Indicative ecological status assessment of the Zapadna Morava River based on aquatic macroinvertebrate community. Water research and management 3 (2), 37–43.

Official Gazzette of RS 74/2011 (2011). Regulation on the parameters of ecological and chemical status of surface waters and parameters of chemical status and quantitative status of groundwaters.

Vicentijevic-Markovic G., Markovic G., Tanaskovic S., Djikanovic V., Skoric S., Nikolic G. (2013). Species newly recorded in Ovcar-Kablar gorge ecosystems. Proceedings of XVIII Conference of Biotechnology, Cacak, 347-352.

IMPLEMENTING MULTIPLE MANAGERIAL AND ENVIRONMENTAL SCENARIOS IN A MEDITERRANEAN LAKE

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Abstract
Ecopath with Ecosim methodology was implemented for the Mediterranean Lake Volvi, to simulate multiple managerial and environmental scenarios, aiming at improving concomitantly the lake's fisheries exploitation and the protection of the ecosystem. These scenarios included the introduction of the alien pumpkinseed (*Lepomis gibbosus*), stocking with European eel (*Anguilla anguilla*) for enhancing fisheries, changes in the fishing effort and in the target species, population increase of Great cormorants (*Phalacrocorax carbo*), etc. In total, nine scenarios were simulated, based on estimated data (by literature and available knowledge) for the period 2015-2030. Scenarios' effects on the biomass of both the functional groups and the fisheries production of the lake are presented. Those revealed that (i) after a high increase that picks around 2025 the biomass of the pumpkinseed would decrease and remain at low levels, (ii) stocking with eels would have nearly no effect on the balance of the lake's food web, but could offer significant economic benefits to professional fishermen, (iii) the cormorants' population and its impact on the commercial fish stocks could be reduced, if the professional fishermen would target mainly European carp (*Cyprinus carpio*) instead of Prussian carp (*Carassius gibelio*), (iv) changes in the fishing effort would have almost no effect in the energy flows and biomasses of the functional groups within the lake's ecosystem, but could cause indirect changes in it (through the increase of piscivorous birds, abandonment of the ecosystem and decrease of its protection) that should not be overlooked.
Keywords: *Aquatic Ecosystem Models, Ecopath with Ecosim, Model Simulations, Lake Volvi, Greece*
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1. Introduction

The implementation of the ecosystem approach, to gain knowledge on the function and, subsequently, on the appropriate management planning of an aquatic ecosystem, is usually driven by societal and scientific needs for assessing and managing the effects of multiple stressors on the different ecosystem's components. For this purpose, the Ecopath with Ecosim (EwE) software package is widely used. The specific software offers great capability for dynamically describing the multiple interactions occurring within a food web (Christensen & Walters 2004). In particular, Ecopath can be used for developing a mass balance model, thus providing a snapshot of an ecosystem, while Ecosim is a time dynamic simulation model enabling the examination of the ecosystems corresponding to different scenarios. Thus, it consists a valuable management tool for both stakeholders and scientists.

Lake Volvi, a large eutrophic lake located in Northern Greece, is the second largest natural lake in Greece. It supports a rich ichthyofauna, with at least 21 native fish species being reported in the lake's catchment (Bobori & Psaltopoulou 2012), thus supporting a long-standing traditional fishery and a quite significant, local fisheries-dependent community (Moutopoulos *et al*. 2020).

In the present study an initial EwE model that was developed for Lake Volvi, based on data of the years 2014-2015 (Moutopoulos *et al*. 2018), was updated with additional data and used for simulating both managerial and environmental scenarios.

2. Material and Methods

Updating the EwE model for Lake Volvi was performed using additional, new information regarding the populations' trends for some functional groups (the model included initially 19 groups, i.e. one group for: phytoplankton, zooplankton, benthic invertebrates, detritus and discards, 11 groups for fish species and three groups of aquatic birds that prey on fish either exclusively, i.e. great cormorants, or occasionally, like pelicans and other aquatic birds, and adding in the Ecosim scenarios the most recent introduction into the lake of the alien *Lepomis gibbosus*, which has apparently established a significant population in a rather short period of time (Bobori *et al*. 2019).

In total, nine scenarios were simulated with the Ecosim module to potentially improve fisheries exploitation and ecosystem protection in Lake Volvi. These scenarios were the following:

1. No change in the biomass of the functional groups and the fishing effort (reference scenario).
2. Increase of pumpkinseed biomass by 20% per year for 5 years (until reaching an increase of 100%), without targeting this species by the local fishery.
3. Increase in the biomass of pumpkinseed by 20% per year (compared to the previous year), until it multiplies sixfold, with targeting the species by professional fishermen (by 15% of biomass) in the 5th year, to check the possibility of controlling its population.
4. Changes in the species targeted by professional fishermen. In particular, a gradual reversal, within 5 years, from targeting the Prussian carp (*Carassius gibelio*), which accounted for 50% of fishery production in 2010-2016, due to its exports to Eastern European countries, to targeting the European carp (*Cyprinus carpio*). The percentage that the latter was contributing to fisheries production, in the aforementioned period, was 10%. The scenario concerned increasing this species' production to 50% of the total fishery production.
5. Stocking with 15,000 eel specimens and start targeting the species.
6. Increase the number of cormorants by 10% per year for 5 years and investigate the effects on biomass estimates of commercial and non-commercial lake fish species.
7. Change in fishing effort by: (7a) reducing the number of professional fishermen by 5% per year for 10 years (final reduction by 50%) and (7b) increasing the number of professional fishermen by 10% per year for 5 years (final increase by 50%), to explore the impacts on commercial and non-commercial lake species.
8. Combining the commercial fishing targeting scenario (scenario 4) with simultaneous stocking with eels (scenario 5).
9. Fisheries' abandonment scenario, with gradual reduction in the number of professional fishermen (scenario 7a) and simultaneous increase in pumpkinseed biomass and targeting of this species (scenario 3) and significant increase in the number of cormorants (scenario 6).

3. Results and Discussion

The estimated, by simulating scenarios, biomass ($t*km^{-2}$) and fisheries production ($t*km^{-2}*y^{-1}$) of each functional group in Lake Volvi for the period 2015-2030 are provided in Figures 1 and 2.

The results of the simulations that were performed, highlighted that:

(a) The lake has the capability to "overcome" the introduction of pumpkinseed maintaining its biomass at low levels, even in cases where its population has multiplied. This is very interesting considering that the species is among the most successful invaders of freshwater ecosystems in Europe, managing to establish populations in many different water bodies, mainly due to its life strategy and plasticity (Copp & Fox 2007, Copp et al. 2017).

(b) Stocking with eels, at least at the simulated level of 15,000 specimens, would have a negligible effect on the balance of the lake's food web, but could provide significant benefits to the professional fishermen, as the species has high commercial value, due to its considerable market demand. In addition, this stocking will result in higher eel biomass until 2030. Eels in the past used to have a higher contribution to the lake's fishery production than the one estimated in 2015, but the local population declined after the connection of Volvi to the sea, through Richios River, was interrupted. This decline was of course inevitable, since for a diadromous species like the eel, maintaining open the aquatic paths that enable the adults to migrate to their reproductive fields at the sea and the juveniles to recolonise the freshwaters is of vital importance. The eel population decline, however, may also be partially attributed to the imposed fishing pressure, since the species suffers population decrease at a pan-European level that is caused by various anthropogenic pressures (e.g., Geeraerts & Belpaire 2010, Kettle *et al*. 2011).

(c) There is competition between fish stocks and cormorants, which affects the most commercial species and consequently the lake's fish stocks through overall mortality (fishing + predation mortality). Cormorant population could be significantly reduced (by 25%) by 2030 through shifting the focus of commercial fishing from *Carassius gibelio* (the main target species in the early 2010s: Perdikaris *et al*. 2012) to *Cyprinus carpio.* Cormorant populations, even though the species is considered as endangered, have increased dramatically in recent decades, due to the implementation of protection measures and abundance in food availability. This increase created intense competition with fisheries in many countries

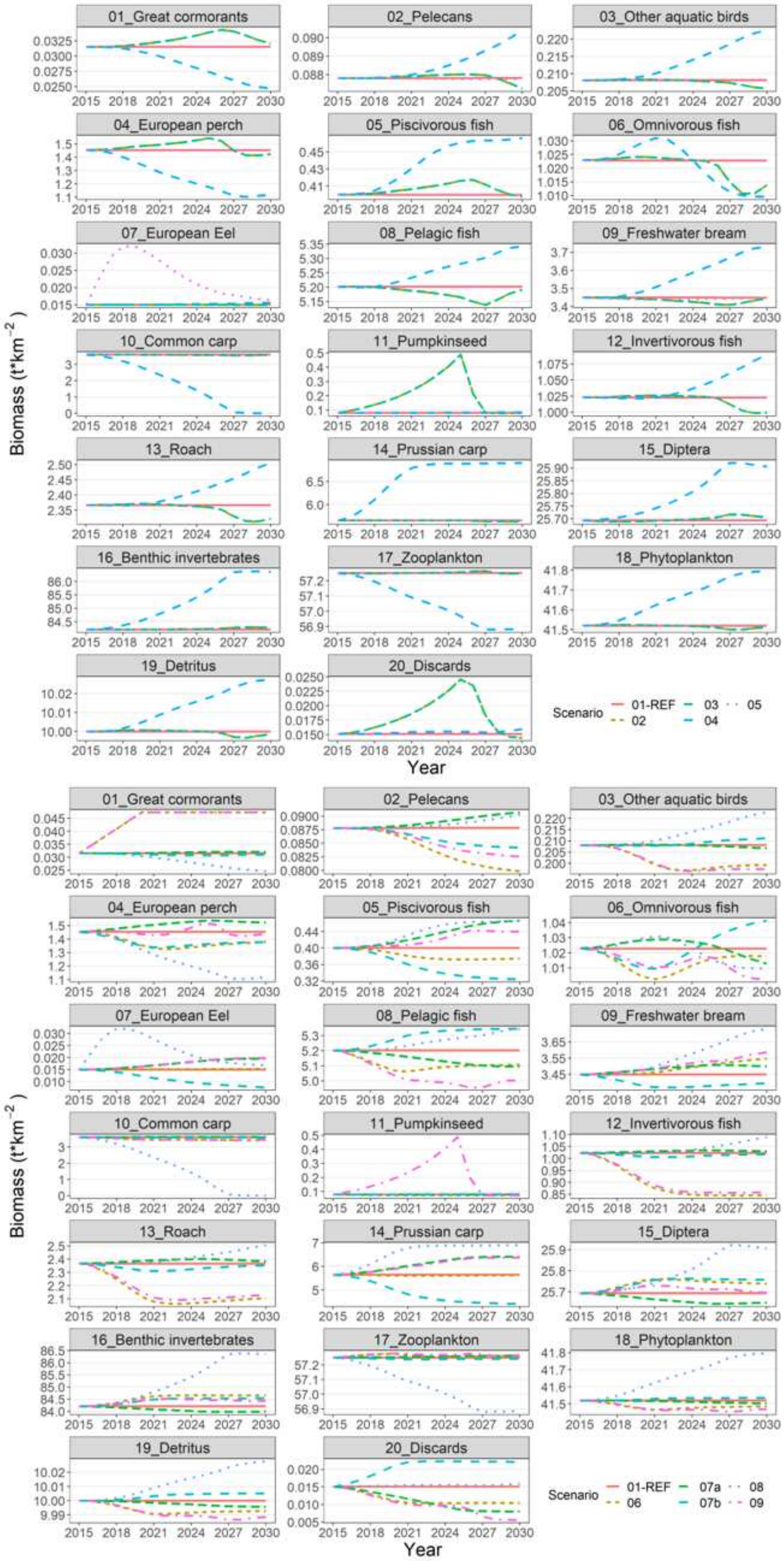

Figure 1. The estimated, by simulating scenarios, biomass (t*km^{-2}) of each functional group in Lake Volvi for 2015-2030. Functional groups are according to Moutopoulos et al. (2018).

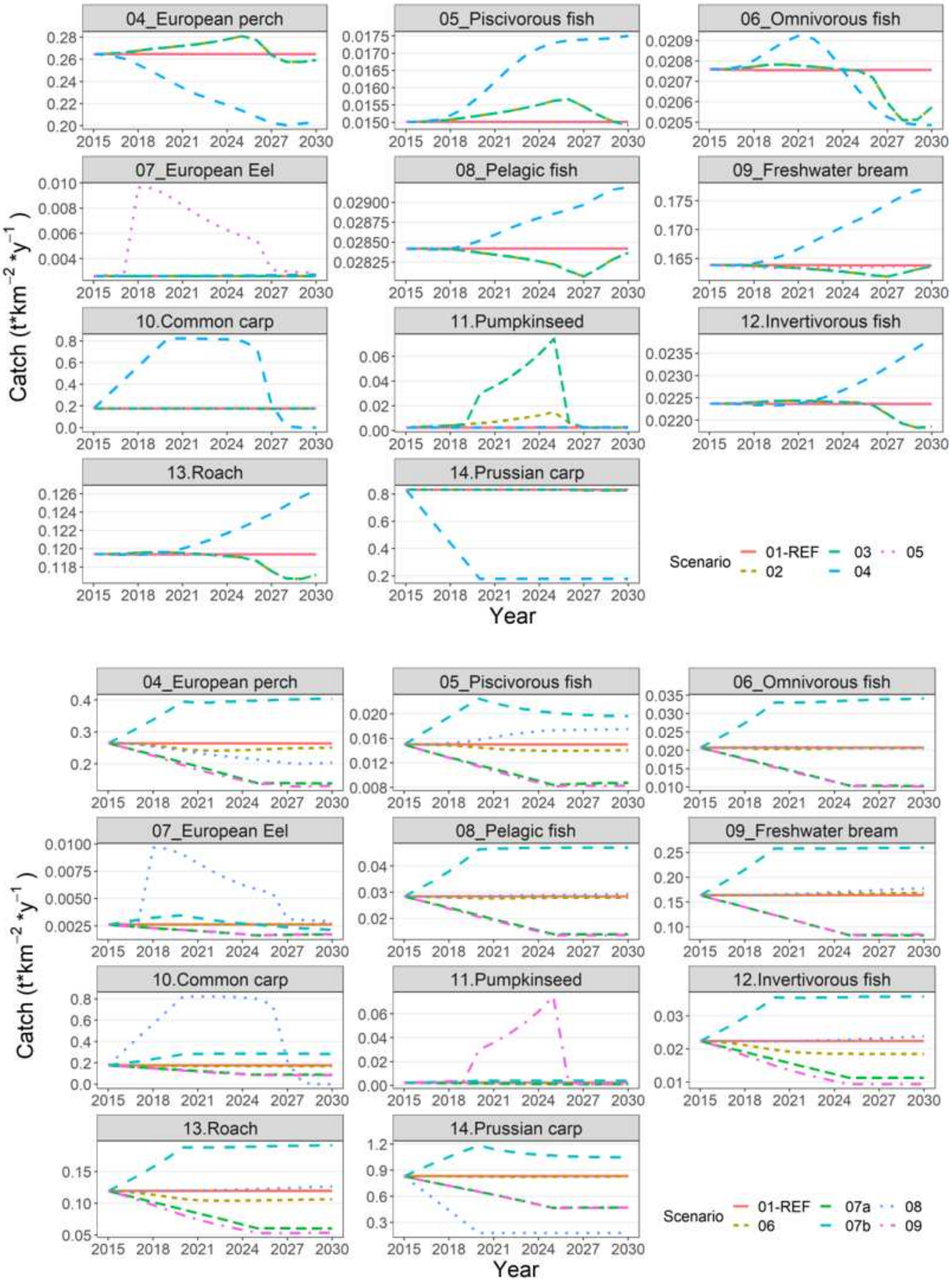

Figure 2. The estimated, by simulating scenarios, fisheries production (t*km^{-2}y^{-1}) of each functional group in Lake Volvi for 2015-2030. Functional groups are according to Moutopoulos et al. (2018).

(Cowx 2013). In Greece, such a competition is also observed, mainly in areas that consist the species' wintering grounds (Liordos *et al*. 2005, Liordos & Goutner 2007, Liordos *et al*. 2014). However, the simulated level of *Cyprinus carpio* fishing (scenario 4) proved unsustainable (Figures 1, 2), therefore, a less intense shift from *Carassius gibelio* to *Cyprinus carpio* could be designed.

(d) Changes (increase or decrease) in the fishing effort would affect the commercial fish species but would have almost no effect in the energy flows and biomasses of the functional groups within the system, although the range of the changes was randomly selected to control direct pressures on the ecosystem.

However, such changes may impose indirect effects in the ecosystem (increase of piscivorous birds, abandonment of the ecosystem and decrease of its protection) that should not be overlooked.

The results of EwE simulation models presented here are indicative of the methodology's potential towards addressing the effects of fisheries exploitation, as well as introductions and stockings of species in a lake ecosystem. Moreover, the method could be used to evaluate changes in a lake's eutrophic conditions and of changes to management and conservation strategies, consisting thus a useful tool for policy development on harvesting strategies for multispecies ecosystem-based fisheries management. Therefore, its implementation to more Greek lake ecosystems should be encouraged.

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References

Bobori D.C., Psaltopoulou Ch. (2012). Fish guide of the National Park of lakes Koronia–Volvi and Macedonian Tempes. Management Authority of lakes Koronia–Volvi, Lagadas, Greece. [In Greek].

Bobori D.C., Petriki O., Aftzi Ch. (2019). Fish community structure in the Mediterranean temperate Volvi Lake at two different stages of pumpkinseed invasion: Are natives in threat? Turkish Journal of Fisheries and Aquatic Science 19 (12), 1039–1048.

Christensen V., Walters C.J. (2004). Ecopath with Ecosim: methods, capabilities and limitations. Ecological Modelling 172, 109-139.

Copp G.H., Fox M.G. (2007). Growth and life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in Europe, and the relevance to its potential invasiveness. In: "Biological invaders in inland waters: Profiles, distribution, and threats", Gherardi, F. (ed.). Springer, Dordrecht., p. 289-306.

Copp G.H, Britton J.R, Guo Z, Edmonds-Brown V.R, Pegg J, Vilizzi L, Davison PI. (2017). Trophic consequences of non-native pumpkinseed *Lepomis gibbosus* for native pond fishes. Biological Invasions 19: 25-41.

Cowx I. (2013). Policy department structural and cohesion policies. Directorate-General for Internal Policies. http://ec.europa.eu/environment/nature/cormorants/files/Cowx_Report_for_Parliament.pdf (accessed 10-7/2021).

Geeraerts C., Belpaire C. (2010). The effects of contaminants in European eel: a review. Ecotoxicology 19, 239–266.

Kettle A.J., Vøllestad L.A., Wibig J. (2011). Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa? Fish and Fisheries 12, 380–411.

Liordos V., Zogaris S., Papandropoulos D. (2005). Great cormorant *Phalacrocorax carbo* food and human perceptions at Amvrakikos Gulf, western Greece. Proceedings of 7th International Conference on Cormorant, 23-26 Nov. 2005, VD, Switzerland, pp. 103-111.

Liordos V., Goutner V. (2007). Spatial Patterns of Winter Diet of the Great Cormorant in Coastal Wetlands of Greece. Waterbirds, 30 (1), 103-111.

Liordos V., Pergantis F., Perganti I., Roussopoulos Y. (2014). Long-term population trends reveal increasing importance of a Mediterranean wetland complex (Messolonghi lagoons, Greece) for wintering waterbirds. Zoological Studies, 53, 12.

Moutopoulos D.K., Stoumboudi, M.Th., Ramfos, A., Tsag, K., Gritzalis, K.C., Petriki, O., Patsia, A., Barbieri, R., Machias, A., Stergiou, K.I., Bobori, D.C. (2018). Food web modelling on structure and functioning of a Mediterranean lentic system. Hydrobiologia 822 (1), 259-283.

Moutopoulos D. K., Petriki O., Ramfos A., Stoumboudi M.T., Stergiou K.I., & Bobori D.C. (2020). Portraying fisheries and ecological status of a Mediterranean lake. Acta Ichthyologica et Piscatoria, 50 (1), 71-83.

Perdikaris C., Ergolavou A., Gouva E., Nathanailides C., Chantzaropoulos A., Paschos I. (2012). *Carassius gibelio* in Greece: the dominant naturalized invader of freshwaters. Review in Fish and Fisheries, 22, 17-27.

HYDROLOGICAL, ENVIRONMENTAL AND TAXONOMICAL HETEROGENEITY DURING THE TRANSITION FROM DRYING TO FLOWING CONDITIONS IN A MEDITERRANEAN INTERMITTENT RIVER

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Abstract
The intermittent rivers and ephemeral streams are considered uniques and relevant aquatic ecosystems that show variations from drying to flowing conditions in a short scale of time. In recent decades, they have recovered major importance for their benefits to the terrestrial and aquatic ecosystems and human well-being. This study considered the hydrological, environmental and taxonomical variability from drying to flowing in an intermittent river from the Chilean Mediterranean. Hydrological states were determined by the THRES Tool to identify the transition of the aquatic states from drying to flowing in the Lonquen River. Significant differences were displayed between the aquatic states finding variations in the composition of the aquatic invertebrates and the environmental variables. High abundance, richness, and diversity were displayed in the drying condition, while the disconnected pools were relevant as a refuge to the lentic taxa, mainly to some endemic species of crustacean, gastropods and bivalves that do not migrate during the drought and extreme events. Instead, the rewetting events allowed the recolonization of the aquatic invertebrates. Further long-term biological, hydrological and environmental monitoring are necessary to establish conservation measures and adaptation to climate change and anthropogenic interventions.
Key words: *Intermittent Rivers and Ephemeral Streams; TREHS Tool; Drying; Rewetting; Disconnected Pools*
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1. Introduction

The intermittent rivers and ephemeral streams (IRES) have been recognised as waterways that cease to flow at a particular space and time (Larned *et al.* 2010, Acuña *et al.* 2014; Datry *et al.* 2014). They are shifting habitat mosaics that provide a complex and dynamic system, variable in time and space (Datry *et al.* 2016). The IRES are present worldwide, and more than 50% of the global river network are non-perennial waterways [Datry *et al.* 2014, Prat *et al.* 2014, Skoulikidis *et al.* 2017). The IRES are understudied and are not recognised in many regions, such as the case of the Chilean Mediterranean Zone (ChMZ), characterised by an arid and semi-arid climate with limited knowledge of the aquatic ecology and expected impacts associated with climate change as an increase of summer mean air temperature (2 to 4ºC) and reduction of the precipitation (20%) (Garreaud 2011, Bonada & Resh 2013 Figueroa *et al.* 2013). Besides the anthropogenic interventions, these considerations could provoke more conversión of the perennial to intermittent rivers in the near decades with the loss of the ecological integrity (Larned *et al.* 2014). The present study aimed to evaluate the hydrological and biological variables during the transition of the flow recession to the resumption in an intermittent river from the ChMZ.

2. Materials and Methods

The intermittent Lonquén river is located in the central-south of Chile. It is represented by an area of 1,178 km^2 with a variation in precipitation from 400 to 1200 mm and evapotranspiration from 944 to 1244 mm, exceeding the average precipitation in the zone (Uribe *et al.* 2004). The aquatic invertebrates were sampled during the dry season (December 2015 to May 2016) and the wet period (July to October 2016). Ninety-six samples were collected using a hand net following the semi-quantitative, multihabitat 3-min kick sampling method (Merrit & Cummins 1996, Stubbington *et al*. 2009a) and preserved in 70% v/v ethanol solution. The aquatic invertebrates were sorted and counted in the laboratory identified to the family level. The statistical analysis used the R software to recognise statistical differences between the aquatic states identified with the THRES Tool (Temporary Rivers Ecological and Hydrological Status) (Gallart *et al.* 2012, 2017).

3. Results

Four aquatic states were identified during the transition from drying the flowing in the Lonquen River: Oligorheic during the drying phase or flow recession (ORD), Arheic with disconnected pools (ARH), Oligorheic during rewetting or flow resumption (ORR) and Eurheic with base flow conditions (EUR). The two calculated metrics: the *six months seasonal predictability of dry period* (Sd6 = 0.9593), and the measure of the *flow permanence* (Mf = 0.6559) performed a recognition to the Lonquén River as an intermittent river with pools. The environmental variables were summarised in Figure 1 and Table 1, indicating that the velocity and temperature contributed most to environmental variability to the EUR and ORD. Instead, conductivity and dissolved solids explained the variability of the ARH states. In contrast, the ORR state represented a transition between the dry and wet conditions with high values of dissolved oxygen and suspended solids. EUR state was represented by high values of dissolved oxygen, nitrates, total nitrogen and total phosphorus (Figure 1).

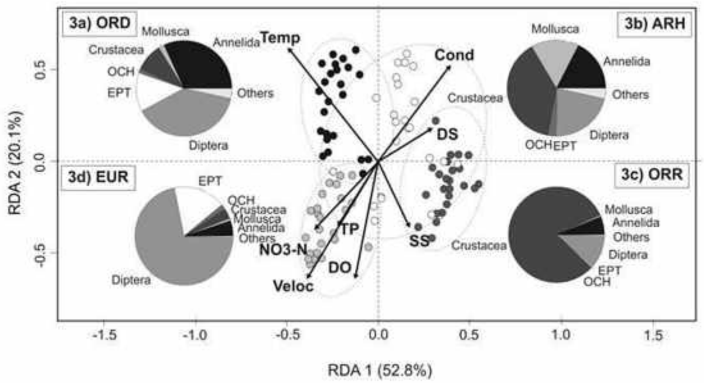

Figure 1. Redundancy analysis (RDA) of the environmental variables: water temperature (Temp), conductivity (Cond), dissolved solids (DS), suspended solids (SS), dissolved oxygen (DO), water velocity (Veloc), nitrate-nitrogen (NO3-N) and total phosphorus (TP). The invertebrate community for the distinguished aquatic states is represented by the composition of the major invertebrate groups (pie charts) in the Lonquén River: (a) ORD (● Oligorheic-drying); (b) ARH (○ Arheic); (c) ORR (◉ Oligorheic-rewetting), and (d) EUR (○ Eurheic).

Table 1. Summary of the environmental variables (mean ± standard deviation) measured in the different aquatic states at the Lonquén River (2015-2016) (see acronyms of aquatic states in Figure 1).

	ORD	ARH	ORR	EUR
Current velocity (m/s)	0.013±0.03	0.0±0.0	0.093±0.08	0.516±0.10
pH	7.64 ±0.40	7.52±0.68	6.65±0.24	7.80±0.27
Temperature (°C)	27.9±3.2	15.7±1.6	10.4±1.5	14.2±0.1
Conductivity (µS/cm)	232±48	289±28	226±38	120±4.5
Dissolved oxygen (mg/L)	5.48±1.53	5.96±2.74	8.72±2.05	10.81±0.23
Suspended solids (mg/L)	9.76±5.19	3.89±1.62	21.4±9.31	15.14±2.83
Dissolved solids (mg/L)	164±63	184±21	172±17	127±7
Nitrate-NO_3-N (mg/L)	0.06±0.10	0.12±0.07	0.02±0.01	0.17±0.02
Total Nitrogen (mg/L)	0.40±0.14	0.27+0.20	0.26±0.10	0.53±0.16
Total Phosphate (mg/L)	0.05±0.01	0.10±0.07	0.06±0.04	0.15±0.06

The aquatic invertebrates identified during the transition was represented by a total of 66,392 individuals that belongs to 49 families. The ORD state displayed the majority of the individuals (51,503; 77.6%), followed by the ORR (10,440; 15.7%), the EUR (2326; 3.5%) and ARH states (2123; 3.2%) hosted the lowest numbers of individuals with the statistical difference between the aquatic states (one-way ANOVA; $F = 22.59$, $p < 0.001$). The family richness was distinguished between the aquatic states with most distinction to ORD (mean±SD; 15.7±4.5; total = 40); followed by ORR (10.0±3.2; total = 32), EUR (9.2±2.8; total = 29) and ARH states (8.2±3.0; total = 28) (Figure 1a). Similarly, the ORD performed the most distinguished aquatic invertebrate community represented by a high proportion of Annelida, crustaceans (cladocerans, copepods and ostracods), EPT (mainly mayflies) and Diptera (chironomids). The crustaceans, annelids, dipterans represented the ARH state with the molluscs that being dominant in the stagnant pools (Figure 1b), the ORR showed a higher abundance of microcrustaceans (Figure 1c). in contrast, the EUR state was distinguished by the abundance of Diptera (Chironomids) and Ephemeroptera (Mayflies) with a reduced presence of Mollusca (Gastropods), Ostracoda, Plecoptera (Grypopterigidae), Coleoptera (Dytiscidae) and non-chironomid Dipterans (Figure 1d). Evidence of significant statistical differences using the test of homogeneity (PERMDISP) were observed in the environmental heterogeneity among aquatic states ($F = 7.087$; $p < 0.001$; Figure 2a), the community composition (presence/absence; $F = 9.026$; $p < 0.001$; Figure 2b) and the community structure (Bray-Curtis; $F = 7.132$; $p < 0.001$; Figure 2c).

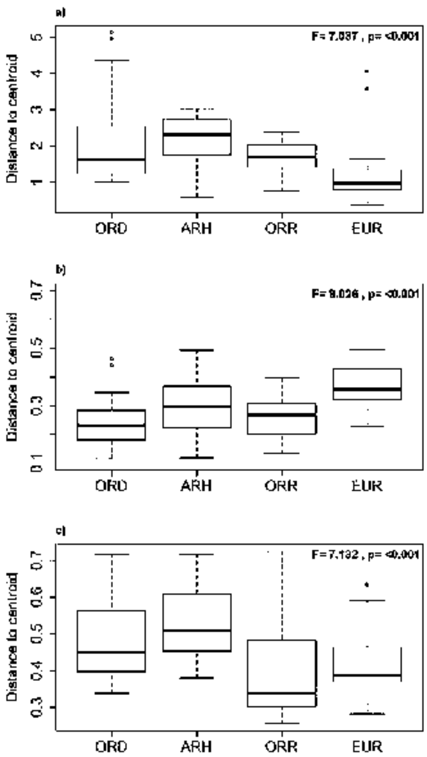

Figure 2. Boxplots of the test of homogeneity (PERMDISP) representing the mean distance from groups centroids of the environmental heterogeneity with Euclidean distance (a); taxonomical structure using abundance and Bray-Curtis dissimilarity (b), and taxonomical community using presence/absence and Sørensen dissimilarity (c) of the aquatic states (see acronyms of aquatic states in Figure 1).

4. Discusion

This study showed the first integration of the hydrological, environmental and biological conditions between the drying to flowing condition in an intermittent river from the Chilean Mediterranean using the THRES Tool development to Mediterranean intermittent rivers. Significant differences were observed between the transition from drying to flowing conditions in the Lonquén River, reflecting variations in the invertebrate community structure and composition. A dominance of tolerant and adapted taxa to the low or no flow conditions prevailed due to the capacity to resist and recover from the drought period with a maximum habitat heterogeneity and complex interactions between the hydrological, physicochemical processes and the variations in the aquatic communities during the flow intermittence (Warfe *et al.* 2014, Arena-Sanchez *et al.* 2016). The presence of disconnected pools is recognised as an essential refuge to drought escape giving the opportunity to colonise and disperse the taxa to search the opportunity to survive and persist through flow cessation (Stubbington *et al.* 2009b; Leigh *et al.* 2016). Our findings showed high proportions of Diptera, Annelida and Ephemeroptera during the drying period, shifting to high abundances of Gastropoda (*Physa chilensis*), Annelida (Lumbriculidae and Naididae), Ostracoda (Cyprididae), Copepoda (Cyclopoida and Harpacticoida) and Branchiopoda (Daphniidae), Coleoptera (Dytiscidae and Hydrophilidae) and Odonata (Aeshnidae). The *Aegla* sp. and *Samastacus spinifrons* (Decapoda) and the *Diplodon chilensis* (Bivalvia) were also represented in the isolated pools during the dry phase, considering an important refuge to endemic species of aquatic invertebrates from the ChMZ.

The flow resumption phase in intermittent rivers allows the recolonisation after the drought event. These routes have been distinguished by aerial dispersal, drift from upstream, redistribution from instream refugia or diapause (hyporheic zone) with increases in abundance and diversity of macroinvertebrates such as Midges (Chironomids) and Blackflies (Simulids) that are abundant in the early stage of flow resumption (Oertli *et al.* 2002). The invertebrate community structure in intermittent rivers typically recovers within a month after flow resumption, except for populations with low resilience, resulting in adverse effects on ecosystem functions (e.g., Molluscs and some Crustaceans) (Arena-Sanchez 2016). However, the resistant propagules in the sediment (often called seedbanks) of dry riverbeds and from the hyporheic zone are crucial for recolonisation after flow resumption and determine the aquatic invertebrate resilience level after drought (Chadd *et al.* 2017). Subsequently, the flowing condition in the Lonquen River evidenced a higher proportion of Branchiopoda (Bosminidae), Ostracoda (Cyprididae), Copepoda (Cyclopoida), and Annelida (Lumbriculidae) occurred during the rewetting and allowing the recolonisation after the drought events. Aerial dispersal, drift from upstream, redistribution from instream refugia, diapause (hyporheic zone) and seedbanks are esentials to the resilience processes that help to recovery the aquatic assemblage in the flow resumption (Oertli *et al.* 2006). Rheophilic taxa (EPT) tends to be dominant with the increase of flow permanence, and the taxa adapted to low flow conditions (OCH) tend to disappear (Hershkovitz & Gasith 2013).

Finally, the limited knowledge, the availability of resources for research and development in most Mediterranean countries have reduced the efforts to conserve the IRES (Skoulikidis *et al.* 2017). Moreover, climate change effects are expected to reduce the aquatic ecosystems with declining endemic populations and loss of freshwater biodiversity and fragmentation of the aquatic ecosystems are already observed today.

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References

Acuña V., Datry T., Marshall J., Barceló, D., Dahm, C. N., Ginebreda, A., McGregor, M., Sabater, S., Tockner, K., Palmer, M.A. (2014). Why should we care about temporary waterways? Science 343(6175), 1080–1081.

Arenas-Sánchez, A., Rico, A., Vighi, M. (2016). Effects of water scarcity and chemical pollution in aquatic ecosystems. Science of the Total Environment 572, 390–403.

Bonada N., Resh V.H. (2013). Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. Hydrobiologia 719, 1–29.

Chadd R.P., England J.A., Constable D., Dunbar M.J., Extence C.A., Leeming D.J., Murray-Bligh J.A., Wood P.-J. (2017). An index to track the ecological effects of drought development and recovery on riverine invertebrate communities. Ecological Indicators 82, 344–356.

Datry T., Larned S.T., Tockner K. (2014). Intermittent rivers: A challenge for freshwater ecology. BioScience 64(3), 229–235.

Datry T., Moya N., Zubieta J., Oberdorff T. (2016). Determinants of local and regional communities in intermittent and perennial headwaters of the Bolivian Amazon. Freshwater Biology 61(8), 1335–1349.

Figueroa R., Bonada N., Guevara M., Pedreros P., Correa-Araneda F., Díaz M.E., Ruiz V.H. (2013). Freshwater biodiversity and conservation in Mediterranean climate streams of Chile. Hydrobiologia 719(1), 269–289.

Gallart F., Cid N., Latron J., Llorens P., Bonada N., Jeuffroy J., Jiménez-Argudo S.M., Vega R.M., Solà C., Soria M., Bardina M., Hernández-Casahuga A.J., Fidalgo A., Estrela T., Munné A., Prat N. (2017). TREHS: An open-access software tool for investigating and evaluating temporary river regimes as a first step for their ecological status assessment. Science of the Total Environment 607–608, 519–540.

Gallart F., Prat N., García-Roger E.M., Latron J., Rieradevall M., Llorens P., Barbera G.G., Brito D., de Girolamo A.M., Lo Porto A., Buffagni A., Erba S., Neves R., Nikolaidis N. P., Perrin J.L., Querner E. P., Quinonero J.M., Tournoud M.G., Tzoraki O., Skoulikidis N., Gamez R., Gomez R., Froebrich J. (2012). A novel approach to analysing the regimes of temporary streams in relation to their controls on the composition and structure of aquatic biota. Hydrological Earth System Sciences 16(9), 3165–3182.

Garreaud R. (2011). Cambio Climático: Bases físicas e impactos en Chile. Revista Tierra Adentro-INIA 93(93), 13–19.

Hershkovitz Y., Gasith A. (2013). Resistance, resilience, and community dynamics in mediterranean-climate streams. Hydrobiologia 719(1), 59–75.

Larned S.T., Datry T., Arscott D.B., Tockner K. (2010). Emerging concepts in temporary-river ecology. Freshwater Biology 55(4), 717–738.

Leigh C., Bonada N., Boulton A.J., Hugueny B., Larned S.T., Vander Vorste R., Datry T. (2016). Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. Aquatic Sciences 78(2), 291–301.

Merrit R.W., Cummins K.W. (1996). An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Oertli B., Joye D. A., Castella E., Cambin D., Lachavanne J. (2002). Does size matter? The relationship between pond area and biodiversity. Biological Conservation 104, 59–70.

Prat N., Gallart F., Von Schiller D., Polesello S., García-Roger E.M., Latron J., Rieradevall M., Llorens P., Barberá G.G., Brito D., De Girolamo A.M., Dieter D., Lo Porto A., Buffagni A., Erba S., Nikolaidis N.P., Querner E.P., Tournoud M.G., Tzoraki O., Skoulikidis N., Gómez R., Sánchez-Montoya M.M., Tockner K., Froebrich J. (2014). The MIRAGE Toolbox: an integrated assessment tool for temporary streams. River Research and Application 30(10), 1318–1334.

Skoulikidis N., Sabater S., Datry T., Morais M. M., Buffagni A., Dörflinger G., Zogaris S., Sánchez-Montoya M.M., Bonada N., Kalogianni E., Rosado J., Vardakas L., de Girolamo A.M., Tockner K. (2017). Non-perennial Mediterranean rivers in Europe: Status, pressures, and challenges for research and management. Science of the Total Environment 577, 1–18.

Stubbington R., Greenwood A.M., Wood P.J., Armitage P.D., Gunn J., Robertson A.L. (2009a). The response of perennial and temporary headwater stream invertebrate communities to hydrological extremes. Hydrobiologia 630, 299–312.

Stubbington R., Wood P.J., Boulton A.J. (2009b). Low flow controls on benthic and hyporheic macroinvertebrate assemblages during supra-seasonal drought. Hydrological Processes 23(15), 2252–2263.

Uribe H., Pérez C., Okuda Y. (2004). Recursos hídricos y manejo del agua para un desarrollo sustentable del secano. Ministerio de Agricultura-Instituto de Investigaciones Agropecuarias. Chillán, Chile.

Warfe D.M., Hardie S.A., Uytendaal A.R., Bobbi C.J., Barmuta L.A. (2014). The ecology of rivers with contrasting flow regimes: identifying indicators for setting environmental flows. Freshwater Biology 59, 2064–2080.

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BIOCHEMICAL CHARACTERIZATION OF MARINE INVASIVE SPECIES *Lagocephalus sceleratus* (Gmelin, 1789), *Pterois miles* (Bennett, 1828) AND *Fistularia commersonii* (Rüpp, 1838) IN THE SOUTHERN AEGEAN

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Abstract
The invasion of marine alien species in the Mediterranean basin, induces negative effects both in socioeconomic aspects and marine ecosystem processes, making the need for their exploitation vital as a measure towards the management of their population. Aim of this preliminary study was to investigate the biochemical characteristics of invasive species from the Southern Aegean Sea. In particular, total fat content, protein content and mineral ash content were determined in flesh and skin of *Lagocephalus sceleratus*, *Pterois miles* and *Fistularia commersonii*. Protein content (Prts) was 22.6, 21.3 and 21.8% for *L. sceleratus, P. miles* and *F. commersonii* respectively, while mineral ash content (MAC) was 11.2 for *L. sceleratus* and 9.5% for both *P. miles* and *F. commersonii*. Total fat content (TF) was found to be of significantly higher concentration in skin tissue of all species. In both flesh and skin tissue, *L. sceleratus* had the highest values of 4.7 and 15.5 % respectively. The assessment of substances of high commercial interest contained in marine invasive species is proposed within the scope of exploring possible prospects of an efficient economic exploitation of the species, and therefore a sustainable management of their population in the Mediterranean.
Keywords: *Marine Invasive Species, Mediterranean Sea, Biochemical characteristics*
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Introduction

Marine alien species of the Mediterranean basin, the majority of which are Lessepsian migrants, have rapidly developed large populations in the Eastern Mediterranean basin, while showing increasing signs of spread in its central and western part (Streftaris & Zenetos 2006; Zenetos *et al*. 2018). *Lagocephalus sceleratus* (Gmelin, 1789) is one of the most famous invasive species, as nowadays it is an acute problem with ecological and economic impact on marine life, fishing industry, human health and safety in local communities (Nader *et al*. 2012). The development of large populations in the Mediterranean, is possible due to its high adaptability and the lack of predators in this area (Kalogirou 2013). On the other hand, it is one of the most toxic species on the planet, as its tissues contain Tetrodotoxin (TTX), a neuroparalytic toxin that can cause death if consumed (Kheifets *et al*. 2012).

At the same time, extensive research has demonstrated the catastrophic ecosystem effects of the invasion of *P. miles* (lionfish), which is another Lessepsian migrant of the Scorpaenidae family (Kletou *et al*. 2016). Although there is not an immediate danger to human life, this species is also venomous and causes a wide range of symptoms including severe pain when its venom is injected through the hard thorns of its fins. Lionfish can reach high abundances in a short time by changing the ecosystem structure and function of its invasion area, mainly due to the predation of other native smaller species (Hüseyinoğlu & Öztürk 2018, Zannaki *et al*. 2019), reinforcing the need to take measures to manage its population.

Invasion of alien species has the potential to dramatically change an ecosystem, affecting ecosystem processes, its composition and interactions within the food chain, even from species without any immediate risk to human health such as *F. commersonii*, which is as well of great interest due to its high abundance in Mediterranean waters. It is a species with more than 70% (% by weight) of its diet consisting of native fish species of high economic value (*Spicara smaris, Boops boops* and *Mullid* spp.). At the same time, it feeds on ecologically important species, such as those of the Gobiidae family (Kalogirou *et al*. 2007). The species has spread throughout the Mediterranean Sea and does not appear to have natural enemies, although further research is needed. In addition, key aspects of its biology as well as its ecological requirements remain unknown, as relevant research is very limited (Bariche *et al*. 2013, Castriota *et al*. 2014, Vitale *et al*. 2016).

Within the scope of exploring possible prospects for their sustainable management and aiming at their most efficient economic exploitation, it is proposed to carry out an assessment of the substances of high commercial interest contained in the specific species. As a first step, the present research aims at the implementation of a primary record of biochemical characteristics for three of the most important marine alien species of the Mediterranean basin, the *L. sceleratus*, the *P. miles* and the *F. commersonii* in the Southern Aegean Sea.

Materials and Methodology

Fish samples were provided from the Hellenic Centre of Marine Research (HCMR) in March 2021 as part of the Project EXPLIAS' partnership. Proximate analysis of edible portion of the fish was carried out in the facilities of the National Technical University of Athens laboratories. More precisely, the following major constituents were determined; mineral ash content (MAC), total fat (TF) and proteins (Prts). TF was determined using the Bligh and Dyer (1959) method for skin tissue and flesh tissue separately, and Prts were determined according to Nurdiyana and Mazlina (2009). MAC was analysed gravimetrically after combustion of the sample in a muffle furnace at 550° C (AOAC). All analyses were performed in triplicate and the calculated mean values are presented.

Results of MAC, TF and Prts are expressed as percentage of wet tissue weight basis (% ww) for each of the three species, and for the biggest and smallest specimen in weight for each species respectively. The weighed specimens of different sizes that were used for this analysis are shown in Table 1. The Microsoft Excel Spreadsheet software was used for the presented figures.

Table 1 Values of fish weight (gr) for different sizes of specimens from the three marine invasive species used for this study

	Weight (g)	
Specimens	S	B
L. sceleratus	169	1200
P. miles	110	261
F. commersonii	193	256

Results

Protein content was 21.3, 22.6 and 21.8% ww for *P. miles, L. sceleratus* and *F. commersonii* respectively as shown in Figure 1. Prts content of the three species was found to be similar.

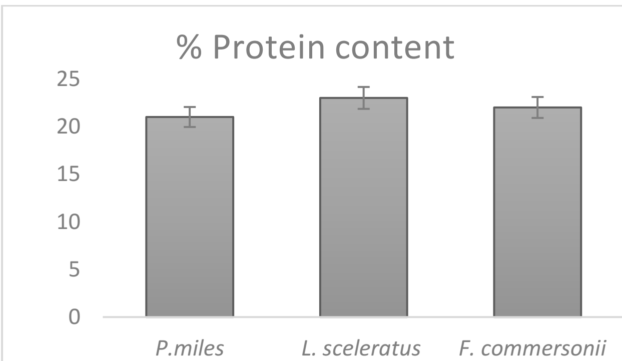

Figure 1 Protein content (Prts) expressed in % wet weight for marine invasive species *L. sceleratus, P. miles* and *F. commersonii*

Mean values of MAC for all fishes examined, was found to be 11.2 and 9.5% ww for *L .sceleratus*, and both *P. miles* and *F. commersonii* respectively. *L. sceleratus* contains the highest amount of MAC (Figure 1). Differences in MAC with fish size, are found to be higher for the *F. commersonii* species. The biggest specimen contains the higher MAC with about a 2.5% increase compared to the smallest specimen's MAC. *P. miles* bigger specimen exhibits an increase of about 1% at its MAC compared to the smaller one. Although *L. sceleratus* contains the highest MAC, differences with size seem to be of minor importance. Values of fish weight (g) for different sizes of specimens for the three species are shown in Table 1.

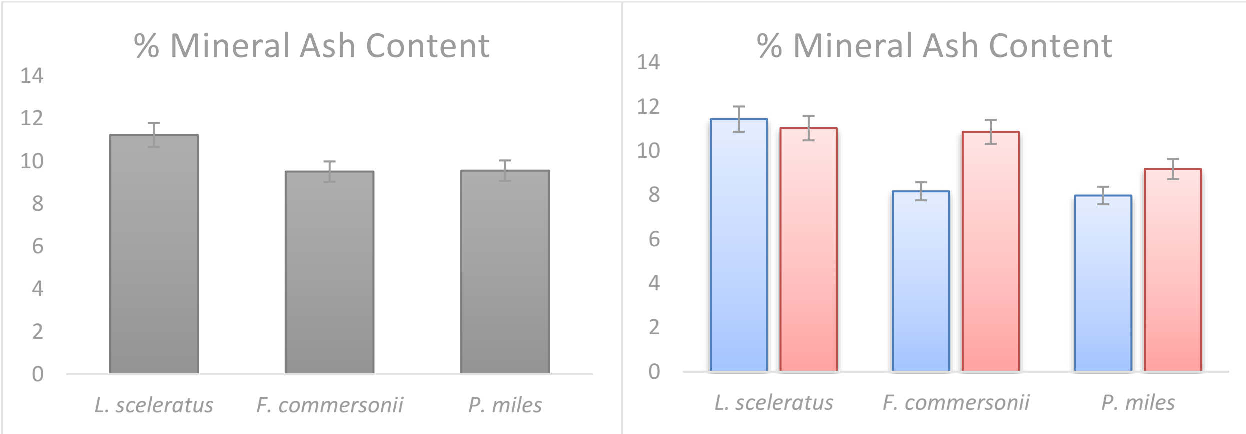

Figure 2 Mean values of Mineral Ash Content (left) expressed in % wet weight for the marine invasive species *L. sceleratus, P. miles* and *F. commersonii*, and Mineral Ash Content (right) for different size specimens (S/B). Values of groups' S and B fish weight (g) are shown in Table 1.

TF was estimated separately for the skin and flesh tissue of all three species examined in this study. Overall, TF was found to be of higher concentration in skin tissue with differences of up to 10% than that of flesh tissue. In both flesh and skin, *L. sceleratus* has the highest values of 4.7 and 15.5 % ww respectively. As shown in Figure 3, *P. miles* follows the same trend with 4.3 and 11.9% ww respectively, while *F. commersonii* exhibits the lowest fluctuation between the two tissue types with 2.7% flesh TF and 4.2% ww skin TF.

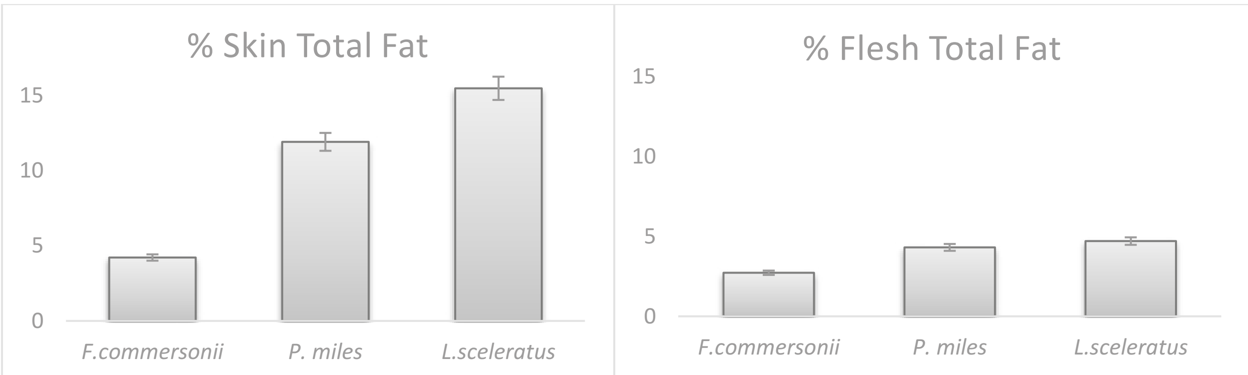

Figure 3 Mean values of Total Fat (% wet weight) from the skin (left) and flesh (right) tissue of marine invasive species *L. sceleratus, P. miles* and *F. commersonii*

As far as size is concerned, in Figure 4, *F. commersonii* skin tissue exhibits no evident differences in TF between the two specimens, while flesh tissue shows a slight decrease in TF for the bigger specimen. On the contrary, *P. miles* and *L. sceleratus* exhibit an increase in flesh TF for the bigger specimen while, skin TF is found to be higher for small *P. miles* and *L. sceleratus* specimens. Between flesh and skin tissue, the higher fluctuations in TF content seem to take place for smaller individuals of *L. sceleratus* and *P. miles.* Between sizes, the highest fluctuations take place for the small and big *L. sceleratus* specimens.

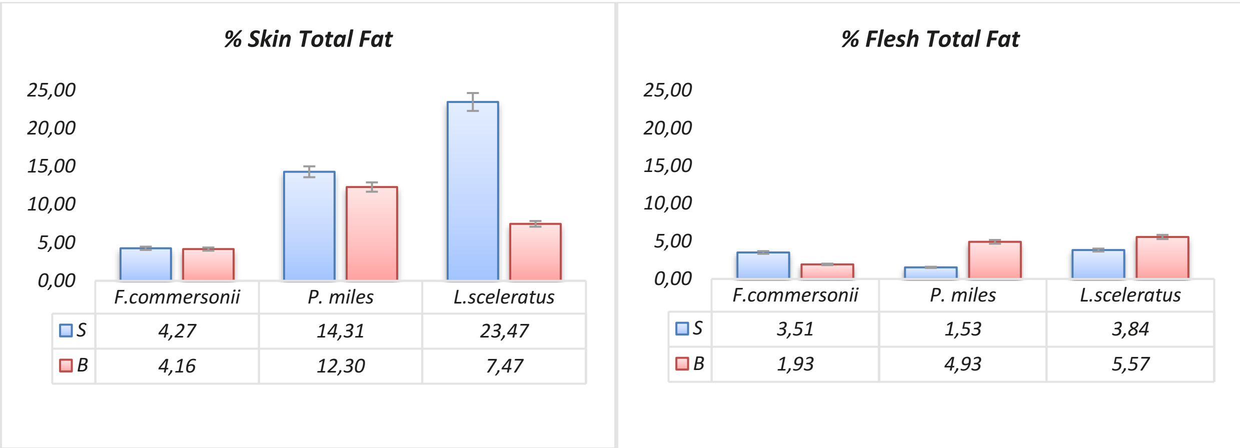

Figure 4 Total Fat (% wet weight) from the skin (left) and flesh (right) tissue for different size speciments (S/B) of marine invasive species *L. sceleratus, P. miles* and *F. commersonii.* Values of groups' S and B fish weight (gr) are shown in Table 1.

Discussion

Rising Mediterranean temperatures due to climate change favour the spread of marine alien species including the ones investigated within this study (Streftaris & Zenetos 2006). With their commercial value and exploitation potential remaining trivial, invasive species such as the pupperfish and lionfish are aggravating factors for Mediterranean fisheries. Finding innovative methods for their economic exploitation is a basic precondition, on one hand, in order to create incentives for their targeted fishing and on the other hand, in order to achieve the control of their populations.

According to our primary record of biochemical characteristics in the Southern Aegean Sea, TF and MAC of the studied invasive species were found to differ from other published records in the Mediterranean region, while Prts seem to be in accordance. The highest fat, protein, and ash contents in *L. sceleratus* have been found to be 0.33, 21.62, and 1.63% ww, respectively for the Mediterranean according to Aydın *et al*. (2013). In the same region, average protein, lipid, and total minerals have also been reported for female individuals to be 20.44%, 0.65%, and 1.43% respectively; while for male individuals 20.58%, 0.84%, and 1.40% ww (Kosker *et al*. 2018). Prts, TF, and MAC in *P. miles* for the Mediterranean region were found to be 20.05-21.08%, 1.11-1.84%, and 1.22-1.54% respectively (Ayas *et al*. 2018). To our knowledge, similar studies for *F. commersonii* for the Mediterranean region have not yet been reported.

It has to be noted that seasonal variation can occur particularly in the fat content of fish species along with regional, age, and sex effects (Aydın *et al*. 2013, Kosker *et al*. 2018, González *et al*. 2019). In addition, differences in biochemical characteristics of marine alien species have been largely connected to biotic factors since these species are of wild and migrating populations (Aydın *et al*. 2013). Therefore, further investigation focusing on the effect of such parameters is suggested.

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References

Ayas D., Sen Agılkaya G., Kosker A., Durmus M., Ucar Y., Bakan M. (2018). The Chemical Composition of the Lionfish *Pterois miles*, Bennett 1828, the New Invasive Species of the Mediterranean Sea. Natural and Engineering Sciences, 3(2), 103-115.

Aydın M., Tufan B., Sevgili H., Köse S. (2013). Seasonal Changes in Proximate Composition and Fatty Acid Profile of Pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) from the Mediterranean Sea of Turkey. Journal of Aquatic Food Product Technology, 22(2), 178-191.

Bariche M., Kajajian A., Azzurro E. (2013). Reproduction of the invasive bluespotted cornetfish *Fistularia commersonii* (Teleostei, Fistulariidae) in the Mediterranean Sea. Marine Biology Research, 9(2).

Castriota L., Falautano M., Battaglia P., Oddo A., Andaloro F. (2012). New biological data on *Fistularia commersonii* in the central Mediterranean Sea. International Journal of Icthyology, 38(1), 15-21.

González I., Aguilar-Perera A., Chel-Guerrero L., Gallegos-Tintoré S., Betancur-Ancona D. (2019). Chemical composition, amino acid and fatty acid profiles of Lionfish *Pterois volitans* from the Alacranes reef, southern Gulf of Mexico. Indian Journal of Animal Research.

Hüseyinoğlu M., n.d. Lionfish invasion and its management in the Mediterranean Sea. Turkish Marine Research Foundation (TUDAV) Publication number: 49, Istanbul, Turkey, 121 pp.

Kalogirou S., 2013. Ecological characteristics of the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) in the eastern Mediterranean Sea – a case study from Rhodes. Mediterranean Marine Science, 14(2).

Kalogirou S., Corsini M., Kondilatos G., Wennhage H. (2007). Diet of the invasive piscivorous fish *Fistularia commersonii* in a recently colonized area of the eastern Mediterranean. Biological Invasions, 9(8).

Kheifets J., Rozhavsky B., Girsh Solomonovich Z., Marianna R., Soroksky A. (2012). Severe Tetrodotoxin Poisoning after Consumption of *Lagocephalus sceleratus* (Pufferfish, Fugu) Fished in Mediterranean Sea, Treated with Cholinesterase Inhibitor. Case Reports in Critical Care, 2012, 1-3.

Kletou D., Hall-Spencer J., Kleitou P. (2016). A lionfish (*Pterois miles*) invasion has begun in the Mediterranean Sea. Marine Biodiversity Records, 9(1).

Kosker A., Ozogul F., Durmus M., Ucar Y., Ozogul Y., Boga E., Ayas D. (2018). Seasonal Changes in Proximate Composition and Mineral-Heavy Metal Content of Pufferfish (*Lagocephalus sceleratus*) from Northeastern Mediterranean Sea. Turkish Journal of Fisheries and Aquatic Sciences, (18), 1269-1278.

Nader M., Indary S., Boustany L. (2013). The puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean. In: FAO - GFCM SAC meeting. volume.TD10.

Nurdiyana H., Mazlina M. (2009). Optimization of Protein Extraction from Fish Waste using Response Surface Methodology. Journal of Applied Sciences, 9(17), 3121-3125.

Sanmartín E., Arboleya J., Villamiel M., Moreno F. (2009). Recent Advances in the Recovery and Improvement of Functional Proteins from Fish Processing By-Products: Use of Protein Glycation as an Alternative Method. Comprehensive Reviews in Food Science and Food Safety, 8(4), 332-344.

Streftaris N., Zenetos A. (2006). Alien Marine Species in the Mediterranean - the 100 'Worst Invasives' and their Impact. Mediterranean Marine Science, 7(1), 87.

Vitale S., Arculeo M., Vaz A., Giusto G., Gancitano S., Ragonese S. (2016). Otolith-based age and growth of the Lessepsian species *Fistularia commersonii* (Osteichtyes: Fistulariidae) in South of Sicily (Central Mediterranean Sea). Italian Journal of Zoology, 83(4), 490-496.

Zenetos A., Corsini-Foka M., Crocetta F., Gerovasileiou V., Karachle P., Simboura N., Tsiamis K., Pancucci-Papadopoulou M. (2018). Deep cleaning of alien and cryptogenic species records in the Greek Seas (2018 update). Management of Biological Invasions, 9(3), 209-226.

Zannaki K., Corsini-Foka M., Kampouris T., Batjakas I. (2019). First results on the diet of the invasive *Pterois miles* (Actinopterygii: Scorpaeniformes: Scorpaenidae) in the Hellenic waters. Acta Ichthyologica et Piscatoria, 49(3), 311-317.

ANTIOXIDANT BIOACTIVE PEPTIDES ENCRYPTED IN MARINE BIVALVE *Mytilus galloprovincialis* PROTEINS: *in silico* ANALYSIS

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Abstract

In the present study, we performed an *in silico* analysis of five muscle and byssus protein sequences of the Mediterranean mussel *Mytilus galloprovincialis* with the use of bioinformatic tools aiming to estimate their antioxidant bioactive potential as indicated by the presence of small bioactive peptides encrypted in their sequence which can be further released by proteolytic enzymes. The physicochemical properties of the proteins and selective antioxidant peptides (AP) were studied. The peptides of interest of the mussel proteins were matched with known APs from the BIOPEP database. The byssal gland adhesive plaque matrix protein 1 (MGFP1) showed the highest frequency of AP fragments in its sequence. All of 14 APs encrypted in paramyosin and analyzed with ToxinPred for their features and toxicity were predicted non-toxic. Three of them were simulated for enzymatic release with potential proteolytic enzymes that could be used to release them into protein hydrolysates.

Keywords: *Mussels, Antioxidant Peptides, In Silico Analysis*

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1. Introduction

The Mediterranean mussel *Mytilus galloprovincialis* is an important bivalve mollusc both for ecosystem functioning and human use. It is farmed widely from coastal Spain and Southern Mediterranean to China (FAO). Generally, world mussel farming production was 2 million tones in 2016 and is generally increasing with the exception of EU where it is decreasing during the last two decades (FAO 2014, Avdelas *et al*. 2021). About a quarter of farmed mussels is discarded as waste by-products not targeted for human consumption (Vareltzis & Undeland 2012). By-products include byssus threads, whole mussels and shells and can be bioprocessed for valuable added ingredients such as bioactive peptides, enzymes, minerals and thus used as functional food ingredients (Naik & Hayes 2019). Bioactive peptides are generally inactive and encrypted into a parent protein, can be typically from 2 to 20 amino acid residues long and show diverse physiological functions such as antidiabetic, antioxidant and hypocholesteremic (Harnedy & FitzGerald 2012).

The protein content as shown in Black Sea *M. galloprovincialis* is high, ranging from approximately 55%-75% of the dry weight of the mussel meat depending on the season (Çelik *et al*. 2012). High molecular weight proteins of the mussel meat include tropomyosin, paramyosin which is found only in invertebrate muscle and transgelin-like protein and the valuable components are released through hydrolysis of the protein fractions which are extracted with methods such as pH shift, electric field treatment, high pressure homogenization, ultrasound treatment (Naik & Hayes 2019, Vercruysse *et al*. 2005). The release of the encrypted APs can be performed either through gastrointestinal digestion or through food processing or both (Harnedy & FitzGerald 2012).Mussels use their byssal threads to attach to surfaces and these threads contain collagen and proteins with bio-adhesive properties which consist a valuable component with industrial applications. The proximal thread matrix protein and the adhesive plaque matrix protein are found in the byssus. Bioinformatic tools, databases and in silico proteolysis can speed up the suitable peptide identification process and assessment of the different sources for its recovery.

The aim of this study was to assess *in silico* the potential of muscle and byssus proteins as sources of APs by estimating their physicochemical and biochemical properties, analysing, and simulating their release and predicting their potential toxicity.

2. Materials and Methods

The amino acid sequences of five *M. galloprovincialis* proteins were retrieved from the UniProtKB database (https://www.uniprot.org/). P91958, O96064 and Q27409 were tagged as reviewed and manually annotated while for Q8T5C2 there is experimental evidence in protein level (unreviewed) and for Q966V3 there is experimental evidence in transcript level (unreviewed), (Table 1). The protein sequences were analyzed in the

BIOPEP database (http://www.uwm.edu.pl/biochemia/index.php/en/biopep) (Minkiewiczi *et al*. 2019), which contains amino acid sequences of 4325 bioactive peptides (accessed in June 2021). The parameter A which is defined as the frequency of bioactive fragments occurred in the protein sequence was calculated using BIOPEP where A=a/N (a - the number of fragments with given activity in a protein sequence, N - the number of amino acid residues of protein). The physicochemical properties of the *M. galloprovincialis* proteins were calculated using the ProtParam tool of ExPASy (https://web.expasy.org/protparam/). The physicochemical characteristics and potential toxicity of the *in silico*-released antioxidant peptides were estimated using the ToxinPred Tool (https://webs.iiitd.edu.in/raghava/toxinpred/algo.php) (Gupta *et al*. 2013). The proteolytic simulation was performed with the BIOPEP analysis tool.

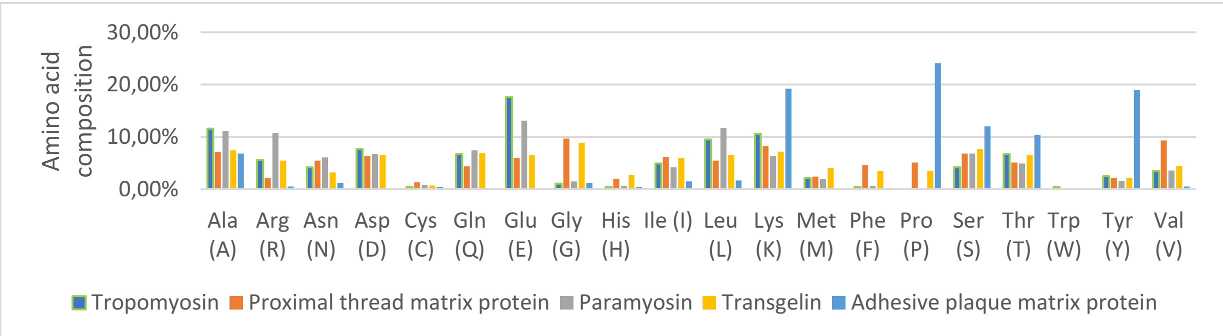
Table 1. *Mytilus galloprovincialis* proteins examined in this study. Physicochemical characteristics. pI: isoelectric point, GRAVY: grand average of hydropathicity

No.	Protein	Accession Number (UniProtKB)	Molecular Weight (kDa)	pI	GRAVY	No. of amino acids
1	Tropomyosin	P91958	32.77	4.62	-1.067	284
2	Proximal thread matrix protein 1	Q8T5C2	48.784	5.59	-0.287	453
3	Paramyosin	O96064	99.572	5.25	-0.98	864
4	Transgelin	Q966V3	44.54	6.85	-0.625	403
5	Adhesive plaque matrix protein	Q27409	85.791	9.99	-1.353	751

3. Results and Discussion

As shown in Figure 1, the Adhesive plaque matrix protein (MGFP1) has a very high composition of proline (approx. 25%), lysine (approx. 20%) and tyrosine (approx. 20%)

Figure 1. Amino acid composition percentages of the investigated proteins. Amino acid codes: Alanine (A), Arginine (R), Asparagine (N), Aspartic acid (D), Cysteine (C), Glutamine (Q), Glutamic acid (E), Glycine (G), Histidine (H), Isoleucine (I), Leucine (L), Lysine (K), Methionine (M), Phenylalanine (F), Proline (P), Serine (S), Threonine (T), Tryptophan (W), Tyrosine (Y), and Valine (V).



Tropomyosin and Paramyosin have a high composition of glutamic acid. The profiles of bioactive peptides encrypted in MGFP1 include indeed proline at a high frequency (data not shown). When bioactive peptides are included as food ingredients a difficulty rises when digested from the receiving organism and there is a chance of deterioration of the bioactivity. Peptides with proline are more resistant to enzymatic digestion and are more prone to conserve their activity (Sarmadi & Ismail 2010). Thus, it can be hypothesised that peptides encrypted in MGFP1 could serve as more effective food ingredients having a higher chance to reach their target. Protein No.5, MGFP1 has the highest number or antioxidant bioactive peptides encrypted in its sequence and the highest frequency of bioactive fragments occurring in the protein. Proteins No. 2, 3 and 4 have a similar A parameter value. (Table 2.) MGFP1is a byssus protein and has bio-adhesive properties. Analogous proteins from *Mytilus edulis* known as Mepf

(Mefp-1 to Mefp-5) are highly resistant to proteolysis (Naik & Hayes 2019) and thus although these proteins are rich in APs, in practice it may be difficult to isolate such peptides.

Table 2. Total count (TC) of antioxidant peptides (APs) encrypted in the analysed proteins. The count includes repeats of APs in the sequence. Parameter A: frequency of occurrence of bioactive fragments in the protein

No.	Protein	APs, TC	A
1	Tropomyosin	15	0.0528
2	Proximal thread matrix protein 1	29	0.0662
3	Paramyosin	48	0.0602
4	Transgelin	25	0.0620
5	Adhesive plaque matrix protein	136	0.1864

Paramyosin is found in very high percentage in oyster and clam smooth adductor muscles and amounts to 38-48% of the myofibril (Harnedy & FitzGerald 2012). Hypothesizing that it can be abundant in *M. galloprovincialis* muscle, it was selected for further analysis (Figure 2).

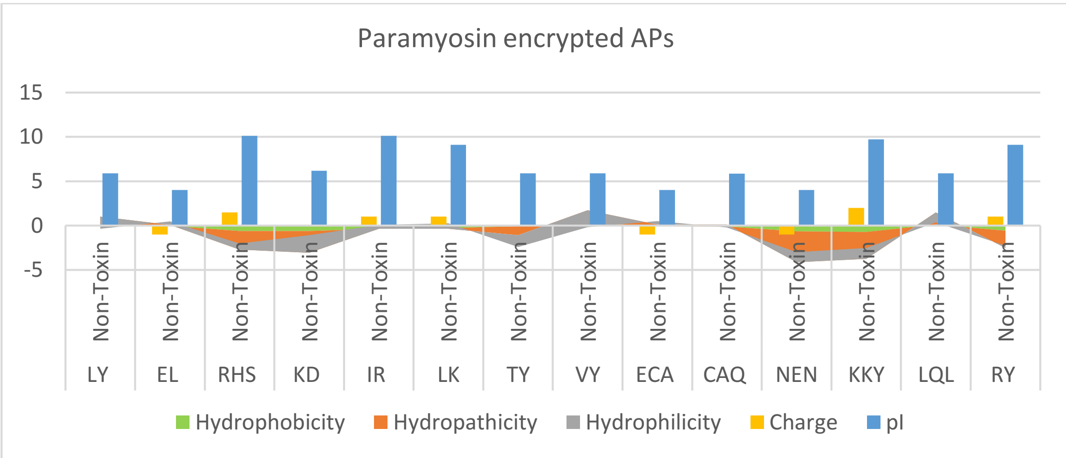


Figure 2. Antioxidant peptides encrypted in Paramyosin: LY, EL, RHS, KD, IR, LK, TY, VY, ECA, CAQ, NEN, KKY, LQL, RY. All peptides are predicted non-toxic.

All APs encrypted in Paramyosin are predicted as non-toxic and their isolectric point ranges from approx. 4-10. As shown in Figure 2, the peptides EL, ECA, NEN have negative charge, while all of the others have a positive or zero charge. Positively charged amino acids inside the peptide sequence is a good indicator of antioxidant activity, together with aromatic and hydrophobic amino acids (Naik & Hayes 2019, Wang *et al.* 2013)

Table 3. Selection of proteolytic enzymes for the isolation of RHS, TY, ECA peptides from Paramyosin, PE: Proteolytic event, N-terminal, C-terminal

Bioactive Peptide, Proteolytic event	Enzyme Name	EC Number	Cutting sequence	Recognition sequence	Location of proteolytic events
RHS, PE N-ter	Proteinase K (Endopeptidase So)	EC.3.4.21.67	V-	V	1N: 18-19
RHS, PE C-ter	coccolysin	EC 3.4.24.30	Y+	Y	1C: 21-22
TY, PE N-ter	trypsin	EC 3.4.21.4	K-	K	2N: 815-816
TY, PE C-ter	subtilisin	EC 3.4.21.62	Y-	Y	2C: 817-818
ECA, PE N-ter	Stem bromelain	EC 3.4.22.32	A-	A	1N: 410-411
ECA, PE C-ter	Stem bromelain	EC 3.4.22.32	A-	A	1C: 413-414

As shown in Table 3, three APs are selected, and a proteolytic event is simulated with specific enzymes. RHS has the basic, positively charged amino acids in physiological pH R, H while ECA contains the hydrophobic amino acid A, properties related to antioxidant activity (Wang et al. 2013). The location of the proteolytic events for the release of the peptides is indicated.

4. References

Avdelas L., Avdic-Mravlje E., Borges Marques A.C., Cano, S., Capelle JJ., Carvalho N., Cozzolino M., Dennis J., Ellis T. et al. (2021). The Decline of Mussel Aquaculture in the European Union: Causes, Economic Impacts and Opportunities. Reviews in Aquaculture 13(1): 91–118. https://onlinelibrary.wiley.com /doi/full/10.1111/raq.12465.

Çelik Meryem Yeşim et al. (2012). Meat Yield, Condition Index, and Biochemical Composition of Mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Sinop, South of the Black Sea. http://dx.doi.org/10.1080/10498850.2011.589099 21(3): 198–205. https://www.tandfonline.com /doi/abs/10.1080/10498850.2011.589099.

FAO (2014). Food and Agriculture Organization of the United Nations, Cultured Aquatic Species Information Programme, *Mytilus galloprovincialis*. http://www.fao.org/fishery/ culturedspecies/ *Mytilus galloprovincialis*/en (accessed: 05-06-2021).

Gupta S., Kapoor P., Chaudhary K., Gautam A., Kumar R et al. (2013). In Silico Approach for Predicting Toxicity of Peptides and Proteins. PLoS ONE 8(9). https://pubmed.ncbi.nlm.nih.gov/24058508/.

Harnedy Pádraigín A., FitzGerald R.J. (2012). Bioactive Peptides from Marine Processing Waste and Shellfish: A Review. Journal of Functional Foods 4(1): 6–24.

Minkiewicz P., Iwaniak A. Darewicz M. (2019). BIOPEP-UWM Database of Bioactive Peptides: Current Opportunities. International Journal of Molecular Sciences 20(23): 5978. https://www.mdpi.com/1422-0067/20/23/5978/htm

Naik A.S., Hayes M. (2019). Bioprocessing of Mussel By-Products for Value Added Ingredients. Trends in Food Science and Technology 92: 111–21.

Sarmadi B.H., Ismael A. (2010). Antioxidative Peptides from Food Proteins: A Review. Peptides 31(10): 1949–56.

Vareltzis P.K., Undeland I. (2012). Protein Isolation from Blue Mussels (*Mytilus edulis*) Using an Acid and Alkaline Solubilisation Technique—Process Characteristics and Functionality of the Isolates. Journal of the Science of Food and Agriculture 92(15): 3055–64. https://onlinelibrary.wiley.com/doi /full/10.1002/jsfa.5723

Vercruysse L, Van Camp J., Smagghe G. (2005). ACE Inhibitory Peptides Derived from Enzymatic Hydrolysates of Animal Muscle Protein: A Review. https://pubs.acs.org/sharingguidelines.

Wang B., Li Li, Chang-Feng C., Jia-Hui M., Hong-Yu L., Yin-feng Xu (2013). Purification and Characterisation of a Novel Antioxidant Peptide Derived from Blue Mussel (*Mytilus edulis*) Protein Hydrolysate. Food Chemistry 138(2–3): 1713–19.

MICROBIAL SPOILAGE AND SHELF-LIFE OF ICE-STORED MEAGRE (*Argyrosomus regius*)

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Abstract
Shelf-life, microbial changes, and microbial communities of whole farmed meagre during storage in ice (two batches, batch A and B, taken in January and July, respectively) were assessed in this study, using sensory, microbiological, and 16S metabarcoding analysis, respectively. Shelf-life found to be 3 days longer for fish caught in January (15 days) compared this observed for fish caught in July (12 days). Total Viable Counts (TVC) at the beginning of storage, was approximately 2-log cfu/g higher in fish caught in July than this found for fish caught in January. Microorganisms reached at population levels >7-log cfu/g at the day 15 for the fish of the batch A and the day 12 for the fish of the batch B. Based on the 16S metabarcoding analysis, two batches presented different microbial composition profiles throughout the storage. Eventually, at the end of fish shelf-life, *Pseudomonas* (47%) and *Psychrobacter* (42.5%) dominated in fish from the batch A (ArW1_D15), while *Pseudomonas* (66.6%) and *Shewanella* (10.5%) dominated in fish from the batch B (ArW2_D12). Our findings will be used to help stakeholders to produce and provide meagre products of a high-quality level in national and international commerce.
Keywords: *Fish, Meagre, Shelf-Life, Spoilage, 16S NGS*
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1. Introduction

Argyrosomus regius (Asso, 1801) is one of the most interesting fish species for the Mediterranean aquaculture due to its biological features e.g., good feed conversion ratio, and quality characteristics e.g., high fillet yield, excellent taste (Duncan *et al.* 2013, Monfort 2010). However, fresh fish spoils very fast mainly due to microbial activity. A consortium of bacteria from the initial fish microbiota (endogenous and exogenous), the so-called Specific Spoilage Organisms (SSOs) can dominate against others from the initial microbial community and produce various metabolites leading to the sensory rejection of the product (Gram & Huss 1996). Next Generation Sequencing (NGS), especially 16S rRNA amplicon-based metabarcoding analysis is now the best way to describe the microbiota of seafood, also including the potential SSOs (Parlapani 2021, Parlapani *et al.* 2018, 2019, 2020).

The aim of this study was a) to record sensory and microbiological changes, and b) to assess microbial communities' composition through 16S metabarcoding, in whole ice-stored meagre. The findings will allow us to help stakeholders (e.g., fish farmers, processors, distributors) towards production and distribution meagre products of a high-quality level in national and international commerce.

2. Materials and Methods

2.1. Provision and storage of meagre

Two different batches of whole meagre of approximately 1-2 kg, were provided from a leading Greek aquaculture company in January (batch A) and July (batch B) 2020. Fish were transferred to the laboratory of Marketing and Technology of Aquatic Products and Foods (University of Thessaly, Volos) in insulated boxes with ice and stored at 0°C.

2.2. Sensory rejection of fish

A panel of five individuals (panelists) evaluated the sensory attributes of the whole meagre using a modification of the Multilingual Guide to EU Freshness Grades for Fishery Products (Howgate *et al.* 1992). More specifically a scale from 5 to 1 was used (5 for the excellent quality (E), 4 for the fresh (A), 3 for the acceptable (B), 2 for the unacceptable (C) and 1 for the totally spoiled fish). As rejection time point was defined the time point that average score was below 3 (which means that at least one out of the five panelists scored with 2).

2.3. Microbiological analysis

Ten grams (10 g) of fish tissue were transferred aseptically to stomacher bags with 90 ml MRD (Maximum Recovery Diluent, 0.1% w/v peptone, 0.85% w/v NaCl) and homogenized for 2 min using a Stomacher (Bug Mixer, Interscience, London, UK). Then, the microorganisms enumerated, using the spread plate technique, as follows: a volume of 0.1 ml of 10-fold serial dilutions was spread on the surface of culture media for the enumeration of Total Viable Counts (TVC) on TSA (Tryptone Soy Agar), incubated at 25°C for 48-72 h, and *Pseudomonas* on cetrimide-fucidin-cephaloridine agar (CFC) incubated at 25°C for 48 h, and using the pour plate technique as follows: a volume of 1 ml was used for the enumeration of H_2S producing bacteria on Iron Agar (IA) by counting only black colonies after incubation at 25°C for 72 h, Enterobacteriaceae on Violet Red Bile Glucose agar (VRBGA), after incubation at 37°C for 24 h and Lactic Acid Bacteria on De Man, Rogosa, Sharpe agar (MRS) after incubation at 25°C for 72 h.

All microbiological media were supplied from LAB M (Lancashire, UK). The results were expressed as mean log cfu/g ± standard deviation (log colony forming unit per g) of four replicates per batch.

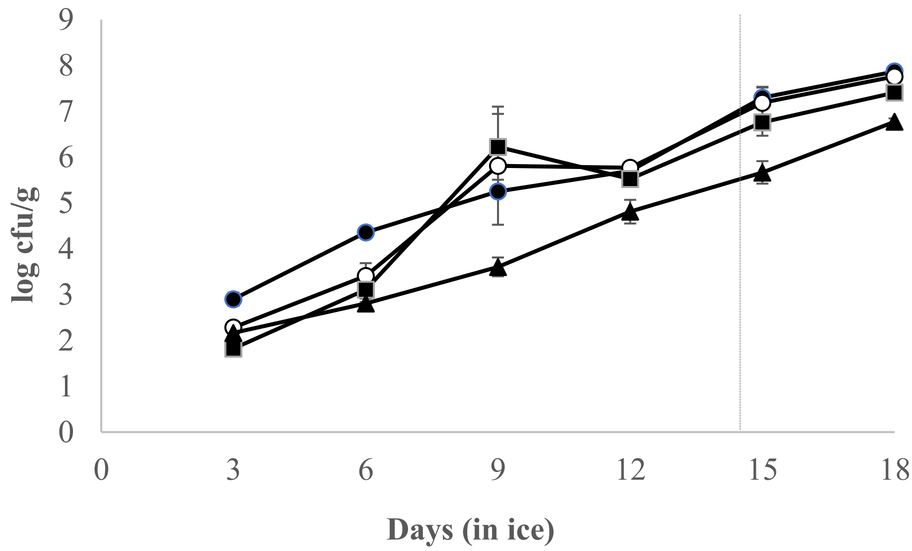
2.4 16S metabarcoding

Before the 16S metabarcoding analysis, fish samples (taken from Day 3, 6, 12 and 17) were prepared according to Parlapani *et al*. (2018). For each sample, 200 µl of diluted pellet were used for bacterial DNA extraction with the NucleoSpin Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). according to the manufacturer's instructions. DNA concentration was measured on a nanodrop Quawell UV-Vis Spectrophotometer Q5000 (Quawell Technology, Inc., USA). The metabarcoding analysis was performed with the Illumina protocols using the primers 27F (AGRGTTTGATCMTGGCTCAG) and 519Rmodbio (GWATTACCGCGGCKGCTG). Sequencing was applied on a MiSeq Illumina platform according to manufacturer's protocols. Bioinformatic analysis was performed using the MR DNA pipelines (MR DNA, Shallowater, TX).

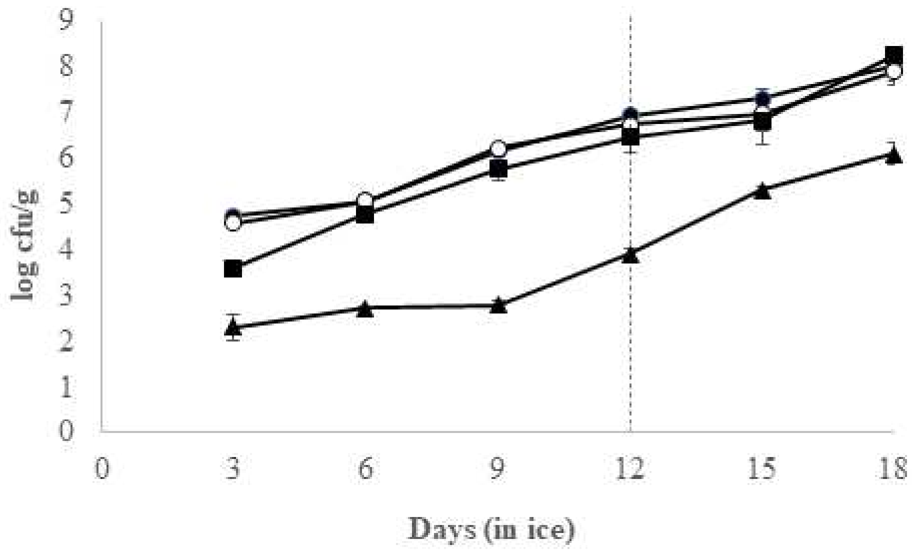
3. Results

Microbial populations of the whole meagre during storage in ice

TVC of the whole meagre was initially 2.90±0.14 log cfu/g and 4.73±0.10 log cfu/g, for fish from the batch A (Fig 1a) and batch B (Fig 1b), respectively. TVC, *Pseudomonas* sp., H_2S-producing bacteria, and LAB presented statistically significant difference ($p<0.05$) in their populations between the two batches, throughout the storage. More specifically, the microbial populations of fish from the batch B exceeded the population level of 7 logs faster than fish from the batch A (Fig 1). Eventually, microorganisms reached at high population levels (>7 logs) at the day 15 for the fish of the batch A (shelf-life of fish batch taken in January, based on sensory analysis) and the day 12 for the fish of the batch B (shelf-life of fish batch taken in July, based on sensory analysis).



(a)



(b)

Figure 1. Microbial population changes of the whole ice-stored meagre taken in (a) January (batch A), and (b) July (batch B). TVC on TSA (●), *Pseudomonas* on CFC (o), H_2S producing bacteria (■) and LAB on MRS (▲). The dashed vertical line indicates the end of shelf life.

Microbial diversity of meagre during storage in ice through 16s metabarcoding

Initially (day 3), *Ralstonia* (37.5%), *Novosphingobium* (24.2%), *Sphingomonas* (16.8%) and *Clostridium* (10.1%) found to dominate in fresh whole meagre from the batch A, while other bacterial genera found at lower abundances (Fig 2). At the end of fish shelf-life (day 15), *Pseudomonas* (47%) and *Psychrobacter* (42.5%) dominated (ArW1_D15). In fish from the batch B, *Clostridium* (43.5%) and *Propionibacterium* (25.9%) were the most abundant bacterial genera. At the end of fish shelf-life (day 12), *Pseudomonas* (66.6%) and *Shewanella* (10.5%) were found to dominate in whole meagre from the batch B (ArW2_D12).

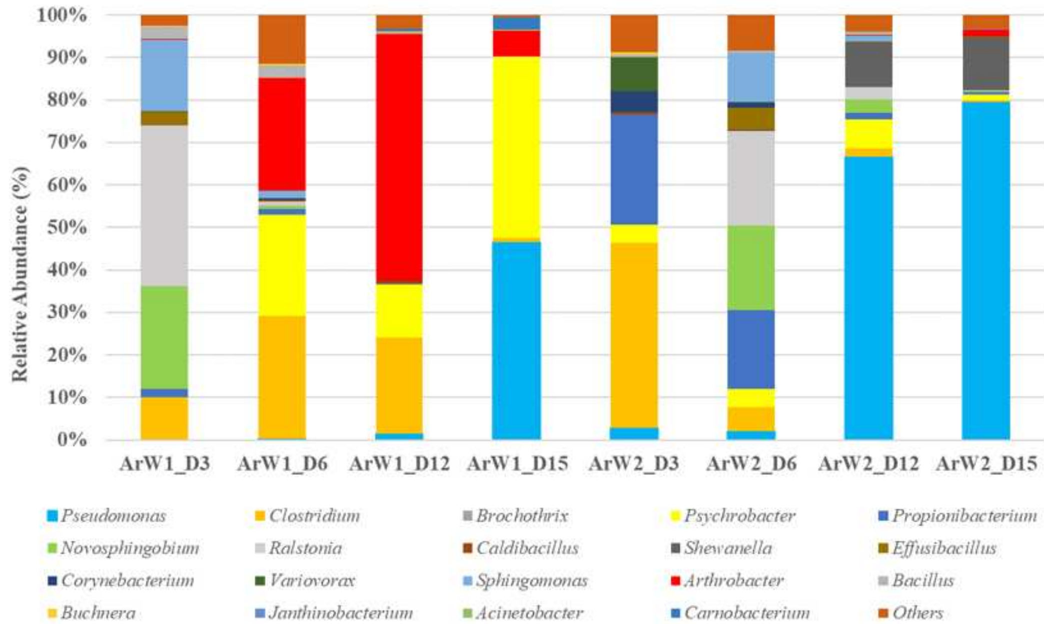


Figure 2. Relative abundance (%) of top-20 bacterial genera of whole ice-stored meagre from the batch A (ArW1) and B (ArW2), using the metabarcoding analysis of 16S rRNA gene.

4. Discussion

Shelf-life, microbial changes, and microbial communities of whole meagre during storage in ice were described herein. Shelf-life found to differ between fish from two batches, batch A and B, taken in January and July, respectively. More specifically, shelf-life was 3 days longer for fish caught in January (15 days) than this observed for fish caught in July (12 days). It is known that shelf-life of fish depends on various factors e.g., the initial microbial population level, the initial microbial composition, the storage conditions (temperature, atmosphere), and so on (Parlapani 2021). Indeed, TVC was approximately 2 logs higher in fish caught in July than this found for fish caught in January. This means that, under the same storage conditions, fish with a higher initial TVC might get spoiled faster than fish with a lower or much lower TVC level.

However, shelf-life also depends on initial microbial composition. The domination of particular bacteria during the storage depends on the interaction behaviors with the rest of the microbiota. Herein, fish from the two batches carried different bacteria as dominants initially. The different microbial composition profiles between the two batches, might lead to the prevailing of different bacterial genera at the end of fish shelf-life in batch A and B. The domination of different bacteria during fish storage is very important since we know that bacteria, even these with a high level of homology each other, can present different spoilage potential and/or activity, thus leading to the production of different metabolites and different sensory changes (Boziaris & Parlapani 2017, Parlapani *et al.* 2017).

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References

Boziaris I.S., Parlapani F.F. (2017). Specific Spoilage Organisms (SSO) in Fish. In: Microbiological Quality of Food: Foodborne Spoilers. Edited by A. Bevilacqua, M.R. Corbo, M. Sinigaglia, Sykes R. Elsevier, Woodhead Publishing, 60-98.

Duncan N.J., Estévez A., Fernández-Palacios H., Gairin I., Hernández-Cruz C.M., Roo J., Schuchardt D., Vallés R. (2013). Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: Woodhead Publishing Series in Food Science, Technology and Nutrition, Advances in Aquaculture Hatchery Technology. Edited by Geoff Allan, Gavin Burnell. Woodhead Publishing, 519-541.

Gram L, Huss HH. (1996) Microbiological spoilage of fish and fish products. International Journal of Food Microbiology, 33(1), 121-37.

Monfort M.C. (2010). Present market situation and prospects of meagre (*Argyrosomus regius*), as an emerging species in Mediterranean aquaculture. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 89. Rome, FAO. 2010. 28p.

Parlapani F.F, Michailidou S. Pasentsis K., Argiriou A., Krey, G., Boziaris I. S. (2018). A meta-barcoding approach to assess and compare the storage temperature-dependent bacterial diversity of gilt-head sea bream (*Sparus aurata*) originating from fish farms from two geographically distinct areas of Greece. International Journal of Food Microbiology 278, 36-43.

Parlapani F.F. (2021). Microbial Diversity of Seafood. Current Opinion in Food Science, 37, 45-51.

Parlapani F.F., Anagnostopoulos D.A., Koromilas S., Kios K., Michailidou S., Pasentsis K., Psomopoulos F., Argiriou A., Haroutounian S.A., Boziaris I.S. (2019). Bacterial communities and potential spoilage markers of whole Blue Crab (*Callinectes sapidus*) stored under commercial simulated conditions. Food Microbiology 82, 325-333.

Parlapani F.F., Ferrocino I., Michailidou S., Argiriou A., Haroutounian S.A., Kokokiris L., Rantsiou K., Boziaris I.S. (2020). Microbiota and volatilome profile of fresh and chill-stored deepwater rose shrimp (*Parapenaeus longirostris*). Food Research International 132, 109057.

Parlapani F.F., Malouchos A., Haroutounian S.A., Boziaris I.S. (2017). Microbial and non-microbial origin volatile organic compounds produced on model fish substrate inoculated or not with gilt-head sea bream spoilage bacteria. LWT - Food Science and Technology 78, 54-62.

MICROBIAL SPOILAGE AND SHELF-LIFE OF ICE-STORED MEAGRE (*Argyrosomus regius*)

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Abstract
Mislabeling of seafood products and marketing of protected species remains a worldwide issue despite the labeling regulations set at local and international level. DNA barcoding has proven to be the most popular and accurate method of detection of fraudulent seafood products. This study investigated the batoid meat market of Greece, themislabeling rates and the protected species occurrence. The cytochrome oxidase subunit I (COI) gene was used toanalyze the samples. The sequences were compared against genetic databases for species identification. At least 13species across nine genera were identified. Moderate mislabeling levels were discovered (13.5%), while low levelsof protected species were recorded (3.5%), confirming an ongoing market for protected species.
Keywords: *Batoidea, Seafood labeling, Mitochondrial DNA, Species identification, Greek market*
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1. Introduction

Global elasmobranchs catches (sharks, rays, and skates) have increased over the last 40 years, threatening many species with the risk of extinction (Davidson *et al.* 2016), even in areas that are historically characterized by high diversity and abundance of elasmobranchs, as the Mediterranean Sea (Serena *et al.* 2020). Their biological characteristics, such as late sexual maturity, prolonged gestation period, low fertility, and long-life span, renders them vulnerable to anthropogenic pressures (Seitz & Poulakis 2006, Dulvy *et al.* 2014). Morphological identification is impossible for the majority of batoid meat sold in the Greek seafood market, due to heavy processing, similar to shark meat (Pazartzi *et al.* 2019). All external traits and characteristics are removed and only the wings (the large, muscular pectoral fins) are kept and sold. The use of broad, "umbrella" labeling terms for batoids is permitted, with a variety of species being grouped under a single category (Official Government Gazette 475/Issue B'/27-3-2015).

Along with poor catch reporting, serious population declines of vulnerable species are effectively masked under more abundant groups (Dulvy *et al.* 2000), whereas mislabeling occurs when one species is substituted and traded under the name of another (Rasmussen & Morrissey 2008). Unintentional and/or fraudulent mislabeling are still under investigation in Greece (Minoudi *et al.* 2020). The aim of this study was to investigate the batoid meat trade in Greece and aid in the detection of illegally traded species that are protected by international and national legislation using DNA barcoding methods.

2. Materials and Methods

A total of 114 tissue samples were collected from products sold under a range of labels. They were obtained from various retailers, located in eight Greek cities (Alexandroupolis, Athens, Kavala, Katerini, Komotene, Mytilene, Rethymno and Thessaloniki).

Genomic DNA was extracted from approximately 25 mg. of tissue, using the Chelex 100 DNA extraction protocol (Walsh *et al.* 1991). The COI gene was utilized (Serra-Pereira *et al.* 2010), and a 670 bp segment of the gene was amplified by polymerase chain reaction (PCR). Sequences were manually checked and edited using ProSeq 3.0 (Filatov 2002) and BioEdit 7.2.6 (Hall 1999). Statistical analyses were conducted in R 3.5.1 (https://cran.r-project.org/) and PAST-6 software (Hammer *et al.* 2001). Any effect of the city/location and the label of patterns of batoids on sale was tested, performing a non-parametric analysis of similarity (ANOSIM)) using the Bray-Curtis distance measure. The contribution of variables to similarity (SIMPER analysis) was also calculated.

3. Results

Species identification

111 batoid products, out of 114 samples in total, were successfully identified with at least 13 species across nine genera detected. This number corresponds to 34.2% of the 38 species currently found in the Mediterranean (Serena *et al.* 2020), and 43.3% of the 30 species recorded in Greece (Chatzispyrou *et al.* 2020). The most frequent species traded was the thornback ray (*Raja clavata,* n = 77, 69.4%), followed by the rough ray (*Raja radula*, n = 10, 8.1%) and individuals of the genus *Dasyatis* (*Dasyatis* spp., n=6) (Figure 1).

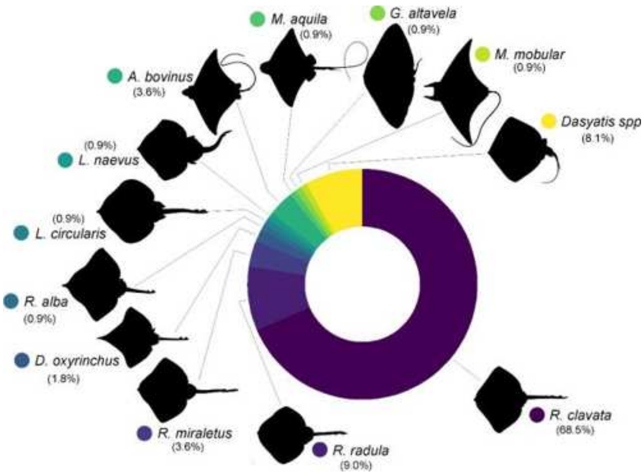

Figure 1: Composition of the identified species in this study. Species belonging to the Dasyatidae family were combined under one category (*Dasyatis* spp.).

Species level identification was possible for 96.4% of the samples proving impossible for most of the Dasyatidae family, mainly due to the taxonomic ambiguity and misnomers in the existing databases. From 111 identified samples, 15 cases (13.5%) of mislabeling were detected between the name reported on the label and the species identified (Figure 2a, Table 1). Previous studies, Pazartzi *et al.* (2019) demonstrated high levels of mislabeling at around 56% in shark filets, while Minoudi *et al.* (2020) reported lower levels of mislabeling among elasmobranchs in Greece. This investigation on rays and skates follows a similar trend.

Table 1. Number of samples, species, protection and mislabeling rates per sampling location. Species of the Dasyatidae family were combined under one category (*Dasyatis* spp.).

City	Number of samples	Number of species	Mislabeled	Protected species
Alexandroupolis (AL)	13	2	-	-
Athens (AT)	9	5	3	2
Kavala (KA)	25	4	-	-
Katerini (KAT)	1	1	-	-
Komotene (KO)	14	4	2	1
Mytilene (MYT)	14	6	7	1
Rethymno (RE)	3	2	3	-
Thessaloniki (TH)	35	4	-	-

Among the samples, species protected by international and national legislation were accounted for 3.6% of the samples (four cases) (Figure 2b). All protected batoids species encountered in the Mediterranean, are listed in the Endangered categories by the Red List of threatened species of the IUCN for the Mediterranean Sea (Dulvy *et al.* 2016). In view of their overall low abundance in the Mediterranean and limited records in the Greek seas, the relatively low number of protected species among our samples may simply reflect their rarity, rather than the successful implementation of the legal protection and prohibitions on landings.

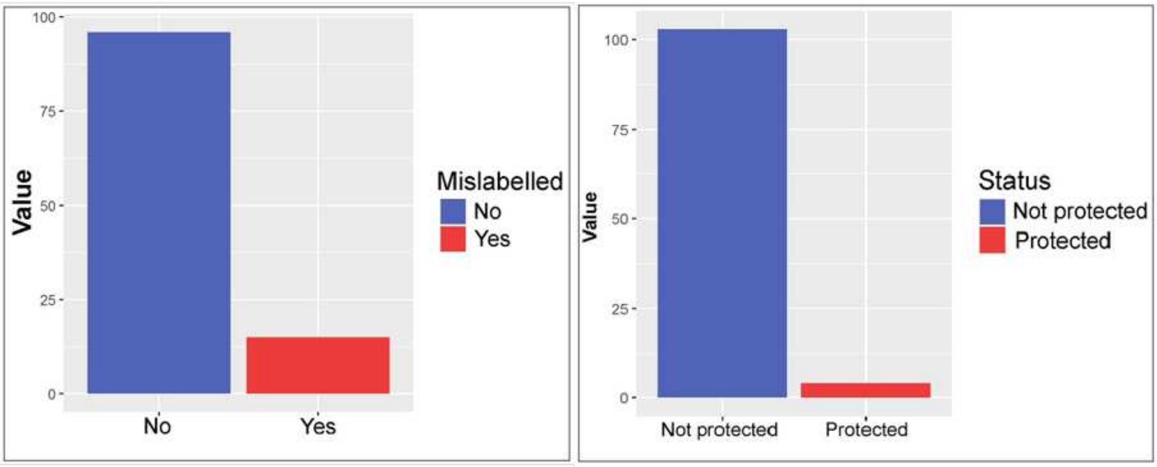

Figure 2. Composition of mislabeled samples (a) and protection status (b) of samples identified in this study

Additionally, any species being listed as "Vulnerable", "Endangered", and "Critically Endangered" in the Red List of threatened species of the IUCN for the Mediterranean Sea (Dulvy *et al.* 2016), were identified and accounted for 19.8% of the samples (22 cases, Figure 3). This study demonstrates that vulnerable, threatened, and protected rays and skates are finding their way into the Greek seafood market. Additionally, current legislation leaves vulnerable species with declining populations unprotected.

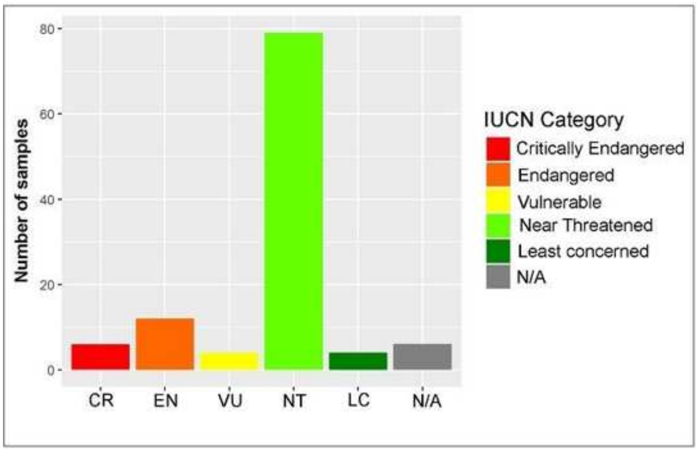

Figure 3. Identified species by conservation status for the Mediterranean according to Dulvy *et al.* (2016). N/A classification refers to the Dasyatis spp. as more than one species are included.

Species comparison among cities and retailer types

Mytilene showed the highest diversity of species identified (number of species = 6), followed by Athens (number of species = 5), whereas Thessaloniki, Kavala and Komotene had the same number (four) (Table 1). The highest level of mislabeling was detected in Mytilene (n = 7), followed by Athens and Rethymno with 3 cases.

Our analysis was not able to identify significant differences between locations, retailers and label types. However, the pairwise comparisons identified significant differences between “salachi” and all other labels, as well as between Athens and all other locations. Interestingly, the labeling of batoid meat in the country appears to be mostly affected by the location rather than the labeling legislation that currently exists. The term “vatos” appears to be more common in the North of the country, while the South (i.e., Athens), the most popular designation is the term “salachi”.

It is important to note that in Southern Greece batoid meat consumption is less common, with some areas not consuming it at all. Similarly, the term “trigona” was only encountered in the city of Mytilene, an island of the North Aegean Sea. As expected, higher mislabeling rates were recorded in Athens and Mytilene compared to all other locations, where a single term is utilized in markets for all batoid meat products (salachi and trigona respectively). Whereas in Northern Greece, the term “vatos” was the most utilized label, which is the legal designation for all *Raja* spp. And *Rostroraja* spp. species, and therefore mislabeling cases were not frequent.

4. Discussion

This study assessed the species composition, level of mislabeling and numbers of protected species in the fish market of Greece, using DNA barcoding. The use of the COI gene was largely successful, as has been demonstrated by previous studies on a range of elasmobranch meat products. The sale of at least 13 species of batoids sold under a variety of commercial terms in Greece were revealed, a number that corresponds to 34.2% of the 38 species currently found in the Mediterranean, and 43.3% of the 30 species recorded in Greece (Chatzispyrou *et al.,* 2020, Papaconstantinou 2014). The thornback ray (*R. clavata*) was the most identified species, representing 69.4% of the samples analyzed. Species level identification was possible in 96.4% of the samples. This was not possible for most samples from the Dasyatidae family, mainly due to the taxonomic ambiguity and misnomers in the existing databases. Due to the scarcity of available research, whether seafood mislabeling is considered common in Greece is not yet known and is currently under investigation. Four species (*Leucoraja circularis*, *Rostroraja alba*, *Gymnura altavela*, and *Mobula mobular*) that are currently protected by international and national legislation were identified, and therefore were illegally caught and landed in Greek markets.

Overall, moderate rates of mislabeling were detected compared to previous studies for elasmobranchs, with the higher rates to be detected in areas where a single term is predominantly used for all batoid meat products. This type of mislabeling could be unintentional and mostly related to marketing practices in the area as well as low consumer awareness. Labeling legislation should become more species specific, leading to the expansion of the list of species protected. In future, it is important to implement similar DNA barcoding studies including more regions and market types, i.e., restaurants, where the lack of labeling regulations renders species substitution easier.

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References

Chatzispyrou A., Gubili C., Laiaki M., Mantopoulou-Palouka D., Kavadas S. (2020). First record of the marbled ray, *Dasyatis marmorata* (Elasmobranchii: Dasyatidae), from Greece (central Aegean Sea). Biodiversity data Journal, 8, e51100

Davidson L.N.K., Krawchuk M.A., Dulvy N.K. (2016). Why have global shark and ray landings declined: improved management or overfishing? Fish Fish, 17, 438-458. https://doi.org/10.1111/faf.12119

Dulvy N.K., Allen D.J., Ralph G.M., Walls R. (2016). The conservation status of sharks, rays and chimaeras in the Mediterranean Sea [brochure]. Malaga, Spain: IUCN.

Dulvy N.K., Fowler S.L., Musick J.A., Cavanagh R.D., Kyne P.M., Harrison L.R., Fordham S.V. (2014). Extinction risk and conservation of the world's sharks and rays. eLife, 3, e00590.

Dulvy N.K., Metcalfe J.D., Glanville J., Pawson M.G., Reynolds J.D. (2000). Fishery stability, local extinctions, and shifts in community structure in skates. Conservation Biology, 14, 283–293.

Filatov D.A. (2002). ProSeq: A software for preparation and evolutionary analysis of DNA sequence data sets. Molecular Ecology, 2, 621–624.

Hall T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

Hammer Ø., Harper D.A.T., Ryan P.D. (2001). PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9.

Minoudi S., Karaiskou N., Avgeris M., Gkagkavouzis K., Tarantili P., Triantafyllidou D., Palilis L., AvramopoulouV., Tsikliras A., Barmperis K., Triantafyllidis A. (2020). Seafood mislabeling in Greek market using DNA barcoding. Food Control, 113.

Pazartzi T., Siaperopoulou S., Gubili C., Maradidou S., Loukovitis D., Chatzispyrou A., Griffiths A.M., Minos D., Imsiridou A. (2019). High levels of mislabeling in shark meat – Investigating patterns of species utilization with DNA barcoding in Greek retailers. Food Control, 98, 179-186.

Rasmussen R.S., Morrissey M.T. (2008). DNA-Based Methods for the Identification of Commercial Fish and Seafood Species. Comprehensive Reviews in Food Science and Food Safety, 7(3), 280-295.

Seitz J.C., Poulakis G.R. (2006). Anthropogenic effects on the smalltooth sawfish (*Pristis pectinata*) in the United States. Marine Pollution Bulletin, 52, 1533–1540.

Serena F., Abella A.J., Bargnesi F., Barone M., Colloca F., Ferretti F., Fiorentino F., Jenrette J., Moro S. (2020). Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea. The European Zoological Journal, 87, 497-536. https://doi.org/10.1080/24750263.2020.1805518

Serra-Pereira B., Moura T., Griffiths A.M., Gordo L.S. Figueiredo I. (2010). Molecular barcoding of skates (Chondrichthyes: Rajidae) from the southern Northeast Atlantic. Zoologica Scripta, 40, 76–84.

Walsh P.S., Metzger D.A., Higuchi R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR- based typing from forensic material. BioTechniques, 10, 506–513.

“PROTECTION” UNDER THE SHADE: LACK OF LABELLING LEGISLATION AND UMBRELLA TERMS ALLOW PROTECTED SPECIES IN BATOID WINGS FROM GREECE

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1. Introduction

Global elasmobranchs catches (sharks, rays, and skates) have increased over the last 40 years, threatening many species with the risk of extinction (Davidson *et al.* 2016), even in areas that are historically characterized by high diversity and abundance of elasmobranchs, as the Mediterranean Sea (Serena *et al.* 2020). Their biological characteristics, such as late sexual maturity, prolonged gestation period, low fertility, and long-life span, renders them vulnerable to anthropogenic pressures (Seitz & Poulakis 2006, Dulvy *et al.* 2014). Morphological identification is impossible for the majority of batoid meat sold in the Greek seafood market, due to heavy processing, similar to shark meat (Pazartzi *et al.* 2019). All external traits and characteristics are removed and only the wings (the large, muscular pectoral fins) are kept and sold. The use of broad, “umbrella” labeling terms for batoids is permitted, with a variety of species being grouped under a single category (Official Government Gazette 475/Issue B’/27-3-2015).

Along with poor catch reporting, serious population declines of vulnerable species are effectively masked under more abundant groups (Dulvy *et al.* 2000), whereas mislabeling occurs when one species is substituted and traded under the name of another (Rasmussen & Morrissey 2008). Unintentional and/or fraudulent mislabeling are still under investigation in Greece (Minoudi *et al.* 2020). The aim of this study was to investigate the batoid meat trade in Greece and aid in the detection of illegally traded species that are protected by international and national legislation using DNA barcoding methods.

2. Materials and Methods

A total of 114 tissue samples were collected from products sold under a range of labels. They were obtained from various retailers, located in eight Greek cities (Alexandroupolis, Athens, Kavala, Katerini, Komotene, Mytilene, Rethymno and Thessaloniki).

Genomic DNA was extracted from approximately 25 mg. of tissue, using the Chelex 100 DNA extraction protocol (Walsh *et al.* 1991). The COI gene was utilized (Serra-Pereira *et al.* 2010), and a 670 bp segment of the gene was amplified by polymerase chain reaction (PCR). Sequences were manually checked and edited using ProSeq 3.0 (Filatov 2002) and BioEdit 7.2.6 (Hall 1999). Statistical analyses were conducted in R 3.5.1 (https://cran.r-project.org/) and PAST-6 software (Hammer *et al.* 2001). Any effect of the city/location and the label of patterns of batoids on sale was tested, performing a non-parametric analysis of similarity (ANOSIM)) using the Bray-Curtis distance measure. The contribution of variables to similarity (SIMPER analysis) was also calculated.

3. Results

Species identification

111 batoid products, out of 114 samples in total, were successfully identified with at least 13 species across nine genera detected. This number corresponds to 34.2% of the 38 species currently found in the Mediterranean (Serena *et al.* 2020), and 43.3% of the 30 species recorded in Greece (Chatzispyrou *et al.* 2020). The most frequent species traded was the thornback ray (*Raja clavata,* n = 77, 69.4%), followed by the rough ray (*Raja radula*, n = 10, 8.1%) and individuals of the genus *Dasyatis* (*Dasyatis* spp., n=6) (Figure 1).

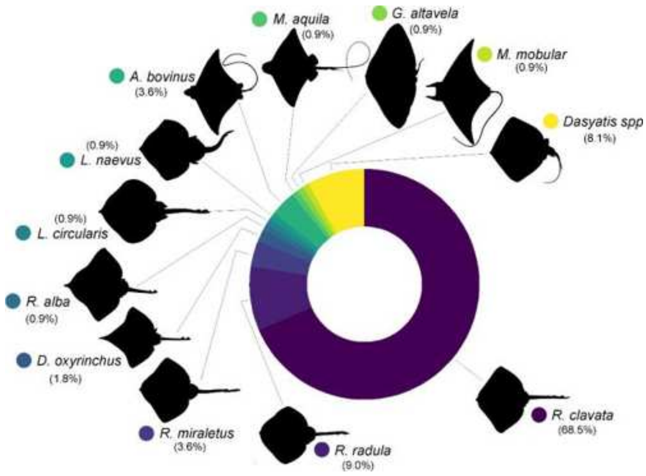

Figure 1: Composition of the identified species in this study. Species belonging to the Dasyatidae family were combined under one category (*Dasyatis* spp.).

Species level identification was possible for 96.4% of the samples proving impossible for most of the Dasyatidae family, mainly due to the taxonomic ambiguity and misnomers in the existing databases. From 111 identified samples, 15 cases (13.5%) of mislabeling were detected between the name reported on the label and the species identified (Figure 2a, Table 1). Previous studies, Pazartzi *et al.* (2019) demonstrated high levels of mislabeling at around 56% in shark filets, while Minoudi *et al.* (2020) reported lower levels of mislabeling among elasmobranchs in Greece. This investigation on rays and skates follows a similar trend.

Table 1. Number of samples, species, protection and mislabeling rates per sampling location. Species of the Dasyatidae family were combined under one category (*Dasyatis* spp.).

City	Number of samples	Number of species	Mislabeled	Protected species
Alexandroupolis (AL)	13	2	-	-
Athens (AT)	9	5	3	2
Kavala (KA)	25	4	-	-
Katerini (KAT)	1	1	-	-
Komotene (KO)	14	4	2	1
Mytilene (MYT)	14	6	7	1
Rethymno (RE)	3	2	3	-
Thessaloniki (TH)	35	4	-	-

Among the samples, species protected by international and national legislation were accounted for 3.6% of the samples (four cases) (Figure 2b). All protected batoids species encountered in the Mediterranean, are listed in the Endangered categories by the Red List of threatened species of the IUCN for the Mediterranean Sea (Dulvy *et al.* 2016). In view of their overall low abundance in the Mediterranean and limited records in the Greek seas, the relatively low number of protected species among our samples may simply reflect their rarity, rather than the successful implementation of the legal protection and prohibitions on landings.

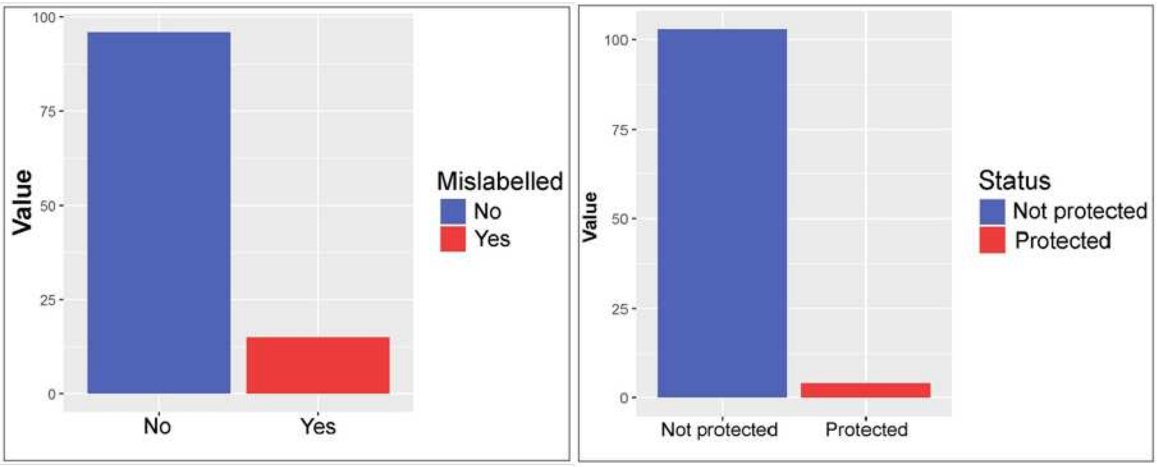

Figure 2. Composition of mislabeled samples (a) and protection status (b) of samples identified in this study

Additionally, any species being listed as "Vulnerable", "Endangered", and "Critically Endangered" in the Red List of threatened species of the IUCN for the Mediterranean Sea (Dulvy *et al.* 2016), were identified and accounted for 19.8% of the samples (22 cases, Figure 3). This study demonstrates that vulnerable, threatened, and protected rays and skates are finding their way into the Greek seafood market. Additionally, current legislation leaves vulnerable species with declining populations unprotected.

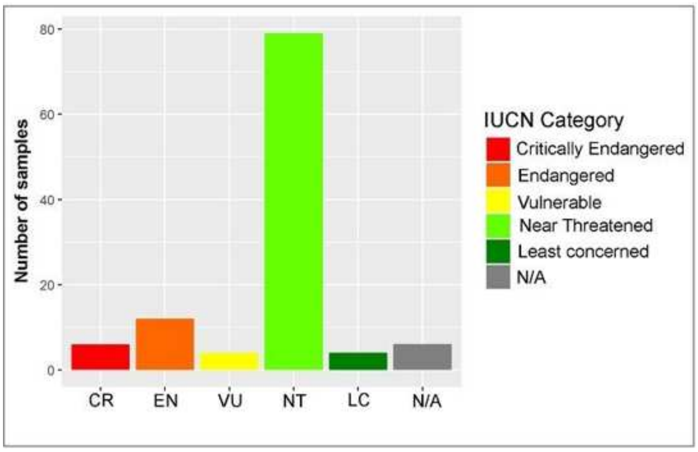

Figure 3. Identified species by conservation status for the Mediterranean according to Dulvy *et al.* (2016). N/A classification refers to the Dasyatis spp. as more than one species are included.

Species comparison among cities and retailer types

Mytilene showed the highest diversity of species identified (number of species = 6), followed by Athens (number of species = 5), whereas Thessaloniki, Kavala and Komotene had the same number (four) (Table 1). The highest level of mislabeling was detected in Mytilene (n = 7), followed by Athens and Rethymno with 3 cases.

Our analysis was not able to identify significant differences between locations, retailers and label types. However, the pairwise comparisons identified significant differences between “salachi” and all other labels, as well as between Athens and all other locations. Interestingly, the labeling of batoid meat in the country appears to be mostly affected by the location rather than the labeling legislation that currently exists. The term “vatos” appears to be more common in the North of the country, while the South (i.e., Athens), the most popular designation is the term “salachi”.

It is important to note that in Southern Greece batoid meat consumption is less common, with some areas not consuming it at all. Similarly, the term “trigona” was only encountered in the city of Mytilene, an island of the North Aegean Sea. As expected, higher mislabeling rates were recorded in Athens and Mytilene compared to all other locations, where a single term is utilized in markets for all batoid meat products (salachi and trigona respectively). Whereas in Northern Greece, the term “vatos” was the most utilized label, which is the legal designation for all *Raja* spp. And *Rostroraja* spp. species, and therefore mislabeling cases were not frequent.

4. Discussion

This study assessed the species composition, level of mislabeling and numbers of protected species in the fish market of Greece, using DNA barcoding. The use of the COI gene was largely successful, as has been demonstrated by previous studies on a range of elasmobranch meat products. The sale of at least 13 species of batoids sold under a variety of commercial terms in Greece were revealed, a number that corresponds to 34.2% of the 38 species currently found in the Mediterranean, and 43.3% of the 30 species recorded in Greece (Chatzispyrou *et al.,* 2020, Papaconstantinou 2014). The thornback ray (*R. clavata*) was the most identified species, representing 69.4% of the samples analyzed. Species level identification was possible in 96.4% of the samples. This was not possible for most samples from the Dasyatidae family, mainly due to the taxonomic ambiguity and misnomers in the existing databases. Due to the scarcity of available research, whether seafood mislabeling is considered common in Greece is not yet known and is currently under investigation. Four species (*Leucoraja circularis*, *Rostroraja alba*, *Gymnura altavela*, and *Mobula mobular*) that are currently protected by international and national legislation were identified, and therefore were illegally caught and landed in Greek markets.

Overall, moderate rates of mislabeling were detected compared to previous studies for elasmobranchs, with the higher rates to be detected in areas where a single term is predominantly used for all batoid meat products. This type of mislabeling could be unintentional and mostly related to marketing practices in the area as well as low consumer awareness. Labeling legislation should become more species specific, leading to the expansion of the list of species protected. In future, it is important to implement similar DNA barcoding studies including more regions and market types, i.e., restaurants, where the lack of labeling regulations renders species substitution easier.

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References

Chatzispyrou A., Gubili C., Laiaki M., Mantopoulou-Palouka D., Kavadas S. (2020). First record of the marbled ray, *Dasyatis marmorata* (Elasmobranchii: Dasyatidae), from Greece (central Aegean Sea). Biodiversity data Journal, 8, e51100

Davidson L.N.K., Krawchuk M.A., Dulvy N.K. (2016). Why have global shark and ray landings declined: improved management or overfishing? Fish Fish, 17, 438-458. https://doi.org/10.1111/faf.12119

Dulvy N.K., Allen D.J., Ralph G.M., Walls R. (2016). The conservation status of sharks, rays and chimaeras in the Mediterranean Sea [brochure]. Malaga, Spain: IUCN.

Dulvy N.K., Fowler S.L., Musick J.A., Cavanagh R.D., Kyne P.M., Harrison L.R., Fordham S.V. (2014). Extinction risk and conservation of the world's sharks and rays. eLife, 3, e00590.

Dulvy N.K., Metcalfe J.D., Glanville J., Pawson M.G., Reynolds J.D. (2000). Fishery stability, local extinctions, and shifts in community structure in skates. Conservation Biology, 14, 283–293.

Filatov D.A. (2002). ProSeq: A software for preparation and evolutionary analysis of DNA sequence data sets. Molecular Ecology, 2, 621–624.

Hall T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

Hammer Ø., Harper D.A.T., Ryan P.D. (2001). PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9.
Minoudi S., Karaiskou N., Avgeris M., Gkagkavouzis K., Tarantili P., Triantafyllidou D., Palilis L., AvramopoulouV., Tsikliras A., Barmperis K., Triantafyllidis A. (2020). Seafood mislabeling in Greek market using DNA barcoding. Food Control, 113.
Pazartzi T., Siaperopoulou S., Gubili C., Maradidou S., Loukovitis D., Chatzispyrou A., Griffiths A.M., Minos D., Imsiridou A. (2019). High levels of mislabeling in shark meat – Investigating patterns of species utilization with DNA barcoding in Greek retailers. Food Control, 98, 179-186.
Rasmussen R.S., Morrissey M.T. (2008). DNA-Based Methods for the Identification of Commercial Fish and Seafood Species. Comprehensive Reviews in Food Science and Food Safety, 7(3), 280-295.
Seitz J.C., Poulakis G.R. (2006). Anthropogenic effects on the smalltooth sawfish (*Pristis pectinata*) in the United States. Marine Pollution Bulletin, 52, 1533–1540.
Serena F., Abella A.J., Bargnesi F., Barone M., Colloca F., Ferretti F., Fiorentino F., Jenrette J., Moro S. (2020). Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea. The European Zoological Journal, 87, 497-536. https://doi.org/10.1080/24750263.2020.1805518
Serra-Pereira B., Moura T., Griffiths A.M., Gordo L.S. Figueiredo I. (2010). Molecular barcoding of skates (Chondrichthyes: Rajidae) from the southern Northeast Atlantic. Zoologica Scripta, 40, 76–84.
Walsh P.S., Metzger D.A., Higuchi R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR- based typing from forensic material. BioTechniques, 10, 506–513.

AMINO ACID COMPOSITION OF SEAWEEDS FROM ÇANAKKALE, TURKEY

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Abstract
In this study, the amino acid compositions of nine macroalgae species distributed along with the costs of Çanakkale, Turkey was evaluated. Total amino acid content was found highest in the *Flabellia petiolata* with 14.719 g/100g, and followed by *Asparagopsis armata* and *Caulerpa racemosa* as 11.031 g/100g and 10.280 g/100g, respectively. The aspartic acid + asparagine and glutamic acid + glutamine levels were high in all species. The percentage of essential amino acids of *Hypnea musciformis* (60.02%) was higher than other macroalgae species. And, according to our results, the analyzed seaweeds could be a good source as an alternative and sustainable source of amino acids for human nutrition and industrial food processing.
Keywords: *Macroalgae, Amino Acids, Extraction, Functional Food, Nutrition*
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1. Introduction

Macroalgae, which are crucial elements for biodiversity, provide feeding and sheltering areas for marine organisms and also, they play an essential role as carbon sequesters (Cirik & Cirik 2011, Froehlich*et al.* 2019). Besides, they contain economically valuable polysaccharides such as agar-agar, alginate, and carrageenan (Ak 2015, Necas & Bartosikova 2013, Öztaşkent & Ak 2021, Rodrigues *et al*. 2015). They are also rich sources of vitamins, minerals, antioxidants, and proteins (Aisha *et al.* 2019, Ak 2015, Ak & Türker 2018; Caf *et al.* 2019). Because macroalgae are valuable food sources and have unique tastes, they have been consumed for centuries in far-east countries (Mouritsen *et al.* 2018, 2021). Today, with the acceleration of studies focused on these photosynthetic organisms' nutritional and functional contents, they are used as functional foods, especially in European countries (Balboa *et al* 2013, Lordan *et al.* 2011, Zbakh *et al*. 2020). Unfortunately, the detailed nutritional composition of these organisms is not fully described yet. Many macroalgae species have high protein levels, some of which are higher than plant-based protein-rich foods, such as soybean and cereals (Harnedy & FitzGerald, 2011).

The amino acid composition is vital for food protein quality in the human diet, especially to achieve an adequate intake of essential amino acids (Cherry *et al.* 2019). Macroalgae proteins are rich in essential amino acids, which consist of almost 50% of their total amino acid composition (Machado *et al.* 2020). Amino acids have a range of critical functions such as DNA replication, catalyzing metabolic reactions, transporting molecules from one location to another (Dumay & Morançais, 2016; Harnedy & FitzGerald, 2011). In addition, they play an essential role in synthesizing hormones and enzymes (Guerrini *et al.* 2000). Also, each amino acids have specific functions in the human body. For example, threonine improves immune function (Bortoluzzi *et al.* 2018); the primary role of valine is the regeneration of muscles (Zeitz *et al.* 2019); isoleucine encourages the muscles (Zeitz *et al.* 2019); Lysine plays a crucial role in hormone and enzyme productions (Yang *et al.* 2020).

The cost line of Çanakkale is very abundant for macroalgae, known as environmentally healthy habitats, and located between the Aegean Sea and Sea of Marmara (40° 8'34.25"N; 26°23'21.83"E). Some species are available throughout the year, whereas others have a markedly seasonal pattern. However, a complete report on the nutritional or functional metabolites content of these macroalgae is unsatisfying. Therefore, this study aims to evaluate amino acid compositions of nine macroalgae species distributed along with the costs of Çanakkale, Turkey.

2. Materials and Methods

Nine macroalgae species (*Ulva rigida* C. Agardh, *Flabellia petiolata* (Turra) Nizamuddin, *Caulerpa racemosa* (Forsskål) J. Agardh, *Halimeda tuna* (J.Ellis & Solander) J.V.Lamouroux, *Codium tomentosum* Stackhouse, *Laurencia obtusa* (Hudson) J.V. Lamouroux, *Asparagopsis armata* Harvey, *Hypnea musciformis*

(Wulfen) J.V. Lamouroux, *Dictyota dichotoma* (Hudson) J.V. Lamouroux) were collected qualitatively by hand from intertidal at four locations from the Assos (39°29'16.16"N; 26°20'43.28"E), Yeniköy (39°55'25.18"N; 26° 9'26.41"E), Ayazma-Bozcaada (39°48'44.71"N; 26° 0'20.87"E) and Bozkent (40°24'3.85"N; 26°53'51.54"E) (Çanakkale/Turkey) were chosen as sampling areas where macroalgae species distributed intensively. Sampling stations were shown in Figure 1. The seaweeds materials were identified according to Guiry & Guiry (2021).

The macroalgae samples were cleaned from their epiphytes, dried at 30 °C, and milled into powder before extraction. The samples were digested with the 3.79 N HCl at 110 °C in 24 hours by drying oven according to Çankırılıgil *et al.* (2020). Amino acid compositions of obtained samples were determined according to Henderson *et al.* (2000). First of all samples were diluted as 10^{-1} and transferred into 2 ml amber vials equipped with PTFE caps. Samples were derivatized with borate buffer, o-phthalaldehyde reagent (OPA), and 9-fluorenylmethyl chloroformate reagent (FMOC) by auto-sampler automatically. Analysis were carried out with HPLC system having diode arrar detector (DAD) and C18 amino acid column as a solid phase. Detection was performed in two wavelengths as 338 nm, 10 nm bandwidth and 262 nm, 16 nm bandwidth. Finally, achieved data were calibrated and expressed as mean values. The sampling map was created Ocean Data View (2021).

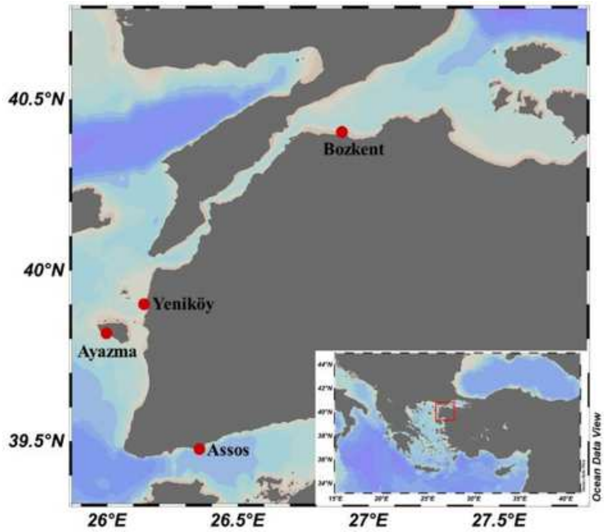

Figure 1. Sampling stations: Assos (39°29'16.16"N; 26°20'43.28"E), Yeniköy (39°55'25.18"N; 26° 9'26.41"E), Ayazma-Bozcaada (39°48'44.71"N; 26° 0'20.87"E) from the North Aegean Sea and Bozkent (40°24'3.85"N; 26°53'51.54"E) from the Sea of Marmara.

3. Results

The amino acid composition of samples collected from three different locations is reported in Table 1. The results of this study showed that seaweeds collected from the coasts of Çanakkale have important essential and non-essential amino acids required for a healthy diet. Total amino acid content was found highest in the *Flabellia petiolata* with 14.719 g/100g and followed by *Asparagopsis armata* and *Caulerpa racemose* as 11.031 g/100g and 10.280 g/100g, respectively. In the amino acid analysis procedure, with the high heat and low pH, asparagine and glutamine were digested to aspartic acid and glutamic acid, respectively, thereby these amino acids were evaluated together. According to our results, aspartic acid + asparagine and glutamic acid + glutamine amounts were high in all species.

The essential amino acids levels showed differences between species and locations. The most abundant essential amino acids were found in green macroalgae *Flabellia petiolata*. Primarily, methionine, phenylalanine, valine and lysine were found highest in this species. The percentage of essential amino acids of *Hypnea musciformis* (60.02%) was higher than other macroalgae species. Conversely, the lowest rate of essential amino acids was found in *Laurencia obtusa* (38.45%) was similar to *Halimeda tuna* (38.84 %). Besides, *Asparagopsis armata* has the highest serine and leucine content, while *Hypnea musciformis* has the highest threonine content with 2.187 g/100g.

Table 1. Amino acid compositions of seaweeds collected from different parts of the Çanakkale, Turkey

Species and Stations	1	2	3	4	5	6	7	8	9	10	11
ASP+ASN	0.378	0.592	1.718	1.197	0.836	0.941	0.753	1.902	0.729	0.756	0.548
GLU+GLN	0.440	0.769	2.013	1.451	1.209	0.628	1.007	1.583	0.869	0.716	0.491
SER	0.275	0.292	0.932	0.593	0.462	0.490	0.523	0.809	0.402	0.333	0.237
HIS	-	-	0.275	0.171	0.157	-	0.878	0.177	-	0.290	0.221
GLY	-	0.478	1.071	0.742	0.570	0.602	0.550	1.092	-	0.473	0.339
THR	0.819	0.500	1.316	0.943	0.680	0.710	0.763	0.736	2.187	0.476	0.363
ALA	0.002	0.063	0.228	0.215	0.266	0.023	0.086	0.161	0.032	0.054	0.024
TYR	0.261	0.476	1.757	1.048	0.844	0.602	0.648	0.386	0.524	0.509	0.434
CYS	-	-	0.335	0.142	0.149	0.104	0.081	0.076	0.091	0.000	0.000
VAL	0.087	0.202	0.652	0.401	0.310	0.270	0.273	0.612	0.220	0.216	0.139
MET	0.107	0.197	0.554	0.392	0.270	0.224	0.260	0.281	0.270	0.266	0.153
TRP	-	-	-	-	-	-	-	-	-	-	-
PHE	0.089	0.348	1.026	0.721	0.454	0.378	0.386	0.695	0.317	0.243	0.225
ISO	0.015	0.061	0.371	0.290	0.152	0.176	0.171	0.456	0.111	0.088	0.089
LEU	0.240	0.397	1.118	0.955	0.481	0.352	0.598	1.140	0.488	0.432	0.350
LYS	0.283	0.499	1.354	1.020	0.754	0.722	0.654	0.924	0.660	0.526	0.409
TOTAL	2.993	4.874	14.719	10.280	7.594	6.222	7.631	11.031	6.899	5.380	4.021

1- *Ulva rigida*- Bozkent, 2- *Ulva rigida*- Bozkent, 3- *Flabellia petiolata* -Ayazma, 4- *Caulerpa racemosa* - Ayazma, 5- *Halimeda tuna* - Ayazma, 6- *Codium tomentosum* - Bozkent, 7- *Laurencia obtusa* - Assos, 8- *Asparagopsis armata* - Ayazma, 9 - *Hypnea musciformis* - Bozkent, 10- *Dictyota dichotoma* Ayazma, 11- *Dictyota dichotoma* Yeniköy
ASP: aspartic acid, ASN: asparagine, GLU: glutamic acid, GLN: glutamine, SER: serine, HIS: histidine, GLY: glycine, THR: threonine, ALA: alanine, TYR: tyrosine, CYS: cystine, VAL: valine, MET:, methionine, TRP: tryptophan, PHE: phenylalanine, ISO: isoleucine, LEU: leucine, LYS: lysine.

4. Discussion

Amino acid compositions of macroalgae showed differences between species and locations. Similar results have been reported Gaillard *et al.* (2018) and Dawczynski *et al.* (2007). Macroalgae are rich in Aspartic acid and Glutamic acid low in Methionine and Histidine (Černá, 2011, Dawczynski *et al.* 2007, Harnedy & FitzGerald, 2011), which is in agreement with our findings. According to Yaich *et al.* (2011), high levels of Aspartic acid and Glutamic acid give macroalgae to the characteristic flavour. Despite the quantitative differences observed among the species, all samples except for Tryptophan presented a complete profile of essential amino acids. Overall, Tryptophan and Methionine were the principal limiting essential amino acids in all the macroalgae. Similar results were reported by Dawczynski *et al.* (2007) and Vieira *et al*. (2018). In addition, lysine was detected in all the analyzed species. According to Vieira *et al.* (2018), the presence of Lysine is of particular interest because this essential amino acid is frequently a limiting amino acid in animal feeds. Results showed that all analyzed macroalgae contain all the essential amino acids at various concentrations. And, according to our results, the analyzed seaweeds could be a good source as an alternative and sustainable source of amino acids for human nutrition and industrial food processing.

References

Aisha Dubey A.R., Haider A. (2019). Seaweed: Nutritional & health benefits. Pharma Innovation J., 8(8), 80-83.
Ak İ. (2015). Sucul ortamın ekonomik bitkileri; makro algler. Dünya Gıda Dergisi, Aralık 2015, 88-97.
Ak İ., Türker G. (2018). Antioxidant properties and phytochemicals of three brown macro algae from the Dardanelles (Çanakkale) strait. Agricultural Science and Technology, 10(4), 354-357.
Balboa E.M., Conde E., Moure A., Falqué E., Domínguez H. (2013). In vitro antioxidant properties of crude extracts and compounds from brown algae. Food Chemistry, 138(2), 1764-1785.

Bortoluzzi C., Rochell S.J., Applegate T.J. (2018). Threonine, arginine, and glutamine: Influences on intestinal physiology, immunology, and microbiology in broilers. Poultry Science, 97(3), 937-945.
Caf F., Şen Özdemir N., Yılmaz Ö., Durucan F., Ak İ. (2019). Fatty acid and lipophilic vitamin composition of seaweeds from antalya and Çanakkale (Turkey). Grasas Y Aceites(3), 1-7. doi: 10.3989/gya.0704182
Çankırılıgil E.C., Berik N., Alp Erbay E. (2020). Optimization of hydrolyzation procedure for amino acid analysis in fish meat with hplc-dad by response surface methodology (rsm). Ege Journal of Fisheries and Aquatic Sciences, 37, 113-123.
Černá M. (2011). Seaweed proteins and amino acids as nutraceuticals.: Academic Press.
Cherry P., O'Hara C., Magee P.J., McSorley E.M., Allsopp P.J. (2019). Risks and benefits of consuming edible seaweeds. Nutrition Reviews, 77(5), 307-329.
Cirik Ş., Cirik S. (2011). Su bitkileri (deniz bitkilerinin biyolojisi, ekolojisi, yetiştirme teknikleri). İzmir: Ege Üniversitesi Su Ürünleri Fakültesi Yayınları.
Dawczynski C., Schubert R., Jahreis G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry, 103(3), 891-899.
Dumay J., Morançais M. (2016). Chapter 9 - proteins and pigments. In J. Fleurence I. Levine (Eds.), Seaweed in health and disease prevention (pp. 275-318). San Diego: Academic Press.
Froehlich H.E., Afflerbach J.C., Frazier M., Halpern B.S. (2019). Blue growth potential to mitigate climate change through seaweed offsetting. Current Biology, 29(18), 3087-3093.e3083.
Gaillard C., Bhatti H.S., Novoa-Garrido M., Lind V., Roleda M.Y., Weisbjerg,M.R. (2018). Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. Animal Feed Science and Technology, 241, 210-222.
Guerrini F., Cangini M., Boni L., Trost P., Pistocchi R. (2000). Metabolicresponces of the diatom achnanthes brevipes (bacillariophycae) to nutrient limitation. Journal of Phycology, 36(5), 882-890.
Guiry M.D., Guiry G.M. (2021). Algaebase. Retrieved 13 February 2021, 2020, from https://www.algaebase.org
Harnedy P.A., FitzGerald R.J. (2011). Bioactive proteins, peptides, and amino acids from macroalgae1. Journal of Phycology, 47(2), 218-232.
Henderson J.W., Robert D.R., Brian A.B., Cliff W. (2000). Rapid, accurate, sensitive and reproducible hplc analysis of amino acids Application Note Publication No: 5980-1193.
Lordan S., Ross R.P., Stanton C. (2011). Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. Marine Drugs, 9(6), 1056-1100.
Machado M., Machado S., Pimentel F.B., Freitas V., Alves R.C., Oliveira M.B.P.P. (2020). Amino acid profile and protein quality assessment of macroalgae produced in an integrated multi-trophic aquaculture system. Foods, 9(10), 1382.
Mouritsen O.G., Rhatigan P., Cornish M.L., Critchley A.T., Pérez-Lloréns J.L. (2021). Saved by seaweeds: Phyconomic contributions in times of crises. Journal of Applied Phycology, 33(1), 443-458.
Mouritsen O.G., Rhatigan P., Pérez-Lloréns J.L. (2018). World cuisine of seaweeds: Science meets gastronomy. International Journal of Gastronomy and Food Science, 14, 55-65.
Necas J., Bartosikova L. (2013). Carrageenan: A review. Veterinarni Medicina, 58(4), 187-205.
Öztaşkent C., Ak İ. (2021). Effect of led light sources on the growth and chemical composition of brown seaweed treptacantha barbata. Aquaculture International, 29(1), 193-205.
Rodrigues D., Freitas A.C., Pereira L., Rocha-Santos T.A.P., Vasconcelos M.W., Roriz M., . . . Duarte A.C. (2015). Chemical composition of red, brown and green macroalgae from buarcos bay in central west coast of portugal. Food Chemistry, 183, 197-207.
Schlitzer R., Ocean Data View, https://odv.awi.de, 2021.
Vieira E.F., Soares C., Machado S., Correia M., Ramalhosa M.J., Oliva-teles M.T., Delerue-Matos C. (2018). Seaweeds from the portuguese coast as a source of proteinaceous material: Total and free amino acid composition profile. Food Chemistry, 269, 264-275.
Yaich H., Garna H., Besbes S., Paquot M., Blecker C., Attia H. (2011). Chemical composition and functional properties of ulva lactuca seaweed collected in tunisia. Food Chemistry, 128(4), 895-901.
Yang, Q., Zhao, D., Liu, Q. (2020). Connections between amino acid metabolisms in plants: Lysine as an example. Frontiers in Plant Science, 11(928).
Zbakh H., Salhi G., Bochkov V., Ciudad C.J., Noé V., Hassoun M., Riadi H. (2020). Insights on the anti-inflammatory and antitumor activities of extracts from the marine green alga codium decorticatum. European Journal of Integrative Medicine, 37, 101170. doi:
Zeitz, J.O., Käding, S.-C., Niewalda, I.R., Most, E., Dorigam, J.C.d.P., Eder, K. (2019). The influence of dietary leucine above recommendations and fixed ratios to isoleucine and valine on muscle protein synthesis and degradation pathways in broilers. Poultry Science, 98(12), 6772-6786.

SEASONAL VARIATION IN THE PROXY-BIOCHEMICAL COMPOSITION OF THE NON-INDIGENOUS PEARL OYSTER *Pinctada imbricata radiata* (LEACH, 1814) FROM THE CENTRAL WEST AEGEAN SEA, GREECE

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Abstract
The proximate biochemical composition of the flesh of the invasive pearl oyster *Pinctada imbricata radiata* (Leach, 1814) sampled (2019-2020) in CE Aegean Sea (Saronikos & Evoikos Gulf) ranged: 64.33% ± 3.04 for protein, 11.41% ± 1.43 for fat, 11.61% ± 3.87 for carbohydrate, 12.65% ± 2.97 for ash and 79.97% ± 3.56 for moisture. The results demonstrate the high nutritional value of the pearl oyster, supporting its suitability as potential seafood source for human consumption.
Keywords: *Pearl Oyster, Biochemical Composition, Aegean Sea*
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1. Introduction

The *Pinctada i. radiata* (Leach, 1814) (pearl oyster) is a benthic species that lives on sandy bottoms and coral reefs (Strack 2008). It originates from the Indo-Pacific Ocean region and has been recorded in the Mediterranean as a non-endemic species since the 19th century (1874), immediately after the opening of the Suez Canal (Zenetos *et al*. 2005). Since then, the pearl oyster has spread and settled in areas of the Central-Eastern Mediterranean with a significant presence in Aegean Sea (Theodorou *et al*. 2019).

Several reports suggest that a seafood diet prevents chronic diseases such as heart disease (Harris & Von Schacky 2004). Bivalves are a source of high quality protein, vitamin, essential amino acid, mineral and low lipid in addition to beneficial ones such as polyunsaturated fatty acid PUFAs, which offer such benefits to the human body (Biandolino *et al*. 2020; Rittenschober *et al*. 2013; Anacleto *et al*. 2014). Protein deficiency worldwide will continue to increase sharply in the coming decades. For now, and in the near future, this deficiency can be partly overcome with effective use of protein-rich molluscs. Molluscs are the largest marine invertebrates and account for 12% of the world's total production. These include species such as gastropods, mussels, and oysters that are popular protein-rich foods and can be used by all cultures as an important part of their diet (Celik *et al*. 2014).

2. Material and Methods

To assess the nutritional value and seasonal variation of the chemical composition of pearl oyster flesh, 4 seasonal samplings were performed (Winter, Spring and Autumn 2019, as well as Summer 2020), in 2 areas: one high (Saronikos Gulf) and one low (Evoikos Gulf) productivity. Specifically, the sampling of the species took place in two coastal areas, in the Saronikos Gulf (sea area of Salamis, 37 59 '113' '/ 23 26' 059 ") and in the Northern Evoikos Gulf (north of Chalkida, 38 30 '783' '/ 23 32' 254 "). The locations of the sampling sites are presented in Figure 1. In the period February 2019 - July 2020, a total of 120 individuals of the species (30 individuals per sampling) were collected from the Saronikos Gulf in 4 samplings (11/2/2019, 1/4/2019, 21/10/2019, and 9/7/2020) and 120 individuals of the species (30 individuals per sampling) from the Evoikos Gulf in 4 sampling (12/2/2019, 2/4/2019, 22/10/2019 and 15/7/2020) (figure 1). The sampling was done by autonomous diving to a depth of 1-4 m and the collection by hand, while each sampling lasted 30 min (Moutopoulos et al., 2020).

The flesh from 30 pearl oysters from each study area was lyophilized and then homogenized to give 5 pooled samples in which protein was measured [Keldahl method (AOAC, 1997)], total fat by extraction (Vareltzis et al., 1997) and ash (from 0.1 g of sample at 550 ºC for 24 hours). Water was measured from the flesh of all 30 oysters (one at a time) by weighing before and after lyophilization).The carbohydrate content (%dry weight) was calculated as:

Carbohydrate (%) =100 - [Lipid (%) + Protein(%) + Ash (%)+ Moisture(%)] (Alkuraieef et al., 2021).

From the results of the biochemical analysis of all samples in each sampling area, the mean value was obtained separately for each biochemical characteristic. The resulting values were used to create the graph in Figure 2.

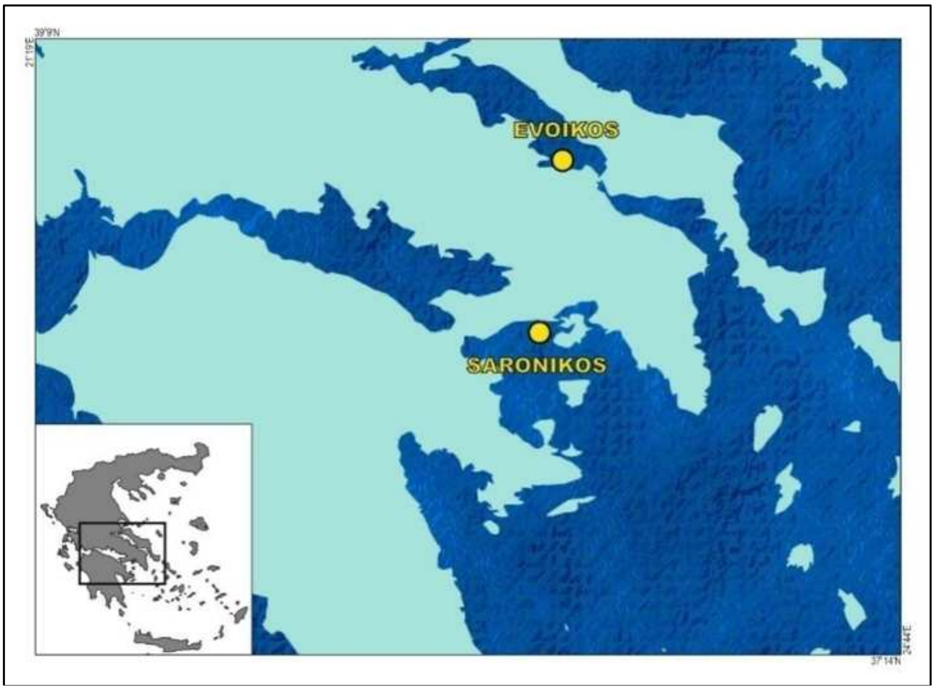

Figure 1. Map of study area. Sampling position Gulf of pearl oyster 37 59 '113' '/ 23 26' 059 '' in the Saronikos Gulf and sampling position 38 30 '783' '/ 23 32' 254 '' in the Evoikos Gulf.

3. Results

Figure 2 shows the seasonal variation of the proxy-biochemical composition of pearl oyster flesh. The total variation of biochemical composition of the flesh of pearl oysters in CE Greece was protein 64.33% ± 3.04, fat 11.41% ± 1.43, carbohydrate 11.61% ± 3.87, ash 12.65% ± 2.97 and moisture 79.97% ± 3.56.

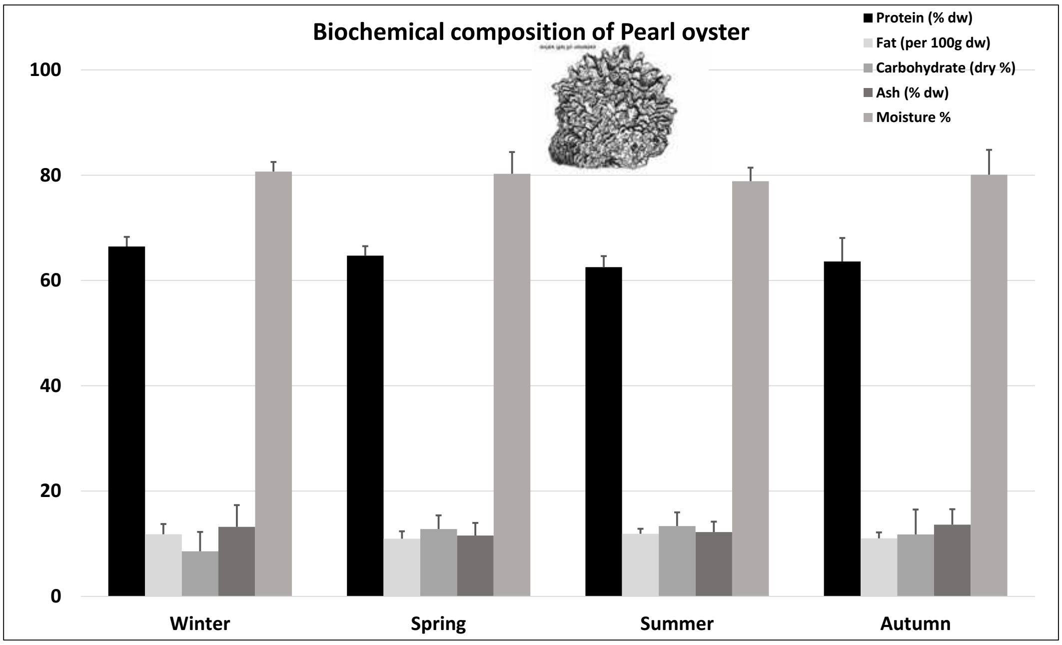

Figure 2. Graph from seasonal variation of the pearl oyster proxy-biochemical composition originated from CE Aegean Sea coast line.

Table 1. Comparison of the biochemical components of *Pinctada i. radiata* studied in the present work and 4 other bivalve molluscs of the eastern Mediterranean Sea and SW Black Sea.

No	Bivalve Species	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	Moisture (%)	Catch location	References
1	*Pinctada imbricata radiata*	64.33± 3.04	11.41 ±1.43	11.61±3.87	12.65± 2.97	79.97±3.56	CE Aegean Sea, Greece	Present study 2021
2	*Ostrea edulis*	48.11± 3.04	8.01± 0.65	29.16±4.62	13.92± 1.89	82.25±2.66	Dardanelles & Marmara Sea, Turkey	Celik et al. 2014
3	*Mytilus galloprovinciallis*	54.03± 2.82	10.52 ±1.22	22.84±4.33	12.61± 2.54	82.45±1.70	Dardanelles & Marmara Sea, Turkey	Celik et al. 2014
4	*Ruditapes decussatus*	56.27± 1.98	5.82± 0.34	23.39±1.62	14.53± 0.66	83.58±0.59	Dardanelles & Marmara Sea, Turkey	Celik et al. 2014
5	*Ruditapes philippinarium*	55.88± 2.04	5.60± 0.19	23.43±1.59	15.10± 0.67	83.28±0.86	Dardanelles & Marmara Sea, Turkey	Celik et al. 2014

Table 1 compares the studied pearl oyster with 4 other species of bivalve molluscs and shows that the pearl oyster has a higher nutritional value since the composition of its flesh is rich mainly in protein and fat.

4. Discussion

Protein, fat, and carbohydrate are the basic building blocks of all living organisms. These molecules change in different organisms depending on their metabolism, give energy and synthesize basic products in them. Pearl oysters from CE Aegean Sea are rich in protein (64.33 ± 3.04%) and fat (11.41 ± 1.43%) with limited carbohydrates (11.61 ± 3.87%) comparing to that of the other native edible molluscs (flat oysters, Mediterranean mussels, clams) in the region. The pearl oyster protein content is maximum in the winter and minimum in the summer. The fat content is maximum during the winter and summer, while the ash shows the maximum values during the winter and autumn.

This superior nutritional profile, of the pearl oyster *Pinctada i. radiata*, is recommended for human consumption in E. Mediterranean.

Acknowledgements

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References

Alkuraieef A.N., Alsuhaibani A.M., Alshawi A.H., Alfaris N.A. Aljabryn D. H. (2021). Chemical and microbiological quality of imported chilled, frozen, and locally cultured fish in Saudi Arabian markets. Food Science and Technology, 2021, 1-11, https://dx.doi.org/10.1590/fst.52520.

Anacleto P., Maulvault A.L., Bandarra N.M., Repolho T., Nunes M.L., Rosa R., Marques A.E. (2014). Effect of warming on protein, glycogen and fatty acid content of native and invasive clams. Food Research International, 64, 439–445, https://doi.org/10.1016/j.foodres.2014.07.023.

AOAC, 1997. AOAC Official Method 981.10. Crude Protein in Meat. In: International, A., (Ed.), Official Methods of Analysis. AOAC International. Vol. Chapter 39., pp. 7-8.

Biandolino F., Parlapiano I., Grattagliano A., Fanelli G., Prato E. (2020). Comparative Characteristics of Percentage Edibility, Condition Index, Biochemical Constituents and Lipids Nutritional Quality Indices of Wild and Farmed Scallops (*Flexopecten glaber*). Water 2020, 12, 1777, https://doi.org/10.3390/w12061777.

Celik M. Y., TürkÇulha S., Çulha M., Yildiz H., Acarli S., Celik I., Celik P. (2014). Comparative study on biochemical composition of some edible marine molluscs at Canakkale coasts, Turkey. Indian Journal of Geo-Marine Sciences, 43 (4), 601-606.

Moutopoulos K.D., Ramfos A., Theodorou A.J., Katselis G. (2021). Biological aspects and population dynamic of Pearl Oyster in the Eastern Mediterranean. Regional Studies in Marine Science 45(5):101821, https://doi.org/10.1016/j.rsma.2021.101821.

Rittenschober D., Nowak V., Charrondiere U.R. (2013). Review of availability of food composition data for fish and shellfish. Food Chemistry, 141, 4303–4310, https://doi.org/j.foodchem.2013.07.007.

Strack E. (2008). Introduction. In: Southgate, P., Lucas, J., The Pearl Oyster. *Elsevier, Oxford*, 1–35.

Theodorou J.A., Perdikaris C., Spinos E. (2019). On the occurrence of rayed pearl oyster *Pinctada imbricata radiata* (Leach, 1814) in Western Greece (Ionian Sea) and its biofouling potential. Biharean Biologist International Journal 13 (1): 4-7, http:// doi.org/biozoojournals.ro/bihbiol/index.html.

Zenetos A., Koutsoubas D., Vardala-Theodorou E. (2005). Origin and vectors of introduction of exotic molluscs in Greek waters. Belgian Journal Zoology, 134 (Suppl. 1), 161–168.

DEVELOPMENT AND APPLICATION OF NOVEL METHODS FOR FISH HARVESTING AND PROCESSING FOR QUALITY PRESERVARTION AND SHELFLIFE EXTENSION

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Abstract
The objective of the study was the design and application of alternative postharvest treatments of marine cultured fish for quality preservation and shelf life extension. Slurry ice was applied as an alternative cooling medium during harvesting and transportation of gilthead seabream and European sea bass, using different concentrations (0.5 or 100%) of slurry ice prepared from sea water, in conventional flake ice. The mild surface disinfection during gutting and filleting of fish by the incorporation of organic acids (i.e. citric acid, lactic acid, peracetic acid) at different concentrations and treatment durations (0-7500 ppm and 0-10 min, depending on the tested acid and application) in the washing water of gutted and filleted fish was also investigated. Whole, gutted and filleted fish were stored isothermally at 0 ± 0.2°C for shelf life evaluation. Quality evaluation was based on microbial growth (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae), colour, texture, lipid oxidation, proteolytic enzyme activity and sensory evaluation. The replacement of conventional flake ice with slurry ice resulted in improved quality and microbial stability during refrigerated storage, resulting in up to 6 days shelf life extension of fish, without affecting the sensory properties of the final products. Fish surface decontamination up to 2.0 log cfu / g was achieved by the addition of citric acid in the washing water, resulting in up to 4 days shelf life extension of gutted fish.
Keywords: *Slurry ice*, *Surface Disinfection, Fish Washing, European Sea Bass, Gilthead Seabream, Spoilage*
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1. Introduction

Fish is highly susceptible to spoilage, which can be caused by both intrinsic chemical reactions and microbial growth. An estimated 25% of primary agricultural and fishery products are lost every year, mostly because of chemical deterioration and microbial spoilage (Baird-Parker 2000). The deterioration process is accelerated by increased temperatures, physical damage and contamination. Therefore, the key to fish preservation is the immediate chilling upon catch or harvest to a temperature slightly above the freezing point and maintaining this temperature throughout the cold chain (Kauffeld *et al.* 2010). New minimal and non thermal food processing methods are sought by the industry in the pursuit of producing better quality fish products with extended shelf life with retention of nutritional and sensory properties (Tsironi & Taoukis 2019).

Slurry ice, is a biphasic system consisting of small spherical ice particles surrounded by seawater at subzero temperature (Cakli *et al.* 2006). Its reported advantages over traditional fresh-water ice (such as flake, tube, and block ice) include its lower temperature, faster chilling (due to a more rapid heat exchange), and lower rate of physical damage (due to its spherical microscopic particles) (Bellas & Tassou 2005; Kauffeld *et al.* 2010). The ability of adjusting the ice concentration up to 60% and the salt content in the range of 2-3% in the ice slurry ensures maximum preservation results without damage to delicate fish and avoids excessive salt uptake by the fish (Kauffeld *et al.* 2010). Ntzimani *et al.* (2021) evaluated the effect of slurry ice as an alternative cooling medium during harvesting and transportation on the quality and shelf life of whole European sea bass.

Sodium salts of the low molecular weight organic acids, as for example acetic, lactic and citric acid, have been applied with the aim to delay microbial growth, preserve sensory attributes and extend the shelf life of various food systems. Several studies have been conducted recently on the efficacy of washing and sanitizing treatments in reducing microbial populations on perishable products. Limited work on the effect on fish has been published and no industrial scaling-up has been reported (Sallam 2007; Thi *et al.* 2015).

The objective of the SlurryFish project (slurryfish.chemeng.ntua.gr, 2018-2022) is to develop and optimize environmentally friendly and cost effective postharvest treatments of marine cultured fish for quality preservation and shelf life extension (Figure 1). The aim of the study was the design and application of slurry ice as an alternative cooling medium during harvesting and transportation and a mild surface disinfection during gutting and filleting of farmed gilthead seabream and European sea bass.

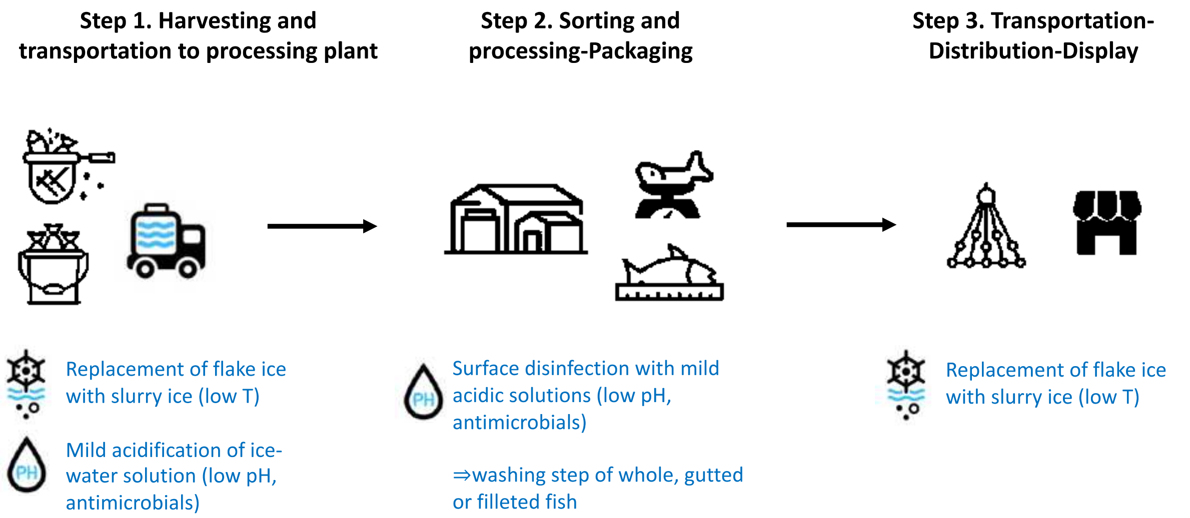


Figure 1. Technological improvements at postharvest fish processing stages developed and optimized within the SlurryFish project.

2. Material and Methods

Slurry ice was prepared from filtered seawater (salinity: 3.5%) using a semi-industrial scale slurry ice machine (ZIEGRA, Germany) in Philosofish S.A. farming facilities (Larymna, Fthiotida, Greece). The temperature of the slurry ice mixture was -3.2°C. Whole gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) was slaughtered and transported in different mixtures of slurry ice and conventional flake ice (C: slaughtered and transported in 100% flake ice-Control samples, SC: slaughtered in 100% slurry ice and transported in 100% flake ice, S50: slaughtered and transported in 50% slurry ice-50% flake ice, S100: slaughtered and transported in 100% slurry ice). Upon receipt at the laboratory (24 hours after harvesting), all fish samples (C, SC, S50, S100) were stored in high-precision low temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Japan) under controlled isothermal conditions at (0 ± 0.2°C). The temperature in the incubators was monitored using miniature data-loggers (COX TRACER, Belmont, NC).

The incorporation of mild organic acids (i.e. citric acid, lactic acid, peracetic acid) at different concentrations and treatment durations (0-7500 ppm and 0-10 min, depending on the tested acid and application) in the washing water of gutted and filleted fish was also investigated. The microbial load reduction of total viable count (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae was evaluated as a function of acid concentration and washing time of gutted fish and fillets.

Whole, gutted and filleted fish were stored isothermally at 0 ± 0.2°C for shelf life evaluation. Quality evaluation was based on microbial population changes (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, H_2S-producing bacteria, yeasts / molds and Enterobacteriaceae), colour, texture, lipid oxidation, proteolytic enzyme activity and sensory evaluation.

TVC was enumerated on plate count agar (PCA, Merck, Darmstadt, Germany) after incubation at 25°C for 72 h, whereas *Pseudomonas* spp. were enumerated on Cetrimide agar (CFC, Merck, Darmstadt, Germany) after incubation at 25°C for 48 h. For H_2S-producing bacteria and Enterobacteriaceae enumeration, the pour-plate method was used. H_2S-producing bacteria were enumerated on Iron Agar (Iron agar with L-cysteine) followed by incubation at 25°C for 48 h. For Enterobacteriaceae enumeration violet red bile glucose agar (VRBG, Merck, Darmstadt, Germany) was used, which was incubated at 37 °C for 18-24 h. Two replicates of at least three appropriate dilutions were enumerated.

The microbial growth was modelled using the Baranyi Growth Model (Baranyi and Roberts 1995). For curve fitting the program DMFit (IFR, Institute of Food Research, Reading, UK) was used (available at http://www.combase.cc/index.php/en/). Kinetic parameters such as the rate (k) and lag phase (λ) of microbial growth were estimated. To evaluate lipid oxidation, 2-thiobarbituric acid reactive substances (TBARs) assay was performed according to the method of Loovas (1992). Color of all fish samples was measured on the dorsal part of the body with the color meter Minolta CR-200 (Minolta Company, Chuo-Ku, Osaka, Japan).Texture parameters were defined using a texture analyzer with a load cell of 5 kg (TA-XT2i, Stable Micro Systems, Godalming, Surrey, United Kingdom). The sensory attributes of raw and cooked fish were evaluated by a sensory panel of eight trained evaluators using descriptive tests with practice evaluation methods of determining spoilage characteristics in fish (Botta, 1995). The proteolytic enzyme activity was assayed by the methods described by Barrett & Kirschke (1981). The protein content of enzyme extracts was quantified with the Bradford (1976) method using bovine serum albumin as a standard. Two replicates per sample were performed.

3. Results

The replacement of conventional flake ice with slurry ice as a slaughtering method led to improved quality stability during subsequent refrigerated storage and shelf life extension, in terms of microbial growth, flesh quality and sensory degradation of fish. The microbial growth (TVC, *Pseudomonas* spp. and H_2S-producing bacteria) in whole sea bass stored isothermally at 0°C is illustrated in Figure 2. The use of slurry ice at slaughter and flake ice in transportation was accompanied by low activities and late peaks of all enzymes that are expected to lead to delayed proteolytic degradation and extended freshness (Figure 3).

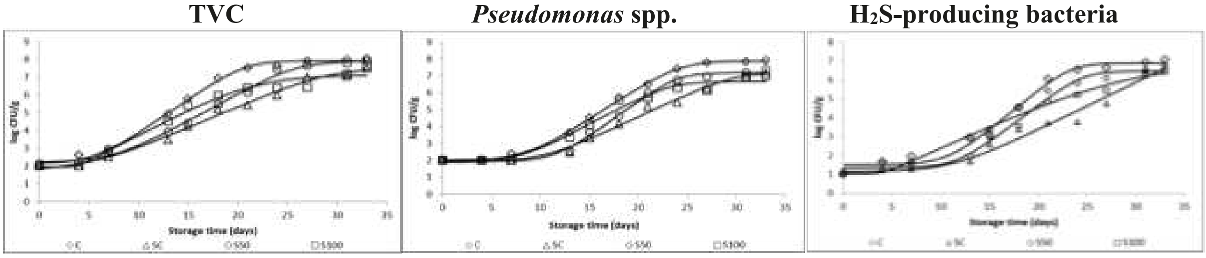


Figure 2. Effect of slurry ice during harvesting and transportation of whole European sea bass on microbial growth during subsequent isothermal storage at 0 °C (◇ C, △ SC, ○ S50, □ S100).

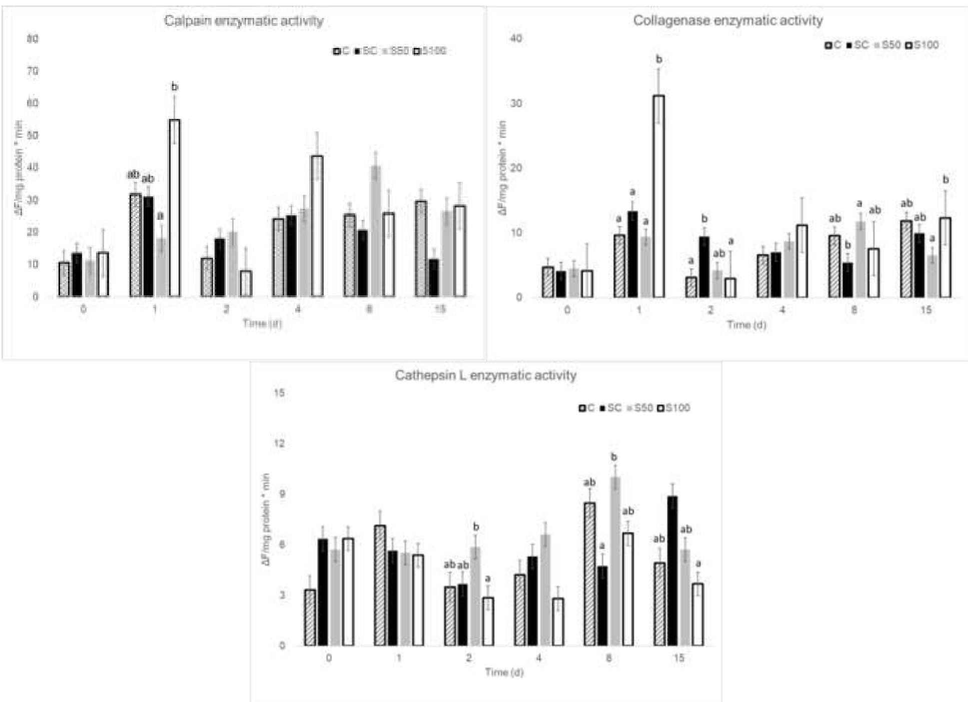
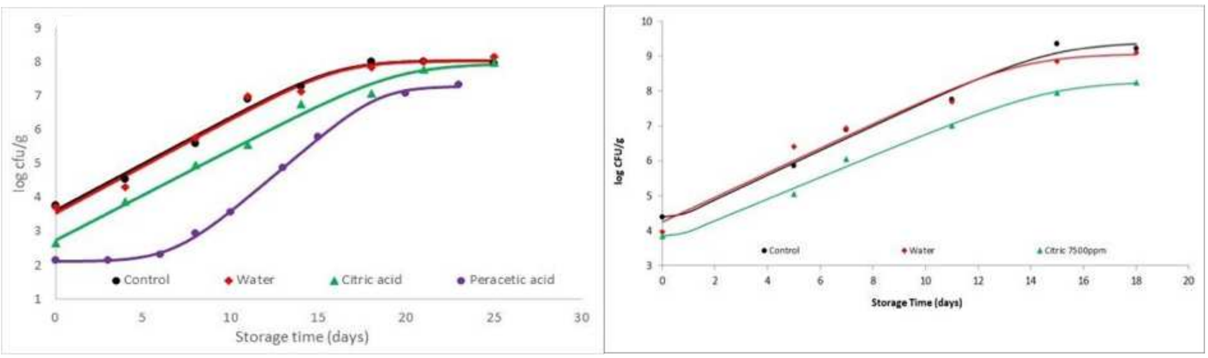


Figure 3. Enzymatic activity of calpain, collagenase and cathepsin L in all slaughter and storage methods. Superscripts indicate statistically significant differences (p<0.05) between treatments on each sampling day

Initial surface decontamination in the range of 1.0-2.0 log cfu / g by the addition of organic acids in the washing water, resulted in 3-4 days shelf life extension of fish stored at 0°C. Increased microbial load reduction was achieved for higher washing solution concentrations and longer treatment. Higher reduction of the initial microbial load was observed after treatment with citric acid for TVC, *Pseudomonas* spp. and H_2S-producing bacteria, with lactic acid for Enterobacteriaceae and with peracetic acid for *Pseudomonas* spp. and Enterobacteriaceae, compared to other bacteria tested. Microbial growth during subsequent refrigerated storage of untreated (Control) and treated fish is illustrated in Figure 4. Limit of sensory shelf life of gutted fish (score 5 by the sensory panel for overall impression) coincided with a level of 10^7 cfu / g of *Pseudomonas* spp. for gutted samples and of TVC for fillets, respectively, stored at 0 °C (Tsironi *et al.* 2019).



(a) (b)

Figure 4. (a) *Pseudomonas* spp. (log cfu/g) in gutted sea bass after surface washing with citric acid (200 ppm for 10 min) and peracetic acid (200 ppm for 4min), or water and Control during storage at 0°C. (b) TVCs (log cfu/g) in filleted sea bass after surface washing with citric acid (7500 ppm for 10 min), water and Control during storage at 0°C.

The results of the study indicated that the application of washing treatment with acids may result in significant deactivation of spoilage microorganisms (*Pseudomonas* spp, H_2S-producing bacteria) in gutted fish and fillets. Washing of fish using organic acids can reduce initial microbial load and significantly extend the shelf life of gutted fish and fillets. The replacement of conventional flake ice with slurry ice as a slaughtering method led to improved quality stability during subsequent refrigerated storage and shelf life extension, in terms of microbial growth, flesh quality and sensory degradation of fish. Shelf life extension of fish could open new distant markets currently inaccessible to fresh fish products and contribute to reduction of food waste. The systematic evaluation of the effect of harvesting, processing and transportation conditions on the quality and shelf life of fish may provide technological solutions for fish handling to improve quality and shelf life of fresh fish and reduce food losses during distribution and storage from harvesting up to the consumer level.

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References

Baird-Parker T.C., (2000). The production of microbiologically safe and stable foods, p. 3-18. In B. M. Lund & T. C. Baird-Parker (ed.), The microbiological safety and quality of food. Aspen Publishers, Inc., Gaithersburg, Md.

Baranyi J., Roberts, T.A. (1995). Mathematics of predictive food microbiology. International Journal of Food Microbiology, 26, 199-218.
Barrett A.J., Kirschke, H. (1981). Cathepsin B, cathepsin H, and cathepsin L. Methods in Enzymology, 80, 535–561.
Bellas I., Tassou S.A. (2005). Present and future applications of ice slurries. International Journal of Refrigeration, 28(1), 115-21.
Botta J.R. (1995). Evaluation of Seafood Freshness Quality. VCH Publishers.
Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1–2), 248–254.
Cakli S., Kilinc B., Cadun A., Tolasa, S. (2006). Effects of using slurry ice on the microbiological, chemical and sensory assessments of aquacultured sea bass (*Dicentrarchus labrax*) stored at 4°C. European Food Research and Technology. 222, 130-38.
Kauffeld M., Wang M.J., Goldstein V., Kasza, K.E. (2010). Ice slurry applications. International Journal of Refrigeration, 33, 1491-1505.
Loovas E.A. (1992). Sensitive spectrophotometric method for lipid hydroperoxide determination. Journal of the American Oil Chemists Society, 69, 777-783.
Ntzimani A., Angelakopoulos R., Semenoglou I., Dermesonlouoglou E., Tsironi T., Moutou K. & Taoukis P. (2021). Slurry ice as an alternative cooling medium for fish harvesting and transportation: Study of the effect on seabass flesh quality and shelf life. Aquaculture and Fisheries (Accepted, in press). DOI: 10.1016/j.aaf.2021.01.006
Sallam K.I. (2007). Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. Food Chemistry, 101(2), 592-600.
Thi A.N.T., Sampers I., Van Haute S., Samapundo S., Nguyen B.L., Heyndrickx M., Devlieghere F. (2015). Decontamination of Pangasius fish (*Pangasius hypophthalmus*) with chlorine or peracetic acid in the laboratory and in a Vietnamese processing company. International Journal of Food Microbiology, 208, 93-101.
Tsironi T. Anjos L., Pinto P.I.S., Dimopoulos G., Santos S., Santa C., Manadas B., Canario A., Taoukis P., Power D. (2019). High pressure processing of European sea bass (*Dicentrarchus labrax*) fillets and tools for flesh quality and shelf life monitoring. Journal of Food Engineering, 262, 83-91.
Tsironi T., Taoukis P. (2019). Advances in conventional and nonthermal processing of fish for quality improvement and shelf life extension. Reference Module in Food Science. Elsevier, pp. 1-7.DOI:10.1016/B978-0-08-100596-5.22618-8

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ASSESSING CONSUMER ATTITUDES TOWARDS THE INVASIVE RAYED PEARL OYSTER *Pinctada imbricata radiata* (LEACH 1814)

Ziou A.[1]*, Douligeri A.[1], Theodorou J.A.[1], Katselis G.[1], Moutopoulos D.K.[1]

Abstract
The paper focuses on the preferences of Greek consumers on the consumption of the non-invasive edible shellfish species, *Pinctada imbricata radiata*. For that to be true, a consumer survey was conducted and a total of 133 randomly selected consumers filled in a specially designed questionnaire. Aspects such as frequency, quantity of fish, points of purchase and criteria when buying fish (e.g. freshness) were covered. The majority of respondents prefer buying bivalve shellfish "once a week" and 1.5-2 kg per family meal. The most preferable points of purchase are traditional fishmonger shops and local markets and freshness is the most important criterion. The 78.2% of the respondents consume bivalves all along the year and prefer a certification of bivalve quality. Preferences vary according to socio-economic factors and participants' origin.
Keywords: *Shellfish Market, Consumer Preferences, Criteria Of Buying Shellfish, Greece*
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1. Introduction

The rayed pearl oyster *Pinctada imbricata radiate*, a non-indigenous species (NIS) of Indo-Pacific origin, has been reported in Aegean since mid-1960 and recently in Ionian Sea (Theodorou *et al*. 2019). Although the species was categorized as is minor commercial interest (Katsanevakis *et al*. 2011), it is harvested for edible purposes, especially in the areas with high natural stocks availability such as Saronikos and Evoikos Gulf (Aegean Sea). However, the commercial exploitation of the species is prohibited, because there is a regulation gap due to its absence on the list of Presidential Decrees 86/98, 227/2003, and 109/2002 regulating the shellfish eradication, even though the species is not treated as endangered (Katsanevakis *et al*. 2011).

The demand for shellfish products has not increased and the per capita consumption in Greece is still low (Theodorou *et al*. 2011). Most of the shellfish production is exported to European markets, mainly to Italy and France. On the other hand, the traditional Greek consumer's attitude has been rapidly changing, mainly due to socio-economic changes such as the improvement of the standards of living, the great expansion of media, the promotion of biological products, the development of the tourism industry, etc. The present study aims to investigate the consumers' preferences and attitudes on the purchase and consumption of the edible pearl oyster. This would prove to be very beneficial for a more rational organization of the distribution roots, which in turn would promote shellfish consumption in Greece.

2. Material and Methods

Research was undertaken through a specially designed questionnaire (Batzios *et al*. 2003) distributed to randomly selected consumers throughout Greece during spring-summer 2020. Apart from demographic questions related to age, social and economic issues of the respondents, other questions examined in the present study were "*Shellfish known and tried at least once*", "*Frequency and quantity of shellfish consumption*", "*Criteria of shellfish freshness*", "*Frequency of shellfish purchase by species*", "*Points of purchase*", "*Preferred pearl-oyster products*", "*Quantity of package preference*", "*Packaging material preference*", and "*Announcements of the veterinary services*".

Data was statistically analyzed addressing variables of respondent's demographic (residence and age) and socio-economic (net monthly family disposable income and education level) status. Chi-square (χ^2) procedure was used and a number of statistical tests of independence were performed to examine the possible relation between each of the above-mentioned characteristics of respondent's demographic and socio-economic status with the questions on the consumer's preferences and attitudes towards shellfish (Zar 2010). Furthermore, in case of significance, the Adjusted Standardized Residuals in the cross-tabulation tables were carefully examined to detect departures from independence. All statistical analyses were carried out using the statistical package IBM SPSS statistics v 26.0.

3. Results

A total of 133 questionnaires were answered and 59.6% of the respondents were female. The mean age of the participants were 41.1 years (SD=16.2) with 2/3 of them living in urban areas (63.2%), whereas almost 1/3 of them inhabits at the Greek coastal cities (30.8%) and the rest at in non-coastal areas (6.0%). The majority of the respondents stated that they had graduated University (69.2%). A percentage of 18.9% of the responders were private employers, 17.8% were University students and 7.3% were public employers. Married participants were slightly higher than single ones (50.8% vs 45.3%) with the majority of the participants had two children (68.7%).

Almost all the responders have tried at least once bivalves (98.4%) and a great percentage of them they have tried at least once smooth clams (87.9%), flat oysters (85.7%), cockles (78.9%), scallops (73.6%) and razor shells (51.1%), whereas less than a quarter of the respondents (24%) knew or have tried at least once the rayed pearl oysters. Bivalve species were more common to consumers of urban areas than to other areas (χ^2, P<0.05). Younger consumers and those of higher education level seemed to know or have consumed shellfish in higher percentages compared to those of lower education (χ^2, P<0.05).

The majority of the responders stated that they usually purchased shellfish from traditional fishmonger shops (36.07%) and from supermarkets (25.1%) and this behavior did not significantly differed (χ^2, P>0.05) with place of residence, age and level of education. More than the half responders stated that they consume bivalves only in special cases (53.3%) and more than a quarter of the respondents reported that their family consumed bivalves once in a month (26.3%) and these attitudes did not differ significantly (χ^2, P>0.05) with the age and the level of education, whereas significantly differed (χ^2, P<0.05) with the place of residence. Consumers from urban area purchased significantly (χ^2, P<0.05) more often bivalves in their family, than the consumers from coastal and non-coastal areas. More than half of the respondents (58.7%) also stated that the amount of bivalves that they usually buy and consume in their family was up to 1kg, whereas a third (35.8%) purchased between 1.5 and 2 kg, whereas only 4.5% consumed more than 2.5 kg. This behavior did significantly differ (χ^2, P>0.05) with the resident place, age and educational level.

With respect to "*Criteria of shellfish freshness*" most of the respondents have stated that the first criterion they examined was "*shellfish odor*" (37.1%), second in priority order was "*shells (closed or not)*" (37.1%), third was "*expiration and shell removal date*" (32.3%) and fourth was "*clarity of packaging water*" (48.4%). The respondents stated that they prefer mussels "*very often*" (47.2%), "*often*" (30.7%) and "*seldom*" (16.5%). For cockle they answered that they buy it "*seldom*" (37.1%) and "*often"*(31.9%). For oysters the corresponding percentages are 38.5% and 27.5%. Scallops are eaten by the consumers "*seldom*" (43.9%), "*often*" (15%) and "*never*" (28%). The majority of the respondents (62.4%) "*never"* prefer pearl-oysters and (12.9%) "*seldom*". Also, 66.3% never prefer horse mussels and "*almost never*" (15.1%, while for smooth clams 43.2% prefer them "*seldom*" and 24.3% "*often*" (Figure 1). The most preferred pearl-oysters product was "*fresh alive in bulk*" (51.4%) and second in order was "fresh *alive, packed in net*" (33.7), and third "*fresh alive in vacuum*" (24.2%). The less preferred product was "*deshelled, breaded nuggets*" (50%). More than half of the respondents, in case of choosing fresh alive pearl-oysters in netting bags, purchased 1 kg (56.8%), a behavior that was not significantly (χ^2, P>0.05) different with the place of residence, age and educational level.

In case of purchased fresh alive pearl oysters in a vacuum consumers preferred a quantity of 6 pieces (29.4%) or 12 pieces (18.5%), which was not significantly (χ^2, P>0.05) different with the place of residence, age and educational level. In case of choosing pearl oysters without shell frozen, the majority of the respondent choose 250gr (46.8%), a behavior that it was not significantly (χ^2, P>0.05) different with age and educational level, whereas was significantly (χ^2, P<0.05) different with the place of residence. In case of choosing pearl oysters without shell breaded nuggets, consumers preferred a quantity of 250gr (38.1%) or 500gr (29.8%), a tension that it no significantly (χ^2, P>0.05) different with the place of residence, age and educational level. In case of choosing pearl oyster "*deshelled in jar with brine*" consumers stated the preferred 250gr (37.8%) or 500gr (29.4%), a behavior that it was significantly (χ^2, P>0.05) different with the place of residence, age and educational level. In case of choosing pearl oyster without shell in jar with olive oil and oregano, most consumers preferred up to 500gr (41.7%) or 250gr (32.5%), a tension which was no significantly (χ^2, P>0.05) different with the place of residence, age and educational level. In case of choosing pearl oyster "*deshelled, smoked in jar*", the most preferred quantity was 250gr (40.2%) or 500gr (33.3%), which was not significantly (χ^2, P>0.05) different with the place of residence, age and educational level. The vast majority of the respondents stated that they preferred a "*transparent container*" (90.4%) or "*glass material*" (90.4%) for the pearl-oyster packaging, whereas the less preferred container was "*vase"* (1.8%) and the less preferred material was "*metal*" (0.8%), behaviors that were not significantly (χ^2, P>0.05) different with the place of residence, age and educational level. The majority of the respondents (81.1%) trusted the announcements of the veterinary services regarding the hygiene of shellfish, an attitude that it was not significantly (χ^2, P>0.05) different with the place of residence, age and education level of the respondents.

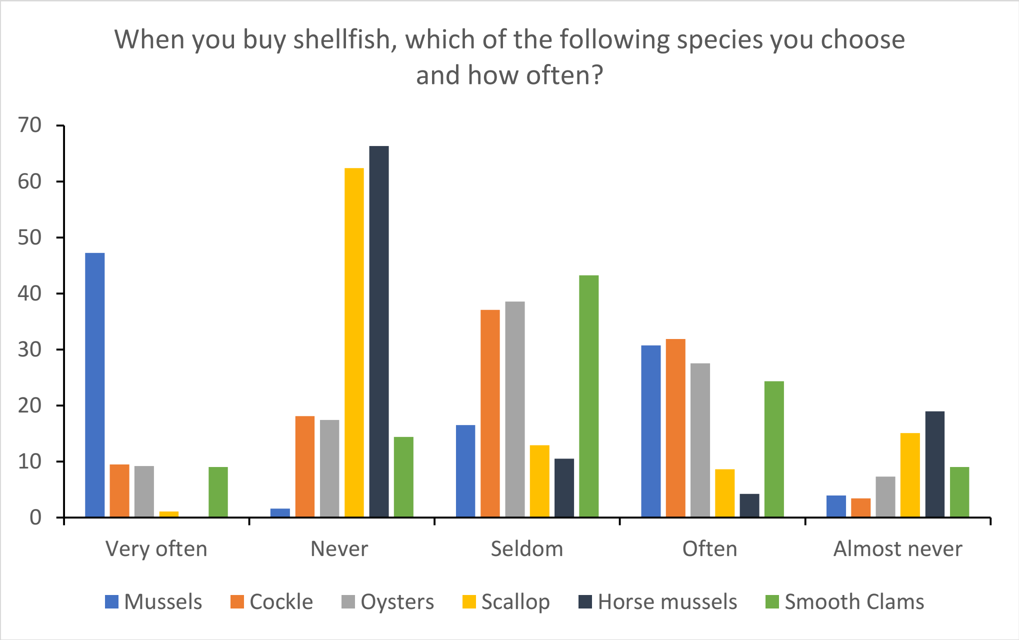

Figure 1. Frequency of shellfish purchase per species.

4. Discussion

There is a lack of tradition in shellfish consumption, in spite of the presence of a wide range of shellfish species in the Greek seas (Theodorou *et al*. 2011). This characteristic preference is related to the lack of confidence Greek consumers exhibit to seafood, owing to the fact of not having adequate information on health and safety issues or considering their taste unsatisfactory (Batzios *et al*. 2003).

The sampling frame regarding the place of residents followed the distribution of the corresponding variables in the entire country. For instance, respondents from urban area contributed more than half of the respondents (66.6%), which is mirror the spatial distribution of the Greek citizens (HELSTAT 2014). The vast majority of Greek consumers knows in higher frequency to purchase and taste mussels than pearl oysters. The need to control an invasive species such as pearl oyster and mitigate its impact to the ecosystem could be harmonized taking also into account the economic benefits gained from this species as a fishery resource (Kleitou *et al*. 2021).

Consumers from urban areas seem much better informed, compared to those from other Greek areas as they were preferred and consumed more frequently and in more quantities bivalves in their family. There is a clear preference of buying shellfish from the traditional fish shops, despite the rapid development and expansion of the Super Markets in Greece over the last years. Traditional fish shops, are considered by the consumers that are routinely inspected by veterinarians who issue a certificate on shellfish sea origin, hygienic condition, freshness and quality (Batzios *et al*. 2003, 2004). This pattern was in line with the statements of the respondents regarding the most critical issues that they examine when purchase shellfish; they first examine "*shellfish odor*", second in priority was "*shells (closed or not)*", and third was "*expiration and shell removal date*".

In the present study more than half of the respondents stated that the most preferred pearl-oyster product was "*fresh alive in bulk*". In this context, food safety and health conditions are of great importance for the consumers. Health is an issue frequently mentioned as a reason for specific food choices and its emphasizing role is a growing trend and has a great influence on attitudes towards eating seafood (Olsen 2003). Greek consumers clearly trust the confirmation provided by the veterinary authorities, concerning the enforcement of E.U. legislation on shellfish hygiene, regardless of their income level or age. The information dispersed by the media on shellfish safety, strongly influences the consumers' purchasing behavior, regardless of their demographic and socio-economic status. However, the media quite often over-emphasize the restriction advice given by the veterinary authorities and the consumers question the shellfish hygiene and their safety status. This situation could be changed whether consumers were properly educated on shellfish safety matters. Thus, the media not only has to play the role of interdiction announcements but should also educate the consumers on issues of shellfish safety and quality.

The findings of this study could prove to be quite helpful for Greek producers and marketers of shellfish in order to identify new business opportunities and plan more efficient marketing strategies, which in turn would promote shellfish consumption. One successful approach to establishing the latter goal could be the organization of local festivities promoting the consumption of various shellfish species with different ways of cooking.

Acknowledgement

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References

Batzios Ch., Angelidis P., Moutopoulos D.K., Anastasiadou Ch., Chrisopolitou V. (2003). Consumer attitude towards shellfish in the Greek market: A pilot study. Mediterranean Marine Science 4(2), 155-174.

Batzios Ch., Angelidis P., Papapanagiotou E.P., Moutopoulos D.K., Anastasiadou Ch., Chrisopolitou V. (2004). Greek Consumer's Image of the Cultured Mussel Market. Aquaculture International 12(3), 239-257.

HELSTAT (2014). Census of population and residence 2011. https://www.statistics.gr/el/statistics/-/publication/SAM03/-

Katsanevakis S., Poursanidis D., Issaris Y., Panou A., Petza D., Vassilopoulou V., Chaldaiou I., Sini M. (2011). "Protected" marine shelled molluscs: Thriving in Greek seafood restaurants. Mediterranean Marine Science 12(2), 429-438.

Kleitou P., Crocetta F., Giakoumi S., Giovos I., Hall-Spencer J. M., Kalogirou S., Kletou D., Moutopoulos D.K., Rees S. (2021). Fishery reforms for the management of non-indigenous species. Journal of Environmental Management 280, 111690.

Olsen S.O. (2003). Understanding the relationship between age and seafood consumption: The mediating role of attitude, health involvement and convenience. Food Quality and Preference 14, 199-209.

Theodorou J.A., Perdikaris C., Spinos E. (2019). On the occurrence of rayed pearl oyster *Pinctada imbricata radiata* (Leach, 1814) in Western Greece (Ionian Sea) and its biofouling potential. Biharean Biologist e181204.

Theodorou J.A., Viaene J., Sorgeloos P., Tzovenis I. (2011). Production and Marketing Trends of the cultured Mediterranean mussel *Mytilus galloprovincialis* in Greece. Journal of Shellfish Research 30(3), 859–874.

Zar J.H. (2010). Biostatistical analysis. 5th eds. Prentice-Hall/Pearson, Upper Saddle River, NJ, USA.

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EVALUATION OF POTENTIAL PREVENTIVE MEASURES AGAINST SEAWATER INTRUSION IN NEA MOUDANIA AQUIFER, GREECE

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Abstract

Seawater intrusion poses a serious threat to coastal regions, since it limits the supply of potable drinking water, while it causes the efficiency of crop production to reduce. In an effort to control seawater intrusion and mitigate its negative effects, various protective measures can be implemented. Within this framework, the present study investigates the utilization of injection barriers, either as a sole solution (SO1) or in combination with groundwater withdrawals decrease (SO2), for controlling seawater intrusion in the coastal aquifer of Nea Moudania, Greece. For each project, two alternative scenarios using different amount of water for injection - and also pumping different amount of water for irrigation in the case of SO2 - were examined and evaluated by combining numerical modeling with a GIS-based economic analysis, thus involving both environmental (i.e. solute concentrations) and economic (i.e. farmers' income) aspects. To further assess the economic impact of the scenarios formed in each project, the ratio of the total economic benefits resulting from their implementation to the total amount of water injected was estimated. The results showed that in the case of SO1 there is no clear-cut "winner" between the two applied scenarios, while in the case of SO2 the scenario with the less amount of injected water - but higher pumping reduction - seems to be more appropriate.

Keywords: *Seawater intrusion, protective measures, numerical modeling, GIS-based economic analysis, Nea Moudania aquifer*

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1. Introduction

Seawater intrusion has evolved into a major environmental issue greatly affecting the groundwater potential of numerous coastal areas worldwide. Not only does seawater intrusion result in an imminent deterioration of groundwater quality, but it also reduces the available and exploitable amount of freshwater (Pool & Carrera 2010; Werner *et al.* 2013; Siarkos & Latinopoulos 2016; Siarkos *et al.* 2017). In addition, seawater intrusion negatively affects local agriculture, since crop productivity is significantly reduced when irrigating with saline water (Zekri 2008; Arslan & Demir 2013). To alleviate its serious consequences on the environment and economy of coastal regions, a number of well-designed preventive measures, such as reduction of pumping rates, relocation of pumping wells, increase of aquifer recharge, construction of injection or/and extraction barriers, construction of subsurface barriers and combination techniques, can be implemented. However, in order to select and apply the most appropriate and efficient solutions for the area of interest, the beneficial effects (both environmental and economic) of the potential measures should be properly investigated and evaluated (Pool & Carrera 2010; Papadopoulou 2011; Kallioras *et al.* 2013; Siarkos *et al.* 2017).

In this perspective, the present study investigates the utilization of injection barriers, either as a sole solution or in combination with groundwater withdrawals decrease, for controlling seawater intrusion in the coastal aquifer of Nea Moudania, Greece, which faces serious water-salinity problems due to seawater intrusion. For each project, two alternative scenarios which differ in the amount of injected water - and the amount of water pumped for irrigation purposes in the case of SO2 - were formed, examined and evaluated by considering not only environmental aspects (i.e. the spatio-temporal evolution of chloride concentrations), but economic as well (i.e. the agricultural income variation). To this task, numerical modeling was combined with a GIS-based economic analysis incorporating the coupling between water-salinity production functions and agro-economic data. Finally, in order to further assess the scenarios developed in each project as far as their economic impact is concerned, the ratio of the total economic benefits resulting from their implementation to the total amount of water used for injection was computed. According to this ratio, higher values indicate that for a certain amount of water injected higher economic benefits are anticipated (over a given time period).

2. Material and Methods

The hydrological basin of Nea Moudania is located in the south-western part of the Chalkidiki peninsula, Northern Greece, while it is a coastal basin bordered to the south by Thermaikos Gulf (Figure 1a).

The basin extends over an area of about 127 km^2, while its mean topographic elevation is about 176 m above sea level and its mean slope is about 12%. The study area is a typical rural area, where agriculture dominates both the local economy and land use, thus leading to increased irrigation needs. These needs are exclusively covered by the Nea Moudania aquifer (Figure 1b), which is characterized by a net deficit in its water balance, since total water demand exceeds natural recharge. Due to this fact, both decline of groundwater levels and intrusion of seawater towards the interior of the aquifer are observed (Latinopoulos 2003; Siarkos & Latinopoulos 2016).

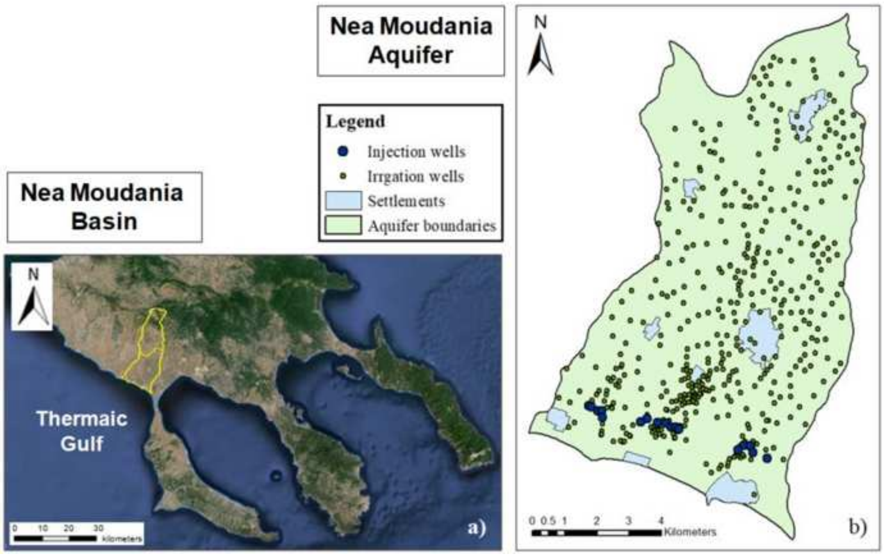

Figure 1. a) Location and boundaries of Nea Moudania basin, and b) boundaries of Nea Moudania aquifer, along with the irrigation wells operating in the region and the wells used for injection.

To control seawater intrusion in the reference area, the present study investigates the utilization of injection barriers either as a sole solution (SO1) or in combination with groundwater withdrawals decrease (SO2), while considering a 30-year implementation period (2020-2050). The reason of forming these two solutions lies on the different source of water used for artificial recharge. More specifically, in the case of SO1, water from the north portion of the aquifer is injected into the recharge wells, while in the case of SO2, water from a reservoir designed to be constructed in a nearby hydrological basin (Olynthios River basin) is used. Moreover, in SO2, water from the reservoir is also used for serving the total domestic water needs and part of the irrigation water requirements in the study area, thus leading to the decrease of groundwater abstractions.
In both projects, two alternative scenarios were formed based on the amount of water used for injection, thus resulting in the development of four scenarios in total (SO1/S1, SO1/S2, SO2/S1 and SO2/S2). In scenarios SO1/S1 and SO2/S1, $2.0*10^6$ m^3 of water was annually injected into the wells, while in scenarios SO1/S2 and SO2/S2 the volume of water injected reached $3.0*10^6$ m^3 per year. Additionally, in the case of SO2, groundwater abstractions for irrigation were reduced by $8.0*10^6$ m^3 in the first scenario, and by $7.0*10^6$ m^3 in the second scenario (pumping for domestic needs was totally ceased in both scenarios). These values were calculated based on the fact that the total volume of water used from the reservoir to serve the artificial recharge and irrigation needs equals to $10.0*10^6$ m^3 per year. Water and Evaluation Planning (WEAP) software (Sieber & Purkev 2005) was applied to determine this specific value by simulating the reservoir's operation, while considering an equitable distribution of its reserves in the broader region and assigning certain priorities (i.e. first priority to domestic water needs so as to be fully covered by the reservoir and secondary priority to rural demand).

With regard to the recharge wells, existing irrigation wells were selected for injection; these wells operate as injection wells only during the non-irrigation period (1st October - 30th April, 212 days), while during the rest of the year they continue to operate for irrigation purposes. In all scenarios, the same irrigation wells - 17 in total - were used (Figure 1b). The selection of their number and location was based on an attempt to protect from future contamination as many irrigation wells as possible, thus preventing farmers' income losses due to crop productivity reduction caused by irrigating with saline water. With respect to the injection rates of recharge wells, they were computed based on the total volume of injected water and the number of recharge wells, assuming that injection rate is the same in all 17 wells.

To simulate all the aforementioned scenarios (including a do-nothing scenario, named as D.N., under which no preventive measures are applied) and study the spatio-temporal evolution of chloride concentrations, a calibrated transient groundwater flow and transport model already developed for the study area (Siarkos and Latinopoulos 2016) was employed. Additionally, to examine farmers' income variation under all scenarios, a GIS-based economic analysis (on the farm-level) incorporating the coupling between water-salinity production functions - created on the basis of the salinity models of Ayers & Wetscot (1985) - and agro-economic data was conducted, while using the results of the transient model (in the form of electrical conductivity values). Finally, in order to define the economic benefits of the protection scenarios, the difference in income between the do-nothing and the protection scenarios was calculated, while in order to assess their economic impact, the ratio of the total benefits over the implementation period to the total amount of water used for injection was computed.

3. Results and Discussion

The differences in chloride concentrations at the end of the simulation period (2050) between the do-nothing and the protection scenarios (Figure 2) clearly illustrate concentration reduction in all cases. More specifically, in the case of SO1, the reduction is mainly observed in the vicinity of the recharge wells, while in the case of SO2, it occurs over a broader area along the injection barriers, thus resulting in the development of a larger zone of influence. Furthermore, in both projects, higher chloride concentration reduction is observed in the second group of scenarios, i.e. SO1/S2 and SO2/S2, in which greater amount of water is injected into the aquifer,

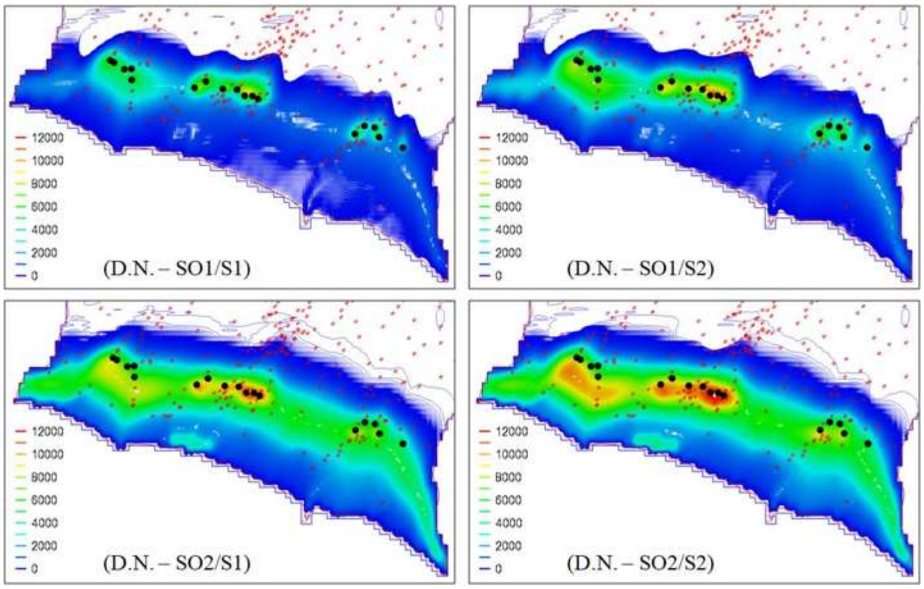

Figure 2. Differences in chloride concentrations (mg/l) between the do-nothing and the protection scenarios at the end of the simulation period (2050), along with irrigation and recharge (black dots) wells.

The same conclusions regarding the effectiveness of the protection scenarios also derive from Figure 3, which shows the change in electrical conductivity values between the do-nothing and the protection scenarios at the end of the simulation period (2050). This change refers to the decrease of electrical conductivity - due to the implementation of preventive measures - below 5.3 dS/m, which is the salinity threshold value of the most resistant crop detected in this specific part of the region (i.e. barley). As it is obvious, in the case of SO2, the changes extend over a larger area than in SO1 for both scenarios. Moreover, in both projects, the implementation of the second group of scenarios, i.e. SO1/S2 and SO2/S2, leads to a greater extent of the total area where changes in electrical conductivity occur.

As regards the results of the economic analysis, Figures 4a and 4b show the aggregate annual agricultural income for all scenarios, and the aggregate annual benefits of the protection scenarios, respectively. As it is apparent, economic benefits gradually increase under all protection scenarios, while the greatest increase is expected to occur under scenario SO2/S2. Furthermore, Figure 5 displays the spatial distribution (on the farm-level) of the annual benefits of the protection scenarios for 2050. Obviously, the results are in full accordance with the chloride concentration and electrical conductivity patterns previously presented. Finally, in Table 1 the results regarding the calculation of the ratio of the total economic benefits of the protection scenarios to the total amount of water injected for the whole implementation period are presented. According to these results, in the case of SO1, the applied scenarios appear to be rather equivalent to each other, while in the case of SO2, scenario S1 seems to prevail over scenario S2, since it results to higher benefits per m^3 of injected water.

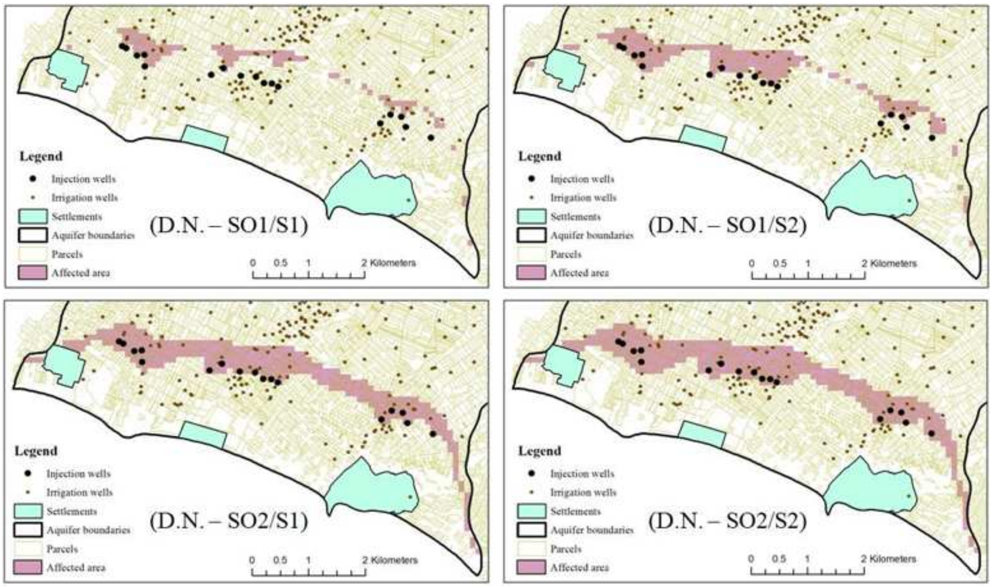

Figure 3. Changes in electrical conductivity (in 2050) between the do-nothing and the protection scenarios (the changes refer to the decrease of electrical conductivity below 5.3 dS/m). The affected area for each scenario is: SO1/S1 = 0.96 km^2, SO1/S2 = 1.93 km^2, SO2/S1 = 3.32 km^2, SO2/S2 = 3.79 km^2.

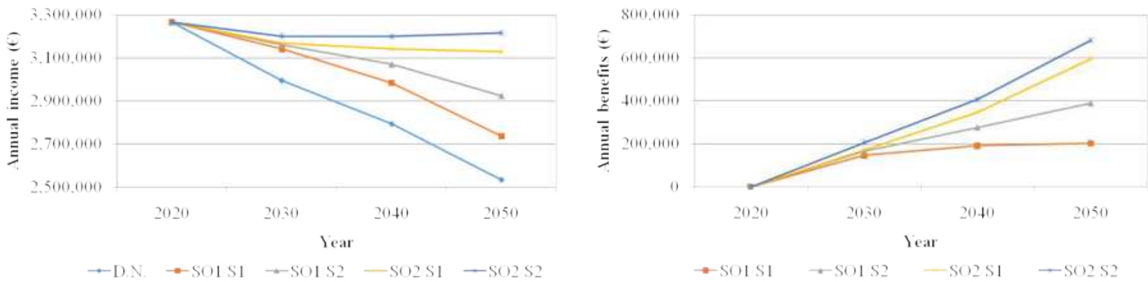

Figure 4. a) Aggregate annual agricultural income for all scenarios, and b) aggregate annual benefits of the protection scenarios.

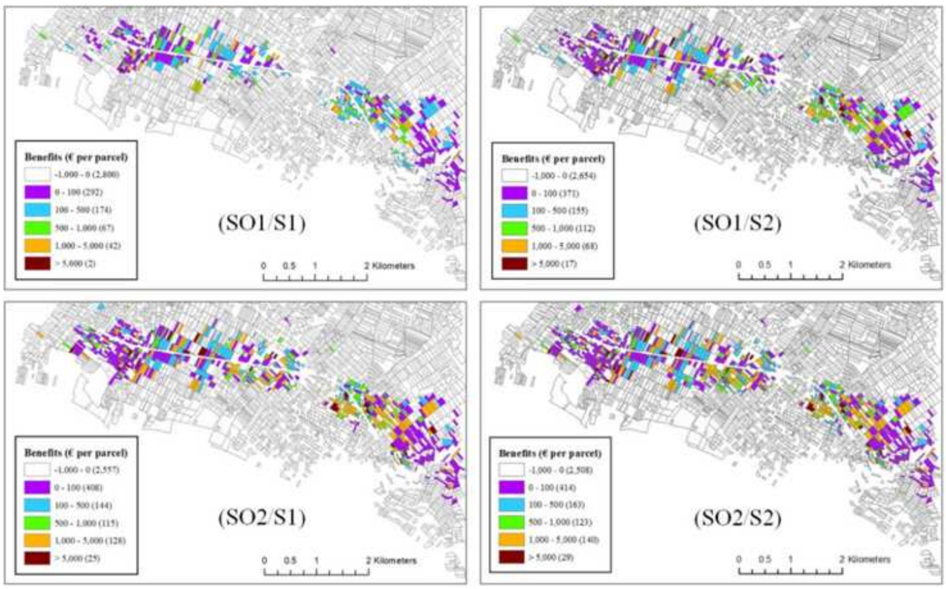

Figure 5. Spatial distribution of the annual benefits (per parcel) of the protection scenarios for 2050 (the number of parcels are given in the parentheses).

Table 1. Results regarding the calculation of the ratio of the total economic benefits of the protection scenarios to the total amount of water injected for the 30-year implementation period (2020-2050).

	D.N.	SO1/S1	SO1/S2	SO2/S1	SO2/S2
Total income (€)	86,879,016	91,253,242	93,251,277	95,030,384	96,401,506
Total benefits (€)	-	4,374,226	6,372,261	8,151,368	9,522,490
Total water injected (m^3)	-	$60*10^6$	$90*10^6$	$60*10^6$	$90*10^6$
Benefits (€) per m^3 of injected water	-	0.073	0.071	0.136	0.106

4. Conclusions

In this study, numerical modeling was coupled with a GIS-based economic analysis in order to investigate and evaluate potential preventive measures against seawater intrusion in Nea Moudania aquifer, Greece. The results showed that the solution involving both artificial recharge and groundwater withdrawals decrease has more positive effects both from environmental and economic point of view. However, for a complete economic evaluation of the projects developed, their implementation costs (i.e. construction and operating costs) should be also taken into consideration. With respect to the scenarios formed in each project and as far as their economic impact is concerned, in the case of SO1, there is no clear-cut “winner” between the two applied scenarios, while in the case of SO2, the scenario with the less amount of injected water - but higher reduction in groundwater withdrawals - seems to be more appropriate.

Acknowledgements

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References

Arslan H., Demir Y. (2013). Impacts of seawater intrusion on soil salinity and alkalinity in Bafra Plain, Turkey. Environmental Monitoring & Assessment 185, 1027-1040.

Ayers R.S., Westcot D.W. (1985). Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome. Irrigation and Drainage Paper 29.

Kallioras A., Pliakas F., Schuth C., Rausch R. (2013). Methods to countermeasure the intrusion of seawater into coastal aquifer systems. In: “Wastewater Reuse and Management”, Sharma S.K., Sanghi R. (eds). Springer Science and Business Media, Dordrecht, p. 479-490.

Latinopoulos P. (2003). Development of water resources management plan for water supply and irrigation. Final Report prepared for: Municipality of Moudania. Dept. Civil Engineering, AUTH, Greece.

Papadopoulou M.P. (2011). Optimization approaches for the control of seawater intrusion and its environmental impacts in coastal environment. Pacific Journal of Optimization 7, 479-502.

Pool M., Carrera J. (2010). Dynamics of negative hydraulic barriers to prevent seawater intrusion. Hydrogeology Journal, 18, 95-105.

Siarkos I., Latinopoulos D., Mallios Z., Latinopoulos P. (2017). A methodological framework to assess the environmental and economic effects of injection barriers against seawater intrusion. Journal of Environmental Management 193, 532-540.

Siarkos I., Latinopoulos P. (2016). Modeling seawater intrusion in overexploited aquifers in the absence of sufficient data: application to the aquifer of Nea Moudania, N. Greece. Hydrogeology J. 24, 2123-2141.

Sieber J., Purkey D. (2005). WEAP - Water Evaluation and Planning System. User Guide for WEAP’ 21. SEI, Stockholm Environmental Institute, Somerville, USA.

Werner A.D., Bakker M., Post V.E.A., Vandenbohede A., Lu C., Ataie-Ashtiani B., Simmons C.T., Barry D.A. (2013). Seawater intrusion processes, investigation and management: recent advances and future challenges. Advances in Water Resources 51, 3-26.

Zekri S. (2008). Using economic incentives and regulations to reduce seawater intrusion in the Batinah coastal area of Oman. Agricultural Water Management 95, 243-252.

SEASONAL OXIDATIVE AND CELLULAR STRESS RESPONSES OF COMMERCIALLY IMPORTANT INVERTEBRATES AT DIFFERENT HABITATS OF THE NORTH AEGEAN SEA

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Abstract
Habitat conditions play a crucial role in food acquisition, energetic and physiological performance of marine organisms during seasonal changes. However, same species' populations exhibiting wide range of distribution might face physiological constraints driven by their habitats' oceanographic and climatological characteristics. As a first step in identifying such marine areas in Greek coasts, we studied the seasonal oxidative and cellular stress responses of *Callinectes sapidus* (blue crab), *Sepia officinalis* (cuttlefish), *Holothuria tubulosa* (sea cucumber) and *Venus verrucosa* (clam), collected from three different Greek gulfs: Thermaikos, Pagasitikos and Vistonikos. Seasonal analysis of lipid peroxidation and apoptosis were estimated. Blue crab and clam exhibited the most significant seasonal changes compared to the sea cucumber and cuttlefish, which exhibited no changes. These results are correlated with the habitats' spatial oceanographic conditions and are discussed based on future projections of global warming in the Mediterranean Sea.
Key words: *Invertebrates, Habitat, Seasonal, Refugia, Oxidative Stress, Apoptosis*
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1. Introduction

Global warming's strong ecological pressure is expected to be loaded on the mid-sublittoral zone ecosystems (Helmuth *et al.* 2006), where the biodiversity of some areas, characterized as "hot-spots", indicates high vulnerability to temperature elevation (Arnell *et al.* 2019). Specifically, elevated temperature, hypoxia and CO_2, according to the oxygen and capacity limited thermal tolerance hypothesis (OCLTT) (Pörtner *et al.* 2017), affect energy turnover and oxygen systems of supply and demand, activating reactive oxygen species (ROS) generation and related phenomena. However, since climate change's effects differ both spatially and temporally, the mapping of oceanographic and climatological characteristics of marine microhabitats is essential, as species' physiological responses are also expected to substantially differ from the ones inhabiting locations characterized by higher environmental risk (Keppel *et al.* 2012). Therefore, the determination of environmental stressors' temporal and spatial forms, and organisms' respective responses is necessary, in order to understand the organisms' physiological responses and how they are related with the microhabitats, subsequently providing effective conservation and aquaculture planning (Helmuth *et al.* 2006, 2010). However, few studies have established clear links between habitat characteristics and physiological performance of the inhabiting species.

The present study's aim was to evaluate and understand the effects of seasonal environmental variations on the physiological stress responses of economically important mid-sublittoral zone invertebrates inhabiting areas of the North Aegean, such as Thermaikos, Pagasitikos and Vistonikos gulf. Specifically, we studied the seasonal changes in indicators of metabolism and antioxidant defense in the species: (a) *Callinectes sapidus* (Rathbun, 1896) (Phylum: Crustacea), (b) *Sepia officinalis* (Linnaeus, 1758) (Phylum: Mollusca, Class: Cephalopoda), (c) *Holothuria tubulosa* (Gmelin, 1791) (Phylum: Echinodermata) and (d) *Venus verrucosa* (Linnaeus, 1758) (Phylum: Mollusca, Class: Bivalvia). The aforementioned gulfs in the North Aegean Sea exhibit diverse water physicochemical characteristics and geomorphology (Raitsos *et al.* 2012). For the purpose of the present work, levels of lipid peroxidation

(Thiobarbituric acid reactive substances - TBARS) as indicators of oxidative stress and Bax/Bcl-2 ratio as an apoptosis indicator were determined.

2. Materials and Methods

2.1. Animal and tissues collection

The examined species were collected from Thermaikos, Pagasitikos and Vistonikos gulfs, under a seasonal prism (Figure 1). Examined species' individuals (N=10) were sampled in April 2018 (10-21 April), June 2018 (7-15 June), November 2018 (4-12 November) and January 2019 (6-11 January). *S. officinalis*, *H. tubulosa, C. sapidus* and *V. verrucosa* samples were collected by divers and local fishermen (0-10 m depth). Immediately after species' sampling, individuals were dissected, and samples of the mantle from *S. officinalis*, the muscle from *H. tubulosa*, the posterior adductor muscle (PAM) from *V. verrucose,* and the muscle from *C. sapidus* were immediately frozen in liquid nitrogen. The frozen samples were transferred to the laboratory, where they were stored at -80°C for the determination of the metabolic and oxidative stress indicators.

2.2. Biophysical variables - Satellite remote sensing

Monthly satellite-derived chlorophyll-a (*Chl-a*) concentrations (mg m^{-3}) were acquired from the Ocean-Colour Climate-Change Initiative (OC-CCI) project. OC-CCI *Chl-a* product is based on multi-satellite sensors such as MODIS-Aqua, SeaWiFS and MERIS, available at the global oceans. Here, the *Chl-a* data at a spatial resolution of 4 km were used. This dataset is the most consistent timeseries of multi-satellite ocean colour data currently available (Racault *et al.* 2015). The monthly climatological *Chl-a* means were computed to assess the overall long-term seasonal cycle (January 1998 to December 2019).

Sea Surface Temperatures (SST) with satellite remote sensing [(GHRSST) SST (v 1.0)] were acquired for Pagasitikos, Vistonikos and Thermaikos Gulfs. Monthly SST climatologies were calculated over 10 years of observations (January 2009 - December 2018) to represent the overall SST seasonal cycle over the regions. GHRSST (*K10 L4*) archived data, available daily at a global 0.1ºC [since April 2008 (NOC, 2008)], were produced at NAVOCEANO (Naval Oceanographic Office) on an operational basis.

Mavropoulou *et al.* (2020) produced a gridded product of salinity over the Mediterranean Sea based on *in situ* observations provided by the World Ocean Database 2013 (WOD13), available at the National Oceanographic Data Center (NODC-NOAA). The NODC complies with international quality control procedures and has established data quality control flags to limit uncertainties and biases among the available data. The Data-Interpolating Variational Analysis (DIVA) software was used to produce a gridded salinity dataset for the time period 1960–2011 (Mavropoulou *et al.* 2020). Here, monthly climatological salinity means were computed to assess the overall long-term seasonal cycle (January 1998 to December 2011).

2.3. Biochemical analyses

Tissues, homogenized in phosphate buffer (50 mmol l^{-1}, pH 7.4), were centrifuged (2,000 *g*, 4ºC, 15 min) and the supernatants were employed for TBARS measurements. In brief, for quantification of TBARS, 250 μL TCA (20%) and 500 μL thiobarbituric acid (0.67%) were added in 250 μL of supernatant. Thereafter, the mixture was boiled (60 min), cooled (room temperature) and centrifuged (3,000 *g*, 15 min) after the addition of 2 mL butanol. TBARS levels were determined following the absorbance at 532 nm (extinction coefficient $\varepsilon = 156\ mM^{-1}\ cm^{-1}$) (Buege & Aust 1978). The preparation of tissue samples for SDS-PAGE and the immunoblot analysis are thoroughly described in Feidantsis *et al.* (2015). In the present study, equivalent amounts of proteins (50 μg) were separated on 10% and 0.275% (w/v) acrylamide and bisacrylamide slab gels and transferred electrophoretically onto nitrocellulose membranes (0.45 μm, Schleicher and Schuell, Keene N. H. 03431, USA). The antibodies used were the following: anti-Bcl2 (7973, Abcam), anti-Bax (B-9) (2772, Cell Signaling) and anti-β-actin (3700, Cell Signaling) as the standard protein for the loading control.

2.4. Statistical analyses

Changes in the biochemical responses were tested for significance ($p<0.05$) using GraphPad Prism 5.0. Specifically, the general linear model (GLM) repeated measures mixed model ANOVA (GLM) has been used with season and location being the independent variables. Post-hoc comparisons were performed using the Bonferroni test.

Due to the small sample size (N=10), homogeneity of variance was not tested, since normality tests have little power to conclude to whether or not a small sample of data comes from a Gaussian distribution.

Temperature variations were also tested for significance (5% level) by using one way (GraphPad Instat 3.0) or two-way (GraphPad Prism 5.0) ANOVA, where season and location were presumed as fixed factors. Homogeneity of variance assumption (big sample size: N=60 recordings per month) was examined with the Welch parametric test.

3. Results

3.1. Biophysical variables

Vistonikos water temperature remained significantly lower compared to Thermaikos and Pagasitikos, and especially in February, July, August, September and January (Figure 1A). Moreover, we analyzed biophysical variables (such as SST, chlorophyll and salinity) to assess their seasonal cycles in three different gulfs (Figures 1B, 1C and 1D respectively). Vistonikos gulf was the coldest region, and the salinity reaches minimum levels (34.7 psu) during September. The Chl-a concentrations of Vistonikos gulf appear to be relatively high, with maximum concentrations during March (1.38 mg m^{-3}), and minimum during summer. In contrast, Pagasitikos gulf was the warmest region throughout the year, with higher salinity and the lowest Chl-a concentrations. Thermaikos gulf depicts by far the highest Chl-a concentrations, relatively colder temperatures and lower salinities. Vistonikos and Thermaikos gulfs appear to have an abrupt increase in chlorophyll during March and April (respectively), whereas Pagasitikos gulf exhibits a smooth transition from low concentrations during summer and mild maxima during winter. Overall, the seasonality of Vistonikos and Thermaikos gulfs appear to have more similar characteristics in comparison to Pagasitikos gulf.

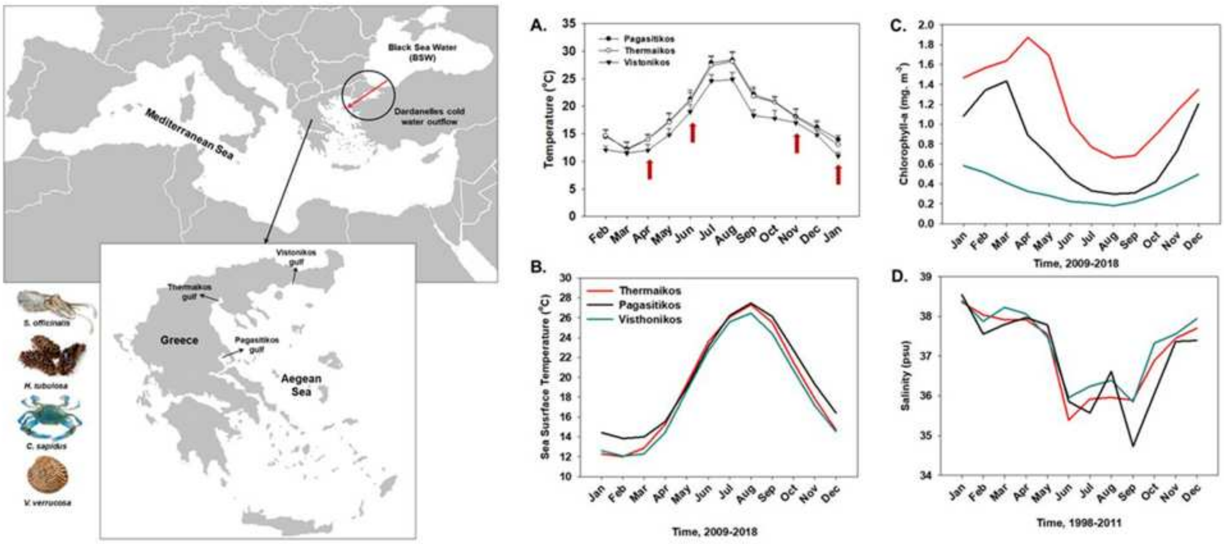

Figure 1. Study samplings of **Pagasitikos, Thermaikos and Vistonikos gulf, where individuals of *C. sapidus* (blue crab), *S. officinalis* (cuttlefish), *H. tubulosa* (sea cucumber) and *V. verrucosa* (clam) were collected. Red arrow points the cold water outflow from the Black Sea to the Aegean Sea. The monthly sea water temperatures Pagasitikos, Thermaikos and Vistonikos gulf (A) where individuals were collected (red arrows point the sampling months), and climatological (long-term mean) seasonal cycles of biophysical variables. The temporal evolution of satellite-derived Sea Surface Temperature (SST) (B) and Chlorophyll-a (C), and *in situ* salinity (D) have been computed for each gulf (Thermaikos, Pagasitikos and Vistonikos).**

3.2. TBARS

In *S. officinalis,* the highest levels of lipid peroxidation (TBARS) were seasonally observed in the mantle of this species in Pagasitikos and the lowest in Vistonikos gulf. Moreover, there are no statistically significant seasonal differences (Figure 2A). Similar to *S. officinalis,* in *H. tubulosa* muscle (Figure 2B) the highest peroxidation levels were observed in Pagasitikos and the lowest in Vistonikos gulf, while there were no significant seasonal differences.

TBARS levels in *C. sapidus* muscle are shown in Figure 2C. In this tissue, the highest peroxidation was exhibited during summer sampling. Between sampling sites, statistically significant differences were observed. Specifically, TBARS levels were higher in Pagasitikos gulf and lower in Vistonikos gulf. In the PAM of *V. verrucosa,* the seasonal and sampling site pattern of peroxidation levels in this species was similar to that of *C. sapidus*. Specifically, TBARS levels in the PAM of *V. verrucosa* were higher during the summer. Moreover, statistically significant differences were exhibited between all three sampling sites. Specifically, TBARS levels were higher in Pagasitikos and lower in Vistonikos gulf (Figure 2D).

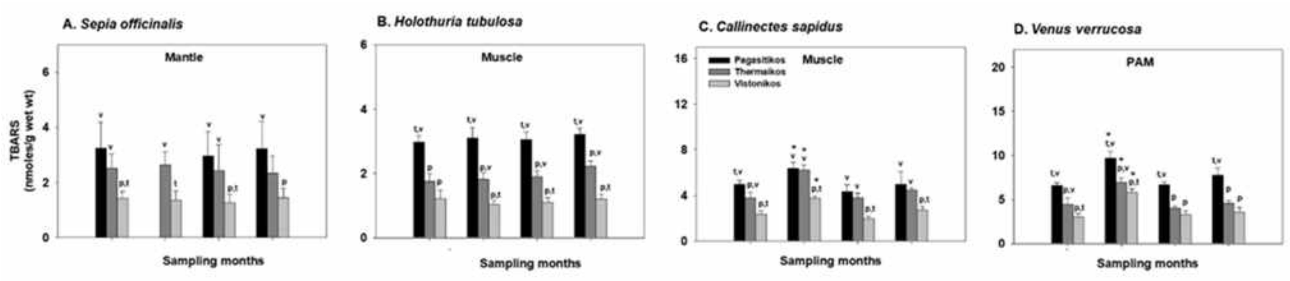

Figure 2. Seasonal variations in the TBARS levels in mantle of *S. officinalis* (cuttlefish) (A), muscle of *H. tubulosa* (sea cucumber) (B), muscle of *Callinectes sapidus* (blue crab) (C) and Posterior Adductor Muscle (PAM) of *Venus verrucosa* (clam) (D) in Pagasitikos, Thermaikos and Vistonikos gulf. Values are means ± SD determined. N=10 preparations from different animals. *p<0,05 compared to the sampling of April, lower case letters denote p<0,05 compared to sampling sites, where p = Pagastitikos gulf, t = Thermaikos gulf, v = Vistonikos gulf.

3.3. Apoptosis

Bax/Bcl-2 levels exhibited their highest values mostly during the summer and the autumn samplings. During the summer months, in *C. sapidus* (Figure 3A) and *H. tubulosa* (Figure 3C) muscle, the highest values are observed in Thermaikos, compared to the other two sampling sites, Pagasitikos and Vistonikos gulf. In the mantle of *S. officinalis* (Figure 3B) and in the PAM of *V. verrucosa* (Figure 3D) the highest Bax/Bcl-2 ratio levels were observed in Pagasitikos individuals and the lowest in individuals obtained from Vistonikos gulf (Figure 3). The main effect of season was significant for the blue crab and clam, whereas the effect of location was significant for all four examined species. Factor interactions were significant only for the blue crab and clam.

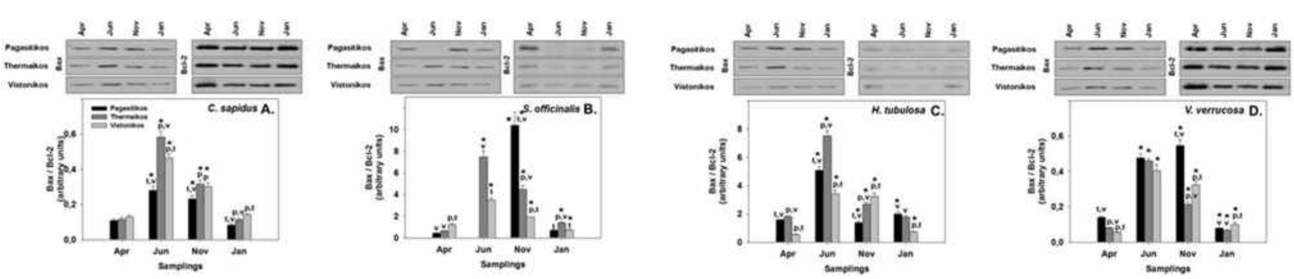

Figure 3. Seasonal variations of Bax/Bcl-2 ratio in *C. sapidus* muscle (A), *S. officinalis* mantle (B), *H. tubulosa* muscle (C) and *V. verrucosa* PAM (D) in Pagasitikos, Thermaikos and Vistonikos gulf. Representative immunoblots are shown. Values are means ± SD determined. n=10 preparations from different animals. *p<0,05 compared to the sampling of April, small letters denote p<0,05 compared to sampling sites, where p = Pagastitikos gulf, t = Thermaikos gulf, v = Vistonikos gulf.

4. Discussion

The data obtained in the present work clearly indicate a close relation between habitats' climatological and oceanographic conditions and physiological stress responses. The latter largely depends on marine animals' biology, since species specific responses between the blue crab and the clam, which integrate more effectively their physiological responses as they face intense environmental variations, and pelagic species, such as the cuttlefish and the sea cucumber, are observed. Oxidative stress indicators, as well as the lower food availability in Pagasitikos gulf, might strongly constrain the energetics of inhabiting marine organisms during long term exposure to increasing of temperature because of global warming. Energy availability and balance is a fundamental requirement for stress adaptation and tolerance of marine organisms (Sokolova 2013). According to OCLTT hypothesis, such constrains in Pagasitikos gulf may bring the examined species towards their higher thermal limits (Pörtner *et al.* 2017). However, it is well known that habitat conditions and sea water quality is a matter of synergistic effects of several environmental factors, including increase in SST and summer heat waves, eutrophication and marine pollution. Frequency and severity of heat waves is expected to increase in Mediterranean Sea, including the Aegean Sea, as a consequence of climate change (Galli *et al.* 2017), with a corresponding increase in fish mortality (Genin *et al.* 2020). On the other hand, the climatological and oceanographic data measured for a 10 ten-year period, indicate that Vistonikos gulf exhibits a habitat with more favorable environmental conditions compared to the other two examined gulfs. However, based on the above mentioned climatic projections in the Mediterranean Sea, it remains unclear in what extent Vistonikos gulf or other coastal habitats could buffer the impacts of climate change in the future.

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References

Arnell, N.W., Lowe, J.A., Challinor, A.J., Osborn, T.J. (2019). Global and regional impacts of climate change at different levels of global temperature increase. Climate Change 155, 377–391.

Buege, J.A., Aust, S.D. (1978). Microsomal lipid peroxidation. Methods in Enzymology 52, 302-310

Feidantsis, K., Pörtner, H.O., Antonopoulou, E., Michaelidis, B. (2015). Synergistic effects of acute warming and low pH on cellular stress responses of the gilthead seabream *Sparus aurata*. Journal of Comparative Physiology Part B 185, 185-205. https://doi.org/10.1007/s00360-014-0875-3

Galli, G., Solidoro, C., Lovato, T. (2017). Marine heat waves hazard 3D maps and the risk for low motility organisms in a warming Mediterranean Sea. Frontiers in Marine Science 4, 136.

Genin, A., Levy, L., Sharon, G., Raitsos, D.E., Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. Proceedings of the National Academy of Sciences of the United States of America 202009748, DOI: 10.1073/pnas.2009748117

Helmuth, B., Broitman, B.R., Blanchette, C.A., Gilman, S., Halpin, P., Harley, C.D.G., O'Donnell, M.J., Hofmann, G.E., Menge, B., Strickland, D. (2006). Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. Ecological Monographs 76, 461-479.

Helmuth, B., Broitman, B.R., Yamane, L., Gilman, S.E., Mach, K., Mislan, K.A.S., Denny, M.W. (2010). Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. Journal of Experimental Biology 213, 955-1003.

Keppel, G., Van Niel, K.P., Wardell-Johnson, G.W., Yates, C.J., Byrne, M., Mucina, L., Schut, A.G.T., Hopper, S.D., Franklin, S.E. (2012). Refugia: identifying and understanding safe havens for biodiversity under climate change. Global Ecology and Biogeography 21, 393-404.

Mavropoulou, A.M., Vervatis, V., Sofianos, S. (2020). Dissolved oxygen variability in the Mediterranean Sea. Journal of Marine Systems 208, 103348.

Pörtner, H.O., Bock, C., Mark, F.C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. Journal of Experimental Biology 220, 2685-2696.

Raitsos, D.E., Korres, G., Triantafyllou, G., Petihakis, G., Pantazi, M., Tsiaras, K., Pollani, A. (2012). Assessing chlorophyll variability in relation to the environmental regime in Pagasitikos Gulf, Greece. Journal of Marine Systems 94, S16–S22.

Sokolova, I.M. (2013). Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate the Effects of Multiple Stressors. Integrative and Comparative Biology 53(4), 597–608. doi:10.1093/icb/ict028.

MODELING SURFACE WATER QUALITY USING ARTIFICIAL NEURAL NETWORKS AND MULTIVARIATE METHODS

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Abstract
This study describes the combination of artificial neural networks and multivariate statistical methods to develop a proper model for forecasting water quality index (WQI) in the Saf-Saf River using water quality parameters. The main objectives of this work were to determine the importance of different input variables and to assess the spatial and temporal water quality variation. MLP models were trained using three different algorithms and tested; these models were compared in terms of efficiency criteria and goodness-of-fit for water quality index modeling. Results show that MLP_{BFGS} model provides the best performance with small root mean square error value (RMSE=0.007) and high coefficient of determination (R^2=0.811) compared with the other types of MLP models. In the meantime, sensitivity analysis reveals that BOD_5 acts as the most important contributor decreasing water quality index. PCA/FA results show relatively spatial and seasonal changes in surface water quality, by generating three groups of sampling sites with similar characteristics. Group I (upstream monitoring sites), group II (midstream sampling sites) and group III (downstream sampling sites) corresponding to relatively low pollution, moderate pollution and very high pollution sites, respectively. Therefore, this approach can provide planners and managers with the right tools and evidence to make decisions about the implementation of sustainable management practices.
Keywords: *Water quality index, Multilayer perceptron, Principal Component Analysis, Factor Analysis, Saf-Saf river basin.*
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1.Introduction

Issues and challenges related to water quality deterioration have generated much debate and discussion on increasing awareness on water quality concerns and the increasing demand to sustainably manage water resources. In the last decades, a rapid industrial development without controlling discharges, the intensive use of fertilizers in agriculture and the over exploitation of water resources destroy river ecosystems and affect human health in different ways. This produces a chemical modification of the water rendering it unusable for other purposes and hence aggravates scarcity of water resources (Lonercan *et al*. 1991). Surface water resources are unfortunately exposed more and more to pollution, in the form of discharges of industrial or domestic effluents, and are gradually becoming unfit for any use, without prior treatment. The latter is often complicated and expensive (Singh *et al*. 2004, 2005). Prevention of river pollution requires effective monitoring of physicochemical and biological parameters (Ramteke *et al*. 1995). The water quality index WQI is a means of summarizing large amounts of water quality data into simple terms (e.g., good, fair, poor) for reporting to policymakers and the public in a comprehensive, consistent manner [Canadian Council of Ministers of the Environment (CCME) 2001].

The Water Quality Index (WQI) is used to characterize the pollution status of hydro-systems, because it represents a single numeric score that describes the water quality condition at a particular location in a specific time (Kaurish & Younos 2007; Gazzaz *et al*. 2012). There are different approaches of WQI used by various countries and institutions around the world to assess the water quality status of their rivers like Argentina (Pesce & Wunderlin 2000), USA (Cude 2001), India (Sargaonkar & Deshpande 2003), Portugal (Bordalo *et al*. 2006), Turkey (Boyacioglu 2007), and China (Song & Kim 2009; Wang *et al*. 2017). In addition, water quality information becomes more easily and quickly interpretable than a list of numeric values. Recently, several actors intervening at different levels in water resources management of Saf-Saf river basin (water quality specialists, other managers, legislators or the general public) need to analyze and process this information in order to effectively fulfill their role. It becomes so imperative to simplify this perception of water quality so that the extension of water quality can serve a technical, social and / or even political purpose (Sakaa *et al*. 2015; Khelfaoui *et al*. 2014).

The objectives of this study, therefore, are twofold; the first objective is to develop WQI for assessing surface-water quality and defining water pollutants. The second objective is to establish a proper model based on artificial neural networks and multivariate statistical techniques; this model aims to assist planners and managers of water resources systems for solving surface water pollution problems.

The ANN model provides a perfect knowledge and understanding about the relationship between water quality parameters and water quality index WQI, and defines the effective water quality parameter influencing the decreasing values of WQI through different sampling sites in Saf-Saf river basin. Artificial neural networks (ANNs) have been successfully applied in a number of diverse fields including water resources (Maier & Dandy 2000; Kumar *et al*. 2004). In the water quality forecasting context, ANNs may offer a promising alternative for water. In our present study, we have applied artificial neural networks ANNs to forecasting water quality index WQI in Saf-Saf river basin based on a cause–effect relationship. Here we have investigated the possibility of building a relationship between water quality parameters (independent variables) with WQI (dependent variable). In addition, the ANNs and decision-makers opinion are used in characterizing and prioritizing the most effective variable. The selected variables have been classified using multivariate statistical techniques including principal components analysis and factor analysis.

2. Materials and Methods

2.1. Data description

A total of thirty-five samples of surface water were collected at various sampling sites along Saf-Saf river basin (Figure 1) during April and September, 2015. All water samples were sampled from a depth of 15cm below the surface and preconditioned in high density polyethylene bottles. They were conditioned by washing initially with 5% nitric acid, and then rinsing several times with distilled water. This was carried out to ensure that the sampling bottles were free from contaminants.

Figure 1. Map indicating the location of sampling points in Saf-Saf river basin.

Each of the surface water samples was analyzed for various physicochemical and biochemical parameters such as water temperature (T, °c), pH, oxygen saturation (OS, %), total dissolved solids (TDS, mgL^{-1}), turbidity (NTU), nitrate (NO_3^-, mgL^{-1}), phosphate (PO_4^{3-}, mgL^{-1}), 5-day biochemical oxygen demand (BOD_5, mgL^{-1}), chemical oxygen demand (COD, mgL^{-1}) and chloride (Cl^-, mgL^{-1}).

2.2. Methodology

The analysis plan is decomposed into four major steps which again are decomposed into many tasks. The contents of the four steps are (Figure 2):

Step 1: the first step aims to create a neural networks model, characterize and prioritize the effective water quality parameters, and establish a relationship between water quality parameters and WQI. The WQI values are calculated using the software CCME Calculator Version 1.0 developed by Canadian Council of Ministers of the Environment (CCME 2001). The CCME WQI was originally developed as the Canadian Water Quality Index (CWQI). The resulting values range between 0 and 100, where 0 represents the worst water quality and 100 represents the best water quality. Once the WQI value has been calculated, water quality is ranked by relating it to one of the following classes: Excellent (95-100), Good (80-94), Fair (65-79), Marginal (45-64), Poor (0-44).

Step 2: this step expresses the analysis of the questionnaire data to examine the decision-makers opinion and judgment of various stakeholders using descriptive statistics.
The results of step 2 were compared with the results of the ANNs in step 1 to explore the understanding and knowledge of the local decision-makers about the health status of surface water in Saf-Saf river basin.
Step 3: the purpose of this step is to transform the variables that were not normally distributed and calculate the correlation matrix of the variables selected from step 1.
Step 4: the multivariate statistical techniques (PCA & FA) were used in this step for the selected water quality parameters, to classify the different sampling sites during wet and dry season.

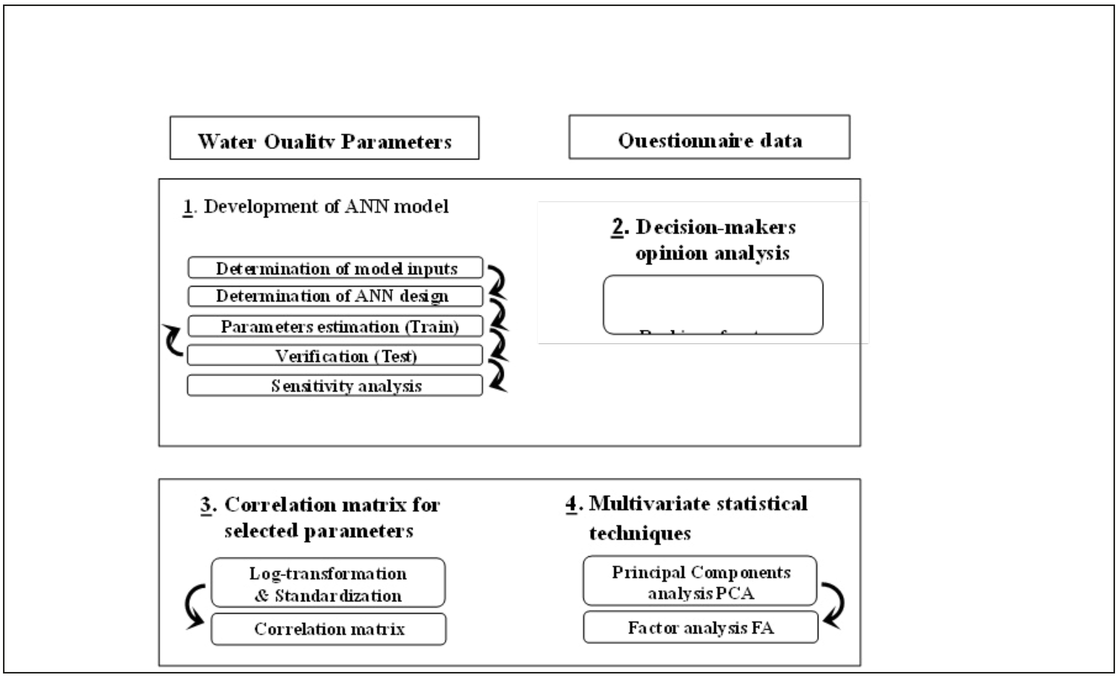

Figure 2. Proposed Water Quality Evaluation Model

3. Results and Discussion

Different MLP models (MLP_{DG}, MLP_{CG} & MLP_{BFGS}) were created and tested in order to determine the optimum number of nodes in the hidden layer. According to Fletcher & Goss (1993), the appropriate number of nodes in a hidden layer ranges from ($2n^{1/2}+m$) to ($2n + 1$), where n is the number of input nodes and m is the number of output nodes. Regarding the results obtained from the 100 MLP models created using three different algorithms, it can be concluded that the best optimal MLP model found is MLP_{BFGS} with 14 hidden nodes and a minimal root mean square error (RMSE) of 0.007 in testing data sets compared with the other types of MLP networks. The MLP_{BFGS} model has very good performance in the two data sets (training and testing) with standard deviation of 15.030 and 13.459, respectively. The corresponding values of coefficient of determination (R^2) and accuracy factor (A_f) for the two data sets are 0.929 and 1.420 for training phase, and 0.811 and 1.210 for testing phase. For the effect of input variables (water quality parameters) on the output (WQI), the MLP_{BFGS} neural network sensitivity analysis was applied to both training and testing phases.

The five most effective water quality parameters for WQI in descending order, are BOD_5, Cl^-, NO_3^-, DCO and TDS. The projection of variables and sampling sites on the factor-plane shows that sampling sites were grouped into three main groups The group I gathers the sampling sites which are typical of average WQI and characterized by low values of chloride, TDS, NO^{-}_{3}, PO^{-3}_{4}, BOD_5 and COD. The sampling sites of group I are located in upstream of Saf-Saf river basin and correspond to relatively low pollution during two seasons (wet and dry season). The group II includes the sampling sites of Saf-Saf valley during dry season and the sampling sites which are located in downstream of Saf-Saf river basin during wet season. This group represents waters with marginal quality based on the WQI. The group III gathers the sampling sites (during dry season) located in downstream area which are characterized by high values of chloride, TDS, NO^{-3}, PO^{-3}_{4}, BOD_5 and COD and very low WQI showing evidence of surface water quality deterioration.

4. Conclusions

In this paper, we developed a new methodology based on combination of artificial neural networks and multivariate statistical techniques to forecast water quality index of surface waters in an unmonitored river basin. MLP neural networks with three different algorithms were trained and tested using datasets (water quality parameters and WQI) measured during wet and dry season in 2015. The predictive capability of the MLP model is determined using three criteria namely RMSE, coefficient of determination (R^2) and the accuracy factor (A_f).

The results obtained in this paper show that MLP_{BFGS} neural network demonstrates the best ANN structure indicating that BOD_5, Chloride, NO_3^-, DCO and TDS are the five most effective water quality parameters influencing WQI in Saf-Saf river. Selecting and ranking water quality parameters assist decision makers and water managers to give a priority to these five water quality parameters in terms of surface water monitoring. PCA combined with FA were used to assess variations in surface water quality of Saf-Saf river basin, both in time and space. Three groups of sampling sites were formed; the group I on the right side of the PC_1 gathers upstream sampling sites that indicate a relatively low pollution during two seasons. The group II characterizes sampling sites of Saf-Saf valley and it represents waters with marginal quality in terms of WQI. The group III includes downstream sampling sites during dry season; it is characterized by very low WQI and it corresponds to high concentration in BOD_5, Chloride, NO_3^-, DCO and TDS reflecting a very polluted surface water.

Therefore, MLP neural network model and multivariate methods enables easy forecasting of surface water quality and allows defining the importance and contribution of water quality parameters to the WQI. In addition, this approach may form a framework to give reliable and trustful knowledge for decision makers in improving river basin sustainability and factual strategies.

References

Bordalo A.A., Teixeira R., Wiebe W.J. (2006). A water quality index applied to an international Shared River basin: the case of the Douro River. Environmental Management 38, 910–920.

Boyacioglu H. (2007). Development of a water quality Index based on a European classification scheme. Water SA 33 (1), 101–106.

Canadian Council of Ministers of the Environment (CCME) (2001). Canadian water quality guide-lines for the protection of aquatic life: CCME water quality index 1.0. Technical Rep., Winnipeg, Canada.

Cude C.G. (2001). Oregon water quality index a tool for evaluating water quality management effectiveness. Journal of the American Water Resources Association 37 (1), 125–137.

Gazzaz N.M., Kamil M., Zaharin A., Juahir H., Firuz M. (2012). Artificial Neural Network Modeling of the Water Quality Index for Kinta River (Malaysia) Using Water Quality Variables as Predictors. Marine Pollution Bulletin 64 (11), 2409-2420.

Kaurish F.W., Younos T. (2007). developing a standardized water quality index for evaluating surface water quality. JAWRA Journal of the American Water Resources Association 43: 533–545.

Khelfaoui F., Zouini D., Tandjir L. (2014). Quantitative and qualitative diagnosis of water resources in the Saf-Saf river basin (north east of Algeria), Desalination and Water Treatment 52:10-12, 2017-2021.

Kumar D.N., Raju K.S., Sathish T. (2004). River flow forecasting using recurrent neural Networks. Water Resources Management 18 (2), 143–161.

Lonercan S., Vansickle T. (1991). Relationship between water quality and human health: a case study of the Linggi river basin in Malaysia. Social Science & Medicin,. 33 (8), 937-946,

Pesce S.F., Wunderlin D.A. (2000). Use of water quality indices to verify the impact of Cordóba City (Argentina) on Suquía River. Water Research 34 (11), 2915– 2926.

Ramteke P.W. (1995). Comparison of standard most probable number method with three alternate tests for detection of bacteriological water quality indicators, Environ. Toxicology Water Quality 10, 173–178.

Sakaa B., Merdas S., Mostephaoui T., Chaffai H., Hani A., Larbi D. (2015). The application of ANNs and multivariate statistical techniques to characterize a relationship between total dissolved solids and pressure indicators: a case study of the Saf-Saf river basin, Algeria, Desalination and Water Treatment 57:28, 12963-12976.

Sargaonkar A., Deshpande V. (2003). Development of an overall index of pollution for surface water based on a general classification scheme in Indian context. Environmental Monitoring & Assessment 89(1), 43–67.

Singh K.P., Malik A., Mohan D., Sinha S. (2004). Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India). Water Research 38, 3980–3992.

Singh K.P., Malik A., Sinha S. (2005). Water quality assessment and appointment of pollution sources of Gomti River (India) using multivariate statistical techniques: Analytica Chimica Acta 538(1), 355–374.

SPATIAL PRIORITIZATION FOR COASTAL MANAGEMENT BASED ON ERODIBILITY ASSESSMENT PAIRED WITH SOCIO-ECONOMIC ELEMENTS

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Abstract

Beach erosion appears to be a major impact of Climate Change, hampering the sustainable development of coastal areas of the wider Mediterranean region. This contribution presents an outline of a methodological framework to prioritize erosion mitigating measures between erosion-prone beaches on the basis of assessing the significance of their touristic (and related economic) activity. The methodology identifies the beaches that are more prone to erosion and prioritize the beaches where urgent erosion mitigation measures have to be undertaken. The study takes place in the Kassandra peninsula (Chalkidiki, Greece), which contains a large number of beaches with a high aesthetic and hedonic value. The study plans to lay the foundation for the development of a Decision Support Systems (DSS), fueled by Earth Observation information.

Key words: *Coastal Erodibility Index, Sea Level Rise, Coastal Management, Touristic Activity*

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1. Introduction

The combined effect of mean Sea Level Rise (SLR) and of coastal storms under Climate Variability and Change (CV&C) puts beaches under an increasing erosion risk and can also have severe impacts on coastal assets/infrastructures and activities (Jimenez *et al,* 2012, IPCC 2019). In Greece, about 30% of the beaches have been found to be under erosion (Alexandrakis *et al,* 2013), whereas more recent studies have predicted that in 2100 between 31% and 88% of all the Aegean Archipelago beaches will be completely eroded (at least temporarily) due to the SLR and extreme events, even under a moderate climatic scenario (Monioudi *et al,* 2017).

At the same time, tourism plays a major role in the Greek economy, accounting (in 2019) for about 20.8 % of the GDP, the 21.7 % of the employment and 30.1 % of the country's exports (https://wttc.org/). In addition, the 3S (Sun-Sea-Sand) tourism model, which is based on beach recreational activities, is dominant in Greece, constituting the main economic output in many areas of the country (SETE 2016). However, it appears that there is a clear shortfall in structured integrated coastal management approaches that can respond effectively to CV&C by incorporating both environmental and economic impact assessments into the design of viable adaptation solutions at a regional scale (e.g., Camus *et al.* 2017, UNFCCC 2020).

In this contribution, a methodological framework is presented for the assessment of the potential beach erosion combined with socio-economic characteristics of a highly touristic Greek coastal region, which aims to support regional sustainable coastal planning under CV&C, through prioritization of the beaches where erosion mitigating solutions are (urgently) required. The framework is implemented on the Kassandra peninsula in Chalkidiki (N. Greece), a coastal stretch with a total coastline length of 180 km (Figure 1). The area has been selected as a case study due to its plethora of sandy beaches with high aesthetic and hedonic value as well as its high 3S touristic activity.

2. Material and Methods

The coastline is divided in 200 m segments which are ranked for several biophysical variables (Gornitz 1997, Klose & Thieler 2001) in order to compute their Coastal Erodibility Index (CEI), a qualitative measure of the erosion risk, in a scale from low (1) to high (5). The biophysical variables collated/computed for each segment are (a) the geomorphological character, (b) the presence of *P. oceanica* meadows, (c) the exposure to waves/winds, and (d) Extreme Sea Levels (ESL). The CEI represents the potential to erosion for each segment and, in the current work it is computed as a function of the wave exposure index, the natural habitats index, the extreme sea level index and the geomorphology index; it was decided to assign zero values (no erosion) on the coastal areas with a geomorphology other than sandy beach. For the visualization and ranking of the variables as well as the computation of the wind, wave and shore exposure the InVest Coastal vulnerability supervisory model was used (Guerry *et al.* 2012).

The coastal geomorphology of the study area was classified for each 200 m segment into five categories: sand beach, cobble beach, low cliff, medium cliff, rocky high cliff (Klose & Thieler 2001).

Categorization of each segment was carried out on the basis of satellite images, in-situ observations and aerial photos.

In order to compute an erodibility index, it is important to consider the coastal habitats and particularly the distribution of the *P. oceanica* meadows, since they provide coastal protection and sediment stabilization (Hemminga & Nieuwenhuize 1990). Following the methodology described in the work of Traganos & Reinartz (2018) the meadows in the NW part of our study area were mapped from Sentinel 2A satellite images (10 m and 20 m bands B2-B5 and band B8) and a supervised habitat classification method was applied. The seagrass mapping included areas down to the 20 m depth contour.

The wave exposure index for each segment (200 m) of the coastline has been computed on the basis of the mean of the strongest 10 % of the winds (in each of 16 equiangular sectors), the maximum fetch distance for each specific segment and the water depth along each fetch-ray. The InVest Coastal Vulnerability model estimates the relative exposure of a shoreline point to waves by assigning it the maximum of the weighted average power of oceanic waves and locally wind-generated waves. In the current work waves were hindcasted from an available wind dataset of 3-hour observations for the years 1985-2004 (National Meteorological Service of Greece) from the station of Thessaloniki, preferred from other meteorological stations more proximal to the study area, due to its longer temporal coverage and quality of the data. The water depth used on wave hind-casting was extracted from the *GEBCO 19 15* arc second bathymetry product.

Projections of extreme sea levels were retrieved from the data-set described in Vousdoukas *et al* (2017) and is available through the JRC open access data-base (http://data.jrc.ec.europa.eu/collection/LISCOAST), for the current decade (2020s) considering an extreme event with a return period of 5 years under the RCP8.5 scenario.

Coastal touristic businesses within 1 km radius from the coastline were digitized from Google maps. Along the area of interest were found approximately 1160 Hotels, 65 Beach Bars and 320 Restaurants from which a density map of the touristic business infrastructure density was constructed. This information, coupled with the CEI index can provide indications for the prioritization of requisite adaptation measures along the Kassandra coastline. In this study beaches with extreme CEI values are prioritized based on their socio-economic characteristics.

3. Results

The Kassandra coastline mostly contains sandy beaches (Figure 1A), with the exception of the rocky high cliffs found in the southeastern part, few cobble beaches in the southern part and small segments of medium cliffs and rip-rap walls in the eastern and western parts of the peninsula. The overall sandy beach length was found to be approximately 110 km, which represents almost 60 % of the overall coastline length. The abundance and length of sandy beaches are some of the main reasons of the high hedonic and aesthetic value of the Kassandra peninsula as well as its high touristic activity.

P. oceanica meadows are found in almost all the length of the coastline under study (Figure 1B). Larger meadows with up to 1 km cross-shore extent are found in the northeastern and northwestern parts of the coastline fronting the sandy beaches, whereas a relative lack of meadows is observed in the cliffy southern edge. Coastal segments fronted by *P. oceanica* meadows were assigned some coastal protection, while those with absence of *P. oceanica* were ranked as unprotected.

Prevailing winds and waves were found to be from the N and NW direction while E and SE winds appear to have similar frequencies, but much lower intensity (Figure 1C). Generally, the wave exposure index follows the pattern of the wind exposure in most cases, with the exception segments of the coastline that are protected, and have small fetches and/or shallow water depths along their fetch rays; these areas show lower wave exposure index. Mean wave heights and periods vary along the coastline segments, from 1.2 m wave height and 4.7 s period in segments fully exposed to northerly winds to very low values of 0.2 m wave height and 2.1 s wave period in segments exposed to easterly winds. The wave exposure index is a highly contributing variable to the CEI.

Finally, the projected ESLs (for the 5-year extreme event, in the 2020s under RCP8.5) obtain the maximum values (1.4 m) along the SW part of the peninsula; similar values (>1.35 m) are projected along the SE part of the peninsula, whereas for the rest of the coastline, the projected ESLs are projected to be lower, ranging between 1.15 and 1.2 m (Figure 1D).

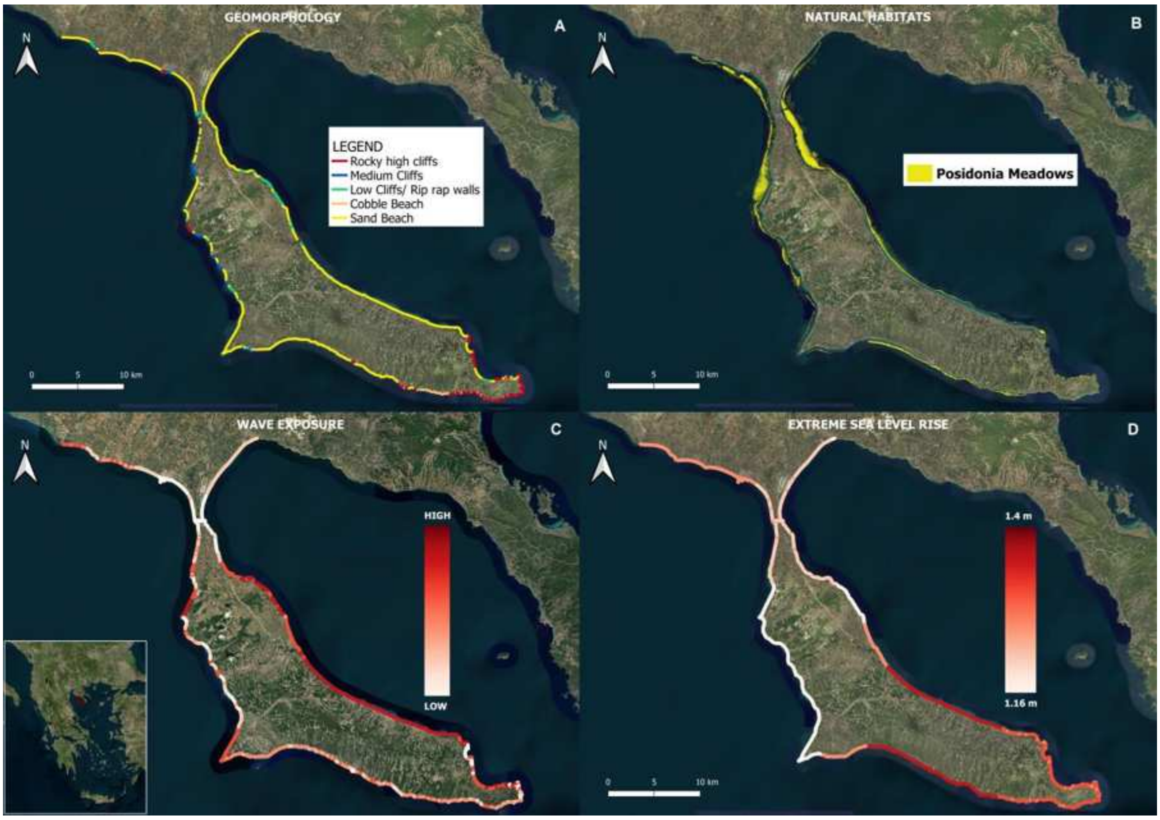

Figure 1: Distribution of the selected variables along the Kassandra coastline: (A) geomorphological character, (B) *P. oceanica* meadows, (C) wave exposure, and (D) extreme sea levels.

The highest density of touristic coastal businesses in the coastline is mostly in the eastern part of the coastline in the villages of Kalithea, Kriopogi, Polichrono, Hanioti and Peukochori (Figure 2A). High densities are also found along the western and the northwestern part of the coastline (Figure 2B).

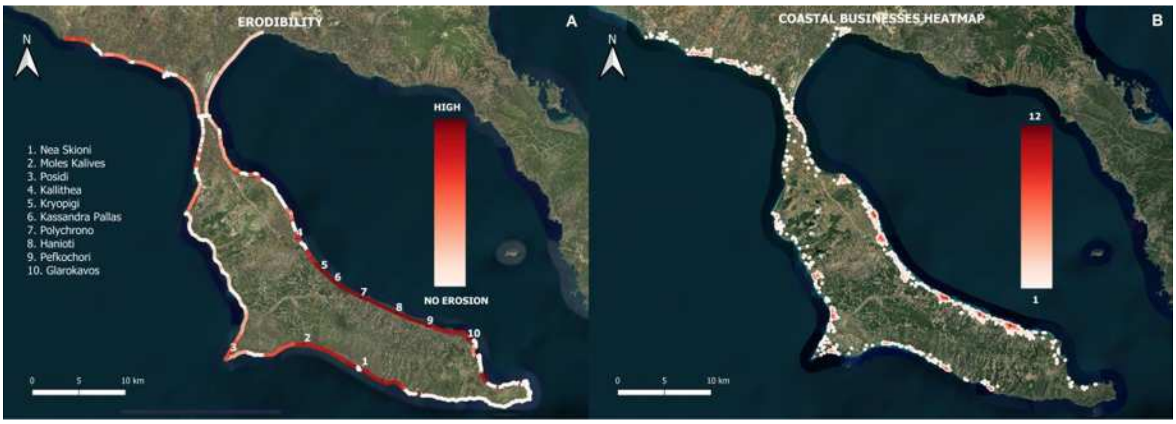

Figure 2: (A) Coastal Erodibility Index (CEI) and (B) coastal touristic business 'heatmap'.

The CEI (Figure 2A) acts as a supervisory indicator of the most vulnerable to erosion beaches in a large segment of coastline. High erodibility values are found in the beaches of the western Kassandra i.e. at the coastal settlements of Nea Skioni (1), Moles Kalives (2) and Posidi (3). Extreme CEI values (higher than 4.5) were estimated for the eastern part of the coastline, and particularly for the beaches of Kalithea (4), Kriopogi (5), Kassandra Pallas (6), Polichrono (7), Hanioti (8), Peukochori (9) and Glarokavos (10) (Figure 2A). These beaches (4-10, Figure 2A) are selected for further consideration, also including more details regarding their economic activity.

In order to prioritize beaches in terms of the erosion mitigation measures, beaches showing the highest erodibility index were compared on the basis of their socio-economic importance using as a proxy the touristic accommodation activity and the annual revenue within 1 km radius from the beach. To obtain this information, the touristic accommodation was categorized as hotels and apartments, on the basis of information collated from touristic booking sites. Then, on each different region the mean price per room for hotels and apartments, the mean room numbers for hotels and apartments and the length (start/end) of season have also been collated considering 100% occupancy (Table 1).

Table 1. Accommodation characteristics for highest CEI ranked beaches

Beach name	Apartments within 1km	Hotels within 1km	Mean room number for apartments	Mean room number for hotels	Mean room price for Apartments (euros)	Mean room price for hotels (euros)	Total Revenue for touristic accommodation per year (season April- October, euros, at 100% occupancy)
Hanioti	36	25	19	84	58	105	54630000
Kalithea	35	18	10	114	62	110	52958000
Peukoxwri	26	17	10	52	65	82	18771000
Kriopigi	27	5	12	120	37	85	13217000
Kassandra Pallas	9	4	12	120	37	85	9407000
Polychrono	52	11	8	24	50	60	7694000

Hanioti and Kalithea appear to have by far the highest touristic activity with over 50 million € annual touristic accommodation revenue when at full occupancy (Table 1); these are followed by Peukoxwri, Kriopigi, Kassandra Pallas Beach and Polychrono, while Glarokavos beach appear to have very limited (close to zero) touristic revenues.

4. Discussion

The current study represents a first approach to build a methodology for regional coastal planning on touristic areas on the basis of a qualitative erodibility assessment coupled with socioeconomic information. Overall, the method appears capable of prioritizing actions to be taken on a coastline of 180 km total length by spatial correlation between identified vulnerable to erosion segments and high touristic activity. A multi-criteria socio-economic analysis (Tzoraki *et al.* 2018) could be further used to integrate the methodology producing more targeted results and also incorporating variables such as coastal road networks (Zhang *et al.* 2020), coastal interventions and rigid structures in the coastline under study (Sauvé *et al.* 2020) and coastal businesses making profit directly from the beach (Aaheim & Orlov 2016). Recent municipality actions, policies and regulations for the coastal areas which can be used as a strong indicator of vulnerability, should be also considered in a future multicriteria socio-economic analysis.

This study proposes concepts for the development of a framework for a Decision Support System, fueled by earth observation data, which can provide decision-makers a tool for designing integrated coastal zone management policies for a sustainable coastline. This tool can be further improved by including more relevant variables and/or details on the already considered variables.

Regarding the indexes that are included on the CEI estimation, most of them are assigned (integer) values between 1 and 5; therefore assignment of weighting factors is required in order to allow for a more realistic representation of the factors that control coastal erosion. For example, in the current approach the *P. oceanica* meadow variable along the beach segments was assigned an equal value in the case that there was present in front of the beach, regardless of the meadow density, extent and seagrass height. Furthermore, there is room for incorporating certain natural processes that affect coastal state such as the sediment input from terrestrial streams.

References

Aaheim A., Orlov A. (2016). Strategies for adaptation to increased risk of erosion on Santorini beaches, CICERO.

Alexandrakis G., Ghionis G., Poulos S., Kampanis A. (2013). Greece, (In:) Coastal erosion and protection in Europe: A comprehensive overview, E. Pranzini, A.T. Williams (Eds.), EARTHSCAN Ltd, London, UK, 355-377.

Camus P., Losada I.J., Izaguirre C. et al. (2017). Statistical wave climate projections for coastal impact assessments. Earth's Future 5, 918–933.

Gornitz V.M., Beaty T.W., Daniels R.C. (1997). A coastal hazards database for the U.S. West Coast. ORNL/CDIAC-81, NDP-043C: Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Guerry A., Ruckelshaus M., Arkema K., et al. (2012). Modeling benefits from nature: using ecosystem services to inform coastal and marine spatial planning. International Journal of Biodiversity Science, Ecosystem Services & Management Vol. 8 (1-2), 107-121.

Hemminga M.A., Nieuwenhuize J. (1990). Seagrass wrack induced dune formation on a tropical coast (Banc Darguin, Mauritania). Estuarine Coastal Shelf Science 31, 499−502.

IPCC SROCC (2019). Special Report on the Ocean and Cryosphere in a Changing Climate [[H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte et al (eds.)], https://www.ipcc.ch/srocc/.

Jiménez J.A., Bosom E., Valdemoro H.I., Guillén J. (2012). Storm induced damages along the Catalan coast (NW Mediterranean) during the period 1958–2008. Geomorphology 143–144, 24–33.

Klose E., Thieler E.R. (2001). Coastal Vulnerability to Sea-Level Rise: A Preliminary Database for the U.S. Atlantic, Pacific, and Gulf of Mexico Coasts. U.S. Geological Survey, Digital Data Series DDS-68, 1 CD-ROM.

Luijendijk A., Hagenaars G., Ranasinghe R., et al. (2018). The State of the World's Beaches. Sci. Rep. 8, 6641.https://doi.org/10.1038/s41598-018-24630-6.

Monioudi I.N., Velegrakis A.F., Chatzipavlis A.E. et al, (2017). Assessment of island beach erosion due to sea level rise: the case of the Aegean archipelago (Eastern Mediterranean). Nat. Hazards Earth Syst. Sci. 17, 449–466.

Sauvé P., Bernatchez P., Glaus M. (2020). The role of the decision-making process on shoreline armoring: A case study in Quebec, Canada. Ocean & Coastal Management, 198, 105358.

SETE (2016). Association of the Greek Touristic Businesses. http://sete.gr/el/statistika-vivliothiki/statistika/(last access: August 2019).

Tragaki A., Gallousi C., Karymbalis E., (2018). Coastal hazard vulnerability assessment based on geomorphic, oceanographic and demographic parameters: The case of the Peloponnese (Southern Greece). Land *7*(2), 56.

Traganos D., Reinartz P. (2018). Mapping Mediterranean seagrasses with Sentinel-2 imagery. Marine Pollution Bulletin 134, 197-209.

Tzoraki O., Monioudi I.N., Velegrakis A.F. et al. (2018). Resilience of Touristic Island Beaches Under Sea Level Rise: A Methodological Framework. Coast. Manag. 46, 78–102.

UNFCCC (2020). Policy Brief: Technologies for Averting, Minimizing and Addressing Loss and Damage in Coastal Zones. United Nations Framework Convention on Climate Change, Executive Committee of the Warsaw International Mechanism for loss and damage. 74 pp.

Vousdoukas, M. I., L. Mentaschi, E.,Voukouvalas, et al, (2017). Extreme sea levels on the rise along Europe's coasts, Earth's Future, 5, 304–323, doi:10.1002/2016EF000505.

WTTC. World Travel and Tourism Council. https://wttc.org/Research/Economic-Impact (accessed 20/04/2021).

Zhang Y., Ruckelshaus M., Arkema K.K., et al. (2020). Synthetic vulnerability assessment to inform climate-change adaptation along an urbanized coast of Shenzhen, China. Journal of Environmental Management 255, 109915.

PRELIMINARY STUDY OF HEAVY METALS IN SEDIMENTS OF VOLOS HARBOR (THESSALY, GREECE)

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Abstract
The aim of this study is to evaluate sediment heavy metal content and possible contamination. The objectives of the study are to determine the content and the distribution of heavy metals in Volos's harbor, (Thessaly region) and to monitor sediment quality. For this purpose, 19 sediment samples were collected on July 2020. The preparation of the samples made by drying at 40-50 °C and sieving and then digested following 3050B method as recommended by the United States Environmental Protection Agency (USEPA). This study focused on the following nine (9) elements As, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn, which showed relatively elevated contents in the sediment and can cause adverse biological effects and the analysis was made using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results were compared with ERL-ERM Sediment Quality Guidelines values and also with the results of other studies from both Greece's and Europe's ports. Ni total content found elevated, exceeding ERM value indicating adverse effects occurrence. Arsenic and chromium content found between ERL-ERM values showing a possible effect range.
Keywords: *Heavy Metals*, *Sediments*, *Volos, Thessaly*
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1. Introduction

The harbor of Volos is located to the west end of the city, in the cove of Pagasitikos Gulf, central Greece. The harbor has a constant traffic of ferries and cruise ships. Volos is a medium-sized commercial and tourist city with around 150.000 inhabitants and with intense agricultural activity. Two industrial areas are located at a distance of a few kilometers to the west and northwest of the city including a steel manufacture. The harbor of Volos is a recipient of both urban and industrial/agricultural waste. Heavy metals in aquatic systems can be sourced from either natural processes or anthropogenic activities: natural sources are mainly through geological weathering and erosion whereas anthropogenic activities include industrial effluents, agricultural runoff, domestic sewage, and atmospheric deposition (Wu *et al*. 2018a).

In aquatic environment, sediments are the main sink and source of heavy metals, playing a significant role in the transportation and storage of potentially toxic metals, due to their physical and chemical properties, such as grain size. Heavy metals have been regarded as serious pollutants and considered a threat in aquatic environment due to their behavior, which cannot be easily degraded and bioaccumulate, in the food chain and may further degrade the ecosystem structure (Zhang *et al*. 2018). Surface sediment is usually collected for the assessment of sediment quality and to determine the distribution of the contaminants in it (Batley 2016). Also, other processes that can affect distribution of heavy metals in sediments involve precipitation, absorption, redissolution as well as biogeochemical processes (Dar *et al*. 2016).

The aim of this study is to evaluate sediment heavy metal content and possible contamination and to determine the distribution of heavy metals in Volos's harbor and to monitor sediment quality.

2. Material and Methods

Sampling was carried out within the Commercial and Tourist Harbor of Volos, Figure 1, in July 2020, using a Van Veen grab sampler. Sediment samples were collected from eighteen locations inside the harbor of Volos and one sediment sample was collected outside the harbor as a reference sample, with depths varying from 2 to 13 m and the contents As, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn were determined. Samples placed in polyethylene bags and transferred to the laboratory and then dried at (40-50°C) and pulverized in a porcelain mortar and then sieved using 1mm sieve. Sample preparation included digestion of the samples with the use of thick acids (HNO_3-

HCl) following 3050B method as recommended by the United States Environmental Protection Agency (USEPA, 1996) and analyses were made using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Figure 1. Sampling locations in Volos's harbor.

3. Results and Discussion

Figure 2 demonstrates the spatial distribution of the elements in the nineteen (19) surface sediment samples.

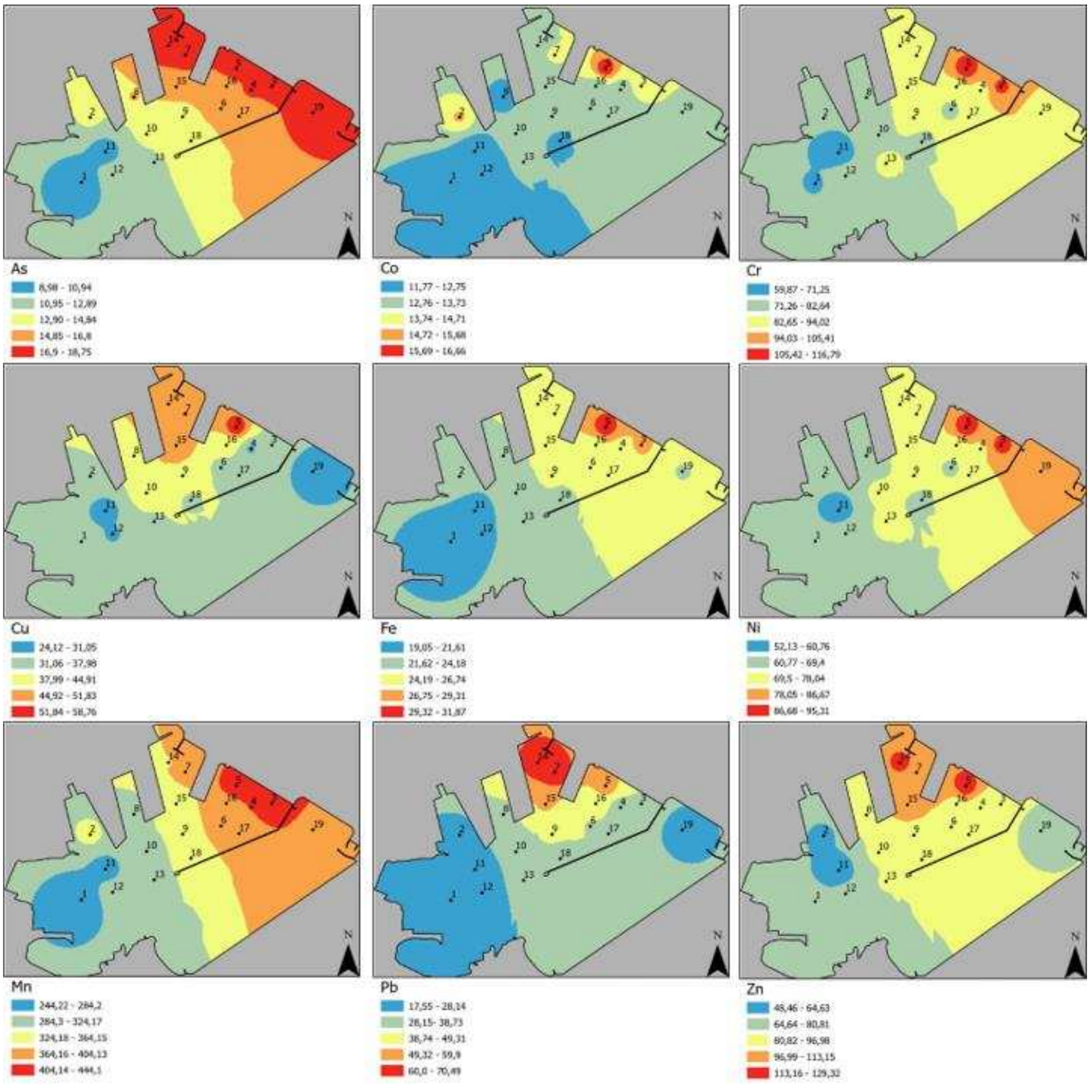

Figure 2. Spatial distribution of heavy metals in Volos's harbor.

As content ranges from 8.9 to 19 mg/kg with a mean value of (15±2.8) mg/kg, Co 12-17 (13±1.2), Cr content varied from 60 to 117 mg/kg, with a mean (84±12.8) mg/kg, Cu 27-59 (40±8.7), Fe content in sediment ranged from 19 mg/kg to 32 mg/kg with a mean content of (24±2.8) mg/kg, Mn content 244-444 (347±53.8), Ni 5-95 (71±9.5), Pb 19-71 (38±14.6) and finally Zn content ranged from 49 to 129 and (88±19.7) mg/kg as a mean content. Maximum contents for both Cr and Zn were measured near the Tourist harbor of Volos. Comparing the content of 9 heavy metals in the sediments inside the harbor with the corresponding content of the reference sample, it appears that arsenic, chromium, nickel and manganese contents are elevated in the reference sample. Fe and Co content is at the same level while Cu, Zn and Pb content found to be higher inside the harbor as expected. As shown in Figure 2, high loads for the majority of the heavy metals, could be a result of the intense urban activity nearby.

Table 1 demonstrates minimum and maximum content as well as mean values of the sediment samples that were analyzed for 9 elements. According to the proposed Sediment Guideline Values by Long & Morgan (1991), there are two assessment values, the Effects Range Low and the Effects Range Median. ERL-ERM values delineate three concentration ranges for a particular chemical. The concentrations below the ERL value represent a minimal effects range, concentrations equal or above ERL and below ERM indicate a range which effects would occasionally occur and finally concentrations above the ERM value represent effects that would frequently appear.

Table 1. Comparative table of heavy metals content in sediments of Volos harbor with other harbors and Sediment Quality Guidelines values.

Location	References	Method	As (mg/kg)	Co (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Volos Harbor	This study	Strong acid digestion	8.9-19 (15)	12-17 (13)	60-117 (84)	27-59 (40)	19-32 (24)	244-444 (347)	52-95 (71)	19-71 (38)	49-129 (88)
Patras Harbor	Papaefthymiou *et al.* 2010	Total digestion	4.16-13.2 (8.15)	13-21 (16)	180-230 (199.6)	30-128 (59)	18-34 (26.9)	494-755 (621)	56-140 (101)	19-128 (37)	55.2-343 (106)
Keratsini Harbor	Galanopoulou *et al.* 2009	Nearly total extraction	66-1813 (471)	-	264-860 (463)	195-518 (288)	-	95-1001 (204)	-	521-1263 (648)	409-6725 (1435)
Heraklion port	Chatzinikolaou *et al.* 2018	Acid digestion	4.9-6.1 (4.55)	-	117-129.1 (125.1)	35.8-133.2 (97.6)	15059-24155 (20144)	162-181 (174)	57.5-113.1 (87.2)	3.2-24.5 (17)	64.7-211.1 (140.5)
Port of Bagnoli	Romano *et al.* 2004	Total digestion	0.5-4 (2.0)	-	4-54 (28)	0.5-126.1 (27.2)	0.2-59.7 (15.4)	457-5947 (1550)	0.01-52.5 (6.93)	52-896 (221)	91-2313 (602)
Barcelona Harbor	Casado-Martínez *et al.* 2009	HNO_3 and aqua regia	17.4-29 (21.5)	-	59.5-105.2 (90.6)	74.9-601.1 (234.5)	-	-	18.9-32.3 (25.63)	86.7-455.3 (184.3)	219.7-1165 (515.6)
SQGs	ERL	-	8.2	-	81	34	-	-	20.9	46.7	150
	ERM	-	70	-	370	270	-	-	51.6	218	410

Arsenic total content was above ERL but below ERM values indicating an effect range that can possibly affect aquatic benthic organisms. Also, Cr total content value ranged between ERL-ERM values showing possible sediment contamination and occasionally effect occurrence. Copper's content in the majority of the sediment samples exceeded the ERL value indicating minimal effects. Ni content found to be significantly higher than the respective ERM value in all sediment samples meaning that adverse effects may occur more frequently and protection measures should be established. In a few cases lead's content found between ERL-ERM values designating possible effect occurrence. Finally, Zn content was notably lower than ERL value so there is no evidence of adverse effects on aquatic benthic organisms.

Compared to other studies of both Greek and Europe harbors, as displayed in Table 1, results showed that the mean content of As was higher than Patras's harbor, port of Bagnoli and Heraklion respectively. Cr mean content exceeded the corresponding contents of Bagnoli port; however, it was significantly lower than Patra's, Keratsini, Heraklion and Barcelona harbors. Copper's content found to be elevated compared with Bagnoli's and Heraklion ports and in all other cases was notably lower. The Fe values of the sediment in Volos's harbor are comparable to those of Patras and Bagnoli harbors, but considerably lower than Heraklion port. Manganese levels were considerably low in Volos and Heraklion harbors compared to Keratsini and Bagnoli harbors. Ni content is higher than those reported for Heraklion, Bagnoli and Barcelona harbors. Lead's content in sediments in harbor of Volos is comparable only with Patra's harbor and considerably lower than the others. The Zn levels in Volos's harbor are lower than those reported in highly polluted harbors.

References

Batley E.G., & Simpson L.S. (2016). Sediment sampling, sample preparation and general analysis. Sediment quality assessment. pp. 17.

Belmer N., Wright I.A., Tipler C. (2015). Urban geochemical contamination of high conservation value swamps, Blue Mountains Australia Water Air Soil Pollution 226:332.

Casado-Martínez M.C., Forja J.M., DelValls T.A. (2009). A multivariate assessment of sediment contamination in dredged materials from Spanish ports, Journal Hazard Material 163, 1353–1359.

Chatzinikolaou E., Mandalakis M., Damianidis P., Dailianis T., Gambineri S., Rossano C., Scapini F., Carucci A., Arvanitidis C. (2018). Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports. Science of the Total Environment 624, 648–660.

Dar M.A., El-Metwally M.E.A, El-Moselhy K.M.I. (2016). Distribution patterns of mobile heavy metals in the inshore sediments of the Red Sea. Arab Journal Geoscience 9, 221.

Galanopoulou S., Vgenopoulos A., Conispoliatis N. (2009). Anthropogenic heavy metal pollution in the surficial sediments of the Keratsini harbor, Saronikos Gulf, Greece, Water Air Soil Pollution 202, 121–130.

Long E.R. & Morgan L.G. (1991). The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52, National Oceanic and Atmospheric Administration, Seattle, WA, pp. 175.

Papaefthymiou H., Papatheodorou G., Christodoulou D., Geraga M., Moustakli A., Kapolos J. (2010). Elemental concentrations in sediments of the Patras Harbour, Greece, using INAA, ICP-MS and AAS. Microchemical Journal 96, 269–276.

Romano E., Ausili A., Zharova N., M. Celia Magno M., Pavoni B., Gabellini M. (2004). Marine sediment contamination of an industrial site at Port of Bagnoli, Gulf of Naples, Southern Italy, Marine Pollution Bulletin 49, 487–495.

USEPA. (1996). Method 3050B: Acid digestion of sediments, sludges, soils. Revision 2. USEPA.

Wu L., Liu G.J., Zhou C.C., Liu R., Xi S., da C., Liu F. (2018a) Spatial distributions, fractionation characteristics, and ecological risk assessment of trace elements in sediments of Chaohu Lake, a large eutrophic freshwater lake in eastern China. Environ Sci Pollution Research 25:588–600.

Zhang H.X, Huo S.L, Yeager K.M, Xi B., Zhang J., He Z., Ma C., Wu F. (2018) Accumulation of arsenic, mercury and heavy metals in lacustrine sediment in relation to eutrophication: Impacts of sources and climate change. Ecological Indicators 93:771–780.

CLIMATE CHANGE IMPACT ASSESSMENT AND NATURE-BASED SOLUTIONS IN THE KALLONI RIVER BASIN, GREECE

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Abstract
The assessment of the climate change impact at a basin scale is essential for developing mitigation and adaptation plans. This study analyses the variation of the hydrologic regime of the Kalloni river in Lesvos Island, Greece by the examination of possible future climate change scenarios from 2021 to 2080 and examines Nature-based solutions (NBS) to provide flood risk mitigation options. In total, eleven sets of GCM-RCP scenarios are considered to predict future rainfall, temperatures and radiation patterns, covering a wide range of uncertainties. Hydrological model HEC-HMS simulations are conducted for the baseline climate and each of the climate scenarios predicting a range of annual and seasonal changes in runoff patterns. Variation was noted in forecasting of long-term average discharges, which show increasing trend in autumn and decreasing in the summer. Furthermore, some Nature-based mitigation measures compatible with the needs and characteristics of the case study are examined for the Kalloni landscape. This paper provides a quantitative framework for policy-makers in small intermittent flow river basins in the Mediterranean, such as Kalloni, to plan and manage the expected future challenges of river discharge and flood occurrence.
Keywords: *Climate Change, Hydrological Regime, Nature-Based Solutions, Lars-WG, HEC-HMS*
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1. Introduction

Addressing of climate change effects in the development of hazardous risk adaptation and mitigation plans has been on the agenda of many governments and institutions (Lavell *et al*. 2012). According to Intergovernmental Panel on Climate Change (IPCC), GCMs are advanced tools currently available for simulating the response of global climate system to increasing greenhouse gas concentrations.

Although GCMs are very important tools for studying the implications of climate change, these contain biases when compared to observed data due to their parameterization systems and large grid size (Sharma *et al*. 2007). Outputs from the GCMs are typically defined at 250–600 km grids, which are quite coarse relative to the scale of exposure units in most regional impact assessments. In order to overcome this problem, many different downscaling methods have been developed over the last few decades (Ebrahim *et al*. 2012). Weather Generator developed by Long Ashton Research Station (LARS-WG) is an effective model for this purpose, which has been widely used to assess possible effects of climate change on hydrological processes (Sharafati *et al*. 2020).

The IPCC defined a series of Representative Concentration Pathway (RCP) emission scenarios for future climate projection based on different 21st century pathways of greenhouse gas (GHG) emissions, population and socio-economic conditions (IPCC 2014). The RCPs include a stringent mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0), and one scenario with very high GHG emissions (RCP8.5) (IPCC 2014). These various climate change scenarios can be used to define the climate change impact on hydrological processes, by downscaling the large-scale GCMs predictions to local scale with the LARS-WG (Sharafati *et al*. 2020).

The main objectives of the current study are the variation analysis of the hydrological regime of the river basin and the examination of Nature-based solutions (NBS) as flood risk mitigation options. The study case concerns a small Mediterranean river, the Kalloni river, which flows over Lesvos Island of Greece. An approach developed based on the integration of spatial downscaling of GCMs and hydrological simulation has been adopted in the study.

2. Materials and Methods

2.1 Spatial downscaling of large-scale climate predictions

Weather Generator version 6.0 from Long Ashton Research Station (LARS-WG 6.0) is a single site numerical model for generating daily time-series of climate variables, namely, precipitation (mm), maximum and minimum temperature (°C) and solar radiation ($MJm^{-2}day^{-1}$). LARS-WG 6.0 is suitable for downscaling coarse resolution climate model simulations in local spatial scale for different climate change scenarios (Sharafati *et al*. 2020). The model, after calibrating site parameters with observed weather data for the baseline period, is capable of simulating synthetic daily time series of weather data that are statistically similar to the observed weather (Wilks & Wilby 1999). LARS-WG 6.0 uses a semi-empirical distribution to calculate the length of wet and dry days (Racsko *et al*. 1991). Moreover, it considers each weather variable as a stochastic variable, and simulates seasonal cycles through Fourier series (Sharafati *et al*. 2020).

The current version LARS-WG 6.0 generates high resolution climate change scenarios over a region using direct outputs from General Circulation Models (GCMs). LARS-WG 6.0 incorporates projections from five GCMs with the different climate scenarios RCP2.6, RCP4.5, and RCP8.5 used in the IPCC. Table 1 summarizes the different GCMs and RCPs that are taken into consideration in this study to forecast futuristic climate data for a period of 60 years from 2021 to 2080. Calibration of site parameters is performed for baseline period of 18 years from 2003 to 2020.

Table 1. **Summary of the five GCMs and corresponding emission scenarios (RCPs)**

GCM	Institution	Grid Resolution	RCP
EC-EARTH	European community Earth-System Model	1.125° × 1.125°	RCP4.5, RCP8.5
GFDL-CM3	NOAA Geophysical Fluid Dynamics Laboratory	2° × 2.5°	RCP4.5, RCP8.5
HadGEM2-ES	Met Office Hadley Center, United Kingdom	1.25° × 1.875°	RCP2.6, RCP4.5, RCP8.5
MIROC5	Atmosphere and Ocean Research Institute (The University of Tokyo), National Institute for Environment Studies and Japan Agency for Marine-Earth Science and Technology, Japan	1.40° × 1.41°	RCP4.5, RCP8.5
MPI-ESM-MR	Max Planck Institute for Meteorology, Germany	1.85° × 1.875°	RCP4.5, RCP8.5

2.2 Hydrological simulation and calibration

Hydrologic Engineering Centre's Hydrologic Modeling System (HEC-HMS) was designed by United States Army Corps of Engineers (USACE) as a software tool for simulating complete hydrological cycle in the contex of solving engineering problem (Scharffenberg *et al*. 2010). HEC-HMS is a deterministic, semi-distributed, conceptual model which is designed to simulate precipitation-runoff processes of dendritic drainage basins. It is applicable to a wide range of geographic areas for solving the widest possible range of problems (USACE 2013). In HEC-HMS, a basin model is constructed by dividing the hydrological cycle (evaporation, surface runoff, infiltration, and groundwater recharge) into individual parts with possibility of processing each one separately. Therefore, each component of the hydrological cycle is represented by a mathematical model.

The inclusion of the evapotranspiration process in the HEC-HMS model is significant for long-term simulations, and it is also necessary when using the deficit and constant loss method. In the case of Kalloni, the method of constant monthly evapotranspiration is selected, which requires from all subbasins a potential monthly evaporation rate (mm/month) and a crop coefficient. The HEC-HMS model is calibrated using observed data (e.g., river discharge) to improve the predictability and reliability of the model. The accuracy and performance of the calibrated model is evaluated by three goodness-of-fit measures, the Nash–Sutcliffe efficiency (NSE) coefficient, the percentage bias error (PBIAS), and the Root Mean Squared Error standard deviation ratio (RMSE Std. Dev.) of observations.

2.3 Consideration of Nature-based solutions

In the final stage of the research, some flood protection measures compatible with the needs and characteristics of the case study are investigated. The measures under consideration are mildly invasive Nature-Based Solutions (NBS) and projects focusing on the mountainous surface water management of the area. As flood protection measures, Nature Based Solutions, referring to measures and actions that are inspired and supported by ecosystem processes to meet human and social needs (European Commission 2015), are rapidly gaining the popularity of science and politics as best alternatives to alleviate the challenges of humans and nature.

Specifically, the nature-based solutions for the area of Kalloni focus on 5 sub-sectors. The focus areas are agriculture, forestry, natural landscape, hydromorphology, urban sector and mountainous surface water management and aim to increase resilience and mitigate flood risk in the wider area of Kalloni. The construction of small dams and ponds in the mountainous part of the watershed for the collection and use of run-off in livestock, but also the increase of soil absorption by reforestation, planting, creation and maintenance of cultivated terraces can enhance the resilience to flooding and eventually replace the need for difficult and costly technical projects.

3. Results and Discussion

3.1 Analysis of precipitation and temperature variables

First step in analyzing effects of climate change on river basin hydrology is to quantify changes in weather variables. Variations in GCMs outputs concerning predicted annual rainfall totals and average temperatures for future simulation period 2021-2080 are shown as boxplots in figures 1a and 1b respectively. The boxplots represent 25th, 50th (median) and 75th percentiles and horizontal lines show mean annual values for Baseline Business as Usual (BaU) scenario. The generated data from BaU scenario for the period 2021-2080 match site statistics based on historical records without taking into account climate change. Variations in GCMs results indicate the range of uncertainty in GCM predictions and they arise mainly from widely rainfall patterns generated by different climate models (Hajian *et al*. 2016, Bates *et al*. 2008). Therefore, studies investigating the potential effects of climate change on water resources using outflows from a single GCM greatly reduce the validity and usefulness of the findings (Hajian *et al.* 2016).

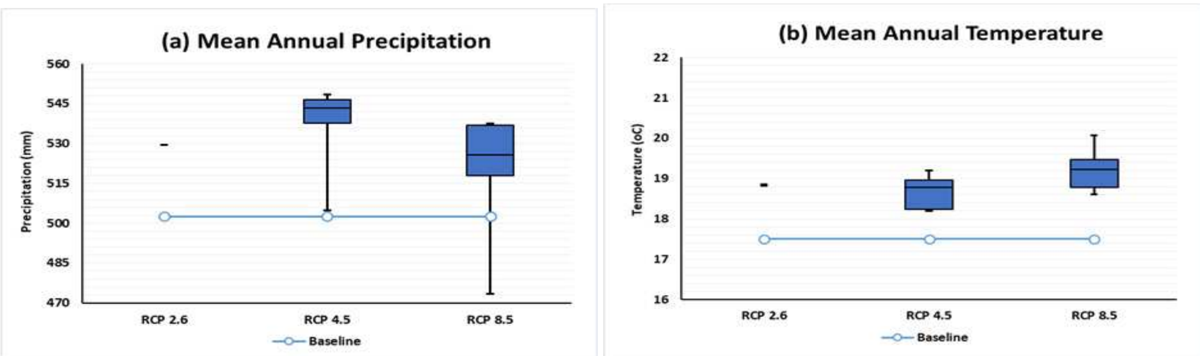

Figure 1. Variations in future (a) mean annual precipitation and (b) mean annual temperature forecasts compared to baseline scenario under the influence of each emissions scenario.

Subsequently, it is important to examine response of these parameters to climate change in a shorter time scale, as they show significant seasonal fluctuations. Results show that rainfall varies significantly between different GCMs, whereas it is observed more uniform results for temperature. As observed, the average cumulative rainfall shows a downward trend in spring and summer and a particularly upward trend in winter and especially autumn. Moreover, there is a shift of peak rainfall from January to February, while January actually shows a drop in cumulative rainfall. Finally, in terms of temperature, there is a general increase in average temperature throughout the year, while the largest changes occur during the winter months.

3.2 Climate change impact on streamflow

Annual peak and average monthly river discharges are computed using calibrated HEC-HMS model based on projected weather data for 2021–2080 period. Although the results from different scenarios vary considerably, a general increasing trend of flow peaks is observed, especially during the long-term period 2040-2080. Figures 2a, b, c show seasonal discharge variations, under all examined GCM models for three RCP scenarios. In terms of monthly variations, January presents the highest peaks in all scenarios as well as in baseline scenario. However, the most critical months are October and November, which show more than double discharge compared to the baseline scenario. Also, in future, dry months August and September are expected to have even lower discharges. This sudden surge of streamflow from summer to autumn months is of particular concern as it increases the risk of flash floods occurrence.

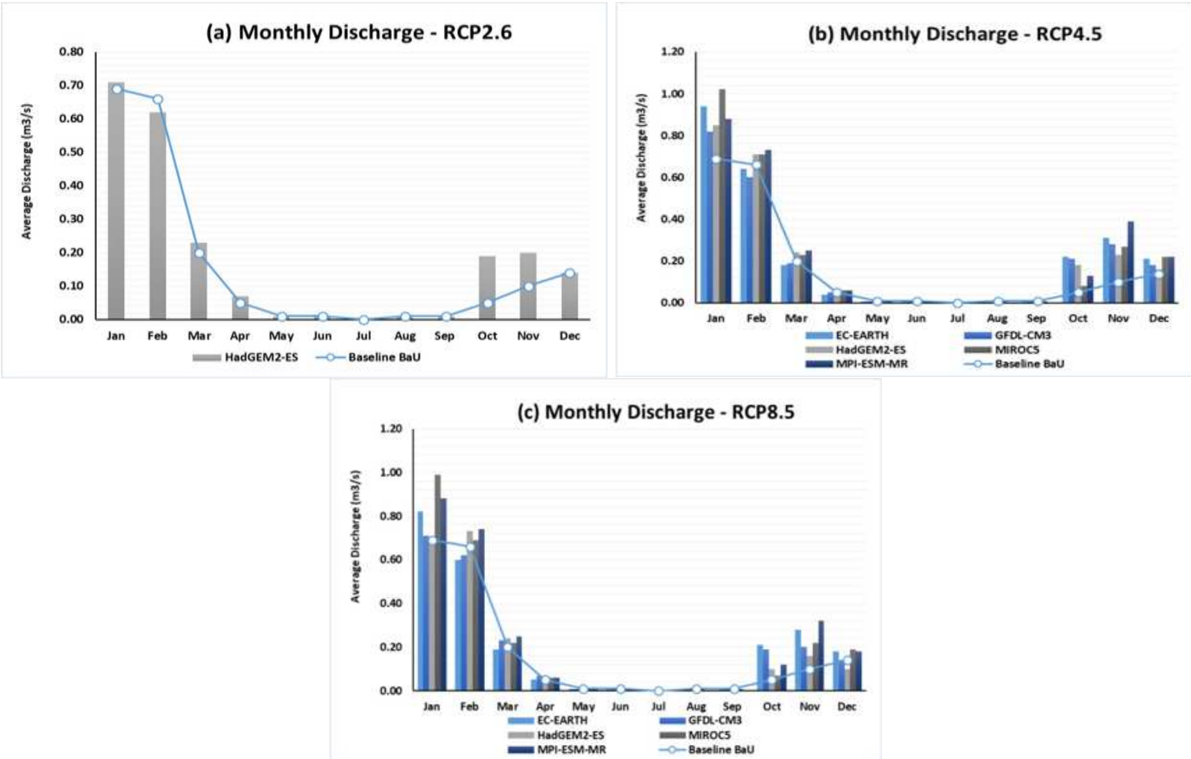

Figure 2. Monthly discharge variations under all examined GCM models for (a) RCP2.6, (b) RCP4.5, and (c) RCP8.5 scenarios.

3.3 Nature Based Solutions

In the present research the construction of five small dams made of gabions and wood is examined. This measure regulates and limits the peak discharge in the mountainous part of the watershed. More specifically, the peak discharge at the catchment outlet is reduced by 40%, with the reducing volume equal to 1000m^3. The dams are located in the basin after a study of the digital elevation model of the area and are dimensioned as shown by the typical cross section of the figure 3. Moreover, the increase of forest cover and water element with the creation of ponds in the mountains is also examined. This measure reduces the total volume of runoff at the entrance of Kalloni by 4%, with a volume of 855m^3 of water.

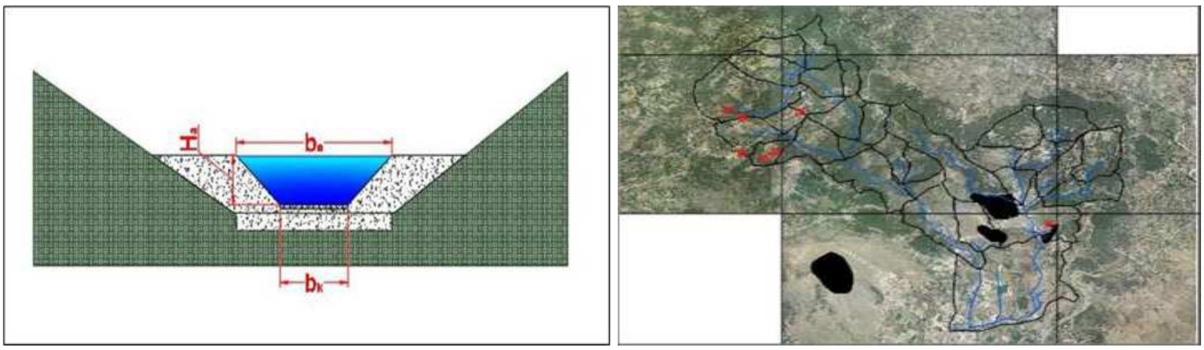

Figure 3. Nature based solution for Kalloni river basin.

4. Conclusions

The present study investigates the climate change impact on hydrologic regime of Kalloni river basin with intermittent flow in response to different climatic models and condition. The results obtained demonstrate that the approach combining hydrological model HEC-HMS and LARS-WG weather generator is efficient for assessing the effects of climate change on the patterns of the river hydrology. Annual predictions of weather variables indicate an increase in mean rainfall and temperature for the region. In seasonal patterns, a significant increase in precipitation is expected in autumn. Slight increase or even decrease can be anticipated in summer and spring rainfall totals. A general increasing trend of mean temperature and a consequent change in evapotranspiration, was observed throughout the year, with the most significant change occurring in winter. The projected decrease in summer precipitation and higher evapotranspiration are expected to cause a reduction in soil water and groundwater recharge in the basin area. This reduction in summer combined with the tremendous increase in autumn discharge leads to a high risk of flooding, particularly in the form of flash floods. Such findings indicate the need for integrated water management and flood mitigation strategies in the area of Kalloni river basin.

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References

Bates B.C., Kundzewicz Z.W., Wu S., Palutikof J.P. (2008). Climate change and water. Technical paper of the Intergovernmental Panel on Climate Change. Geneva: IPCC Secretariat.

Ebrahim G. Y., Jonoski A., van Griensven A., Di Baldassarre G. (2012). Downscaling technique uncertainty in assessing hydrological impact of climate change in the Upper Beles River Basin, Ethiopia. Hydrology Research. doi: 10.2166/nh.2012.037.

European Commission (EC) (2015). Nature-Based Solutions & Re-Naturing Cities. Final Report of the Horizon 2020 Expert Group on 'Nature-Based Solutions and Re-Naturing Cities'. Directorate-General for Research and Innovation–Climate Action, Environment, Resource Efficiency and Raw Materials. p. 74.

Hajian F., Dykes A. P., Zahabiyoun B., Ibsen M. (2016). Prediction of climate change effects on the runoff regime of a forested basin in northern Iran, Hydrological Sciences Journal, 61:15, 2729-2739, DOI: 10.1080/02626667.2016.1171870.

Intergovernmental Panel on Climate Change (IPCC) (2014). Climate Change 2014: Synthesis Report; Core Writing Team, Pachauri, R.K., Meyer, L.A., Eds.; Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; IPCC: Geneva, Switzerland.

Lavell A., Oppenheimer M., Diop C., Hess J., Lempert R., Li J., Muir-Wood R., Myeong S. (2012). Climate change: new dimensions in disaster risk, exposure, vulnerability, and resilience. In: Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation [Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (eds.)]. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, UK, and New York, NY, USA, pp. 25-64.

Racsko P., Szeidl L., Semenov M. (1991). A serial approach to local stochastic weather models. Ecological Modeling 57:27–41. https://doi.org/10.1016/0304-3800(91)90053-4

Sharma D., Gupta A.D., Babel M.S. (2007). Spatial disaggregation of bias-corrected GCM precipitation for improved hydrologic simulation: Ping River Basin, Thailand. Hydrology and Earth System Sciences, 11, 1373–1390. www.hydrol-earth-syst-sci.net/11/1373/2007/

Scharffenberg W., Ely P., Daly S., Fleming M. and Pak J. (2010). Hydrologic Modeling System (HEC-HMS): Physically-Based Simulation Components. 2nd Joint Federal Interagency Conference, June 27-July 1, Las Vegas, NV.

Sharafati A., Pezeshki E., Shahid S., Motta D. (2020). Quantification and uncertainty of the impact of climate change on river discharge and sediment yield in the Dehbar river basin in Iran. Journal of Soils and Sediments. Hillslope And River Basin Sediment Dynamics. https://doi.org/10.1007/s11368-02-02632-0

U.S. Army Corps of Engineers (USACE) (2013). HEC-GeoHMS Geospatial Hydrologic Modeling Extension, Hydrologic Engineering Center, User's Manual, Version 10.1.

Wilks D.S., Wilby, R.L. (1999). The weather generation game: A review of stochastic weather models. Progress Physics Geography 23, 329–357.

A COMPARATIVE STUDY OF IDENTICATION METHODS FOR BACTERIAL PATHOGENS ISOLATED FROM THE RAINBOW TROUT *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract
In this study, four different methods were compared for the identification of some bacterial pathogen isolated from diseased rainbow trout (*O. mykiss*). For this purpose, two different rainbow trout farms (A, B) located in Fethiye/ Turkey was visited three times in different seasons (spring, summer and autumn) and bacteriological analysis was applied to identify the causative agents of disease. One hundred twenty seven isolates were recovered from 58 diseased fish. All isolated bacteria were analyzed by conventional bacteriological methods, commercial bacterial identification test kit (API), automated bacteria identification system Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), BD Phoenix™) and 16S rRNA sequence analysis. The isolated bacteria were identified as *Pseudomonas* sp., *Lactococcus garvieae, Vibrio anguillarum, Yersinia ruckeri, Aeromonas veroni* and *Pseudomonas* sp. by conventional bacteriological method. These results have been confirmed with 16S rRNA sequence analysis. Although similar results were obtained in the rapid diagnosis kits, except for *Aeromonas veroni and Pseudomonas* sp., and in the BD Phoenix, except for *V. anguillarum* and *A. veroni,* it was determined that the results closest to the conventional bacteriological methods and 16S rRNA sequence analysis results were similar to the MALDI-TOF MS. In comparison of all identification methods used in the present study, it was demonstrated that the MALDI-TOF MS provides better results than other methods in terms of cost and identification time, and it could be used as an alternative to conventional method to identification of bacterial fish pathogens.
Keywords: *Rainbow trout, Bacterial Pathogen, Conventional Method, API, 16S rRNA, MALDI-TOF MS, BD Phoenix*
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1. Introduction

Bacterial diseases constitute one of the most important problem identified in cultured freshwater and marine fish species in the world. It has been reported that bacterial infections leading to mass mortalities cause economic losses in the trout production. Pathogenic bacteria such as *Lactococcus garvieae, Vibrio anguillarum, Aeromonas hydrophila, Pseudomonas fluorescen* and *Flavobacterium psychrophilum*, have been frequently reported to cause disease and mortality in rainbow trout (Austin & Austin 2016).

Identification of bacteria on species level is critical for epidemiological research, antimicrobial therapy and control in the aquaculture. The diagnosis of bacterial fish diseases has progressed from traditional culture-dependent methods involving the recovery of pathogens on agar-containing media and identification by conventional method. Today, this method is frequently used in many laboratories (Austin 2019). But, the identification of bacterial fish pathogens using conventional methods is time consuming, expensive, complicated and requires expertise (Frans *et al*. 2008; Hameed *et al*. 2018). Therefore, alternative identification methods are needed for bacterial fish pathogens.

The aim of the present study was to compare of different diagnostic methods to identify of the bacterial pathogens isolated from diseased rainbow trout.

2. Material and Methods

Field Sampling and Bacteriological Examination

Two rainbow trout farms (A, B) located in Fethiye/ Turkey was visited three times (spring, summer and autumn). A total of 37 diseased fish were supplied from farm A, while a total of 21 fish were supplied from farm B. Bacteriological examination method was applied to a total of 58 diseased fish. For this purpose, samples of kidney, liver, spleen, and blood were streaked onto Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA). Plates were incubated at 19-20^{o}C for 24-48 hours. All isolates (n=127) recovered from diseased rainbow trout were identified by using different identification methods such as conventional method, commercial identification test kits (API 20E, Rapid ID 32 Strep) and automated identification systems (MALDI-TOF MS and BD Phoenix).

Identification by Conventional Bacteriological Method

Morphological, physiological and biochemical characterization of all filed isolates was performed using tests such as Gram staining, motility, cytochrome oxidase and catalase activity, fermentative degradation of glucose (O/F), methyl red, Voges-Proskauer, indole, citrate, nitrate, aesculine, urease, gelatin and starch hydrolysis, carbohydrate fermentations (lactose, fructose, arabinose, cellobiose, xylose trehalose, galactose, mannitol mannose, maltose), amino acid decarboxylation (ornithine, lysine and arginine).

Identification by Commercial Bacterial Identification Test Kit

API 20E for Gram-negative isolates and radip ID32 Strep kit for suspected Gram-positive streptococci were used according to the manufacturer instruction except incubation temperature, which was 19-20 C° (Buller 2004).

Identification by Automated Bacteria Identification System

Two different BD Phoenix panels (Gram negative/ (n=49) and Gram-positive/ (n=78) were used in this study. Each bacterial sample was added into Phoenix ID broth and the suspension matched a McFarland 0.5 standard. Each suspension was inoculated into the Phoenix system panel and were logged and loaded into the Phoenix system instrument (Funke & Funke 2004).
Also for MALDI-TOF MS, a single bacterial colony from each tested isolates were placed onto a 96-spot polished steel UV-absorbing MALDI-TOF plate. One drop of MALDI matrix (saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) which absorbs laser energy was added to each bacterial colony and they were allowed to air dry. The prepared plate was then put on a fixed, pulsed laser beam (Bruker Biotyper MALDI Automation Control software) for analyzing the samples (Akter 2017; Pečur Kazazić *et al*. 2019). All results were recorded electronically in system database.

Identification by 16S rRNA Sequence Analysis

DNA isolations from bacteria were performed with Bacteria Lysis Buffer in accordance with the manufacturer's instructions. The DNA purity of the isolated bacteria was rated at 230/260 nm wavelengths and the results were found to be between 1.8 <DNA<2.0. The 16S rRNA sequence analysis of each bacterium from the obtained bacterial DNAs was carried out by purchasing service from a commercial company (Lontek/Istanbul).

3. Results and Discussion

In the sampling study carried out in farm A, a total of 37 diseased rainbow trout were examined; while in farm B, a total of 21 fish were examined bacteriologically (Figures 1,2). After bacteriological isolation, a total of 127 (n=72 farm A/ n= 55 farm B) isolates were obtained from the all diseased fish samples (Table 1).

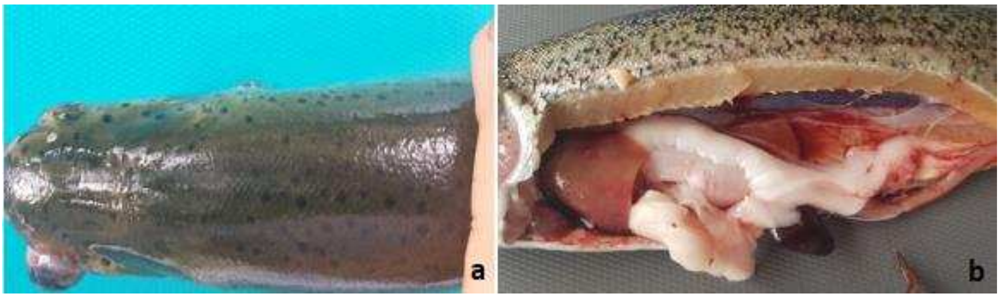

Figure 1: Diseased rainbow trout obtained from farm A. (a) unilateral exophthalmus, (b) splenomegaly (2. sampling)

As a result of the conventional bacteriological method *Pseudomonas* sp., *L. garvieae, V. anguillarum, Y. ruckeri* and *A. veroni* were identified as agents of disease (Table 1). In addition, *L. garvieae* (61.41%) was found to be the most common bacteria among these pathogenic bacteria. There are various reports that these bacteria cause disease in rainbow trout (Vendrell *et al*. 2006). Moreover, some reports have reported that *L. garvieae* causes mixed infections with different bacteria such as *V. anguillarum* (Tanrıkul & Gultepe 2011), *Frigoribacterium faeni*

(Akaylı *et al*. 2020) and *A. sobria* (Al Khaziri *et al*. 2018). Similarly, in this study, *L. garvieae* caused mixed infections with members of *Aeromonas, Vibrio, Yersinia* and *Pseudomonas* genus, which were defined as opportunistic bacteria.

Conventional bacteriological method including morphological, physiological and biochemical tests and also molecular techniques based on 16S rRNA sequencing have been reported to be the gold standard for identification of bacterial species (Laupland & Valiquette 2013). However, these techniques require a substantial amount of time and expensive reagents (Buller 2004). In the present study, approximately 2 months of laboratory work was performed for the identification of all bacteria by using conventional bacteriological method.

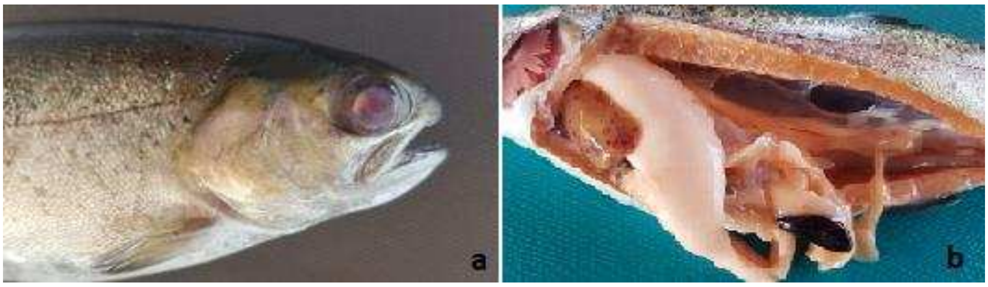

Figure 2: Diseased rainbow trout obtained from farm B. (a) opacification of the eye (Sample 2), (b) ecchymosis of the liver and splenomegaly (3. Sampling)

According to the rapid diagnostic kit results, isolated bacteria were identified as *P. aeruginosa/ fluorescens/ putida, L. garvieae, V. anguillarum Y. ruckeri* and *A. hydrophila* (Table 1). It was observed that all isolates were correctly identified on species level, except *Pseudomonas* sp. and *A. veroni*. Although rapid diagnostic kits (API) are frequently used in the identification of pathogenic fish bacteria by many researchers (Coz-Rakovac & Strunjak-Perovic 2007), it has been reported that some strains may be incorrectly identified as the API system is not included in the database (Coz-Rakovac & Strunjak-Perovic 2007; Topić Popović *et al*. 2007). In this study, *Pseudomonas* sp. and *A. veroni* among our isolates could not be identified on species level by using API 20E. In addition, it has been reported that Rapid ID 32 Strep kit are not sufficient for identification of *L. garvieae* strains and *L. garvieae* is identified as *Enterococcus faecalis* (Teker *et al*. 2019).

In this study, all identified bacteria with API kits were identified based on genus. However, it was found to be insufficient for identification on the *Aeromonas* and *Pseudomonas* species.

Table 1: Number of isolates and identification results of bacteria identified by using different methods.

Conventional Method	API kit	MALDI-TOF MS	BD Phoenix	Number of isolates (n)	Percentage amount (%)
Pseudomonas sp.	*P. aeruginosa* *P. fluorescens*	*P. chlororaphis*	*Pseudomonas sp,*	24	18.89
L. garvieae	*L. garvieae*	*L. garvieae*	*L. garvieae*	78	61.41
V. anguillarum	*V. anguillarum*	*V. anguillarum*	*Vibrio holisae,*	7	5.51
Y. ruckerii	*Y. ruckerii*	*Y. ruckerii*	*Y. ruckeri,*	6	4.72
A. veroni	*A. hydrophila*	*A. veroni*	*A. cavieae*	12	9.77

The isolated bacteria were identified by BD Phoenix system as *Pseudomonas* sp., *L. garvieae*, *V. holisae, Y. ruckerii* and *A. cavieae*. Saticioglu *et al*. (2021) reported that BD Phoenix panels was used in the identification of *Shewanella* sp., which causes lens atrophy in rainbow trout trout eyes. It has been reported that *V. anguillarum* (4-37°C) and *A. veroni* (4-42°C) grow in a wide temperature range (Buller 2004). In addition, although *A. veroni* is present in the BD Phoenix system, *V. anguillarum* is not present in the system, so the isolated bacteria may be misidentified.

In the present study, isolated bacteria were identified as *P. chlororaphis*, *L. garvieae*, *V. anguillarum*, *Y. ruckerii* and *A. veroni* by using MALDI-TOF MS system. It has been reported that Gram-negative bacteria such as *Y. ruckeri* (Jansson *et al*. 2020), *V anguillarum* (Jansson *et al*. 2020) and *A. veroni* (Piamsomboon *et al*. 2020), can be successfully identified by MALDI-TOF MS in a short time. In addition to these bacteria, it was observed that *L. garvieae* (n=78) from diseased rainbow trout was identified on the basis of species level. Rapid identification methods were used in the identification of bacteria isolated from diseased rainbow trout herein. It can be concluded that MALDI-TOF MS provides more advantageous results than other methods in terms of time, labor and cost.

References

Akaylı T., Çanak Ö., Yardımcı R., Ürkü Ç., Ökmen D. (2020). A mixed *Frigoribacterium faeni* and *Lactococcus garvieae* infection in cultured rainbow trout (*O. mykiss*). Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 23(6), 1569-1577.

Akter T. (2017). Morphological and biochemical characterization of two fish pathogenic bacteria: *Aeromonas salmonicida* and *Yersinia ruckeri* for rapid diagnosis of fish disease. Asian Journal of Medical and Biological Research 3(1), 44-51.

Al Khaziri B., Al Sulimani A., Al Mandhari N., Yoon G., Al-Busaidi A. (2018). Bacterial infection in farmed barramundi juveniles, *Lates calcarifer*. Journal of Agricultural and Marine Sciences 23, 76-80.

Austin B. (2019). Methods for the diagnosis of bacterial fish diseases. Marine Life Science & Technology 1(1), 41-49.

Austin B., Austin A. (2016). Bacterial fish pathogens: Disease in farmed and wild fish. Springer, UK, pp. 552.

Buller N.B. (2004). Bacteria from fish and other aquatic animals: A practical identification manual. Oxfordshire, Cabi Publishing, UK, pp. 725.

Coz-Rakovac R., Strunjak-Perovic I. (2007). Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria: A review. Veterinarni Medicina 52(2), 49.

Frans I., Lievens B., Heusdens C., Willems K.A. (2008). Detection and identification of fish pathogens: What is the future? The Israeli Journal of Aquaculture Bamidgeh 60(4), 213-229.

Funke G., Funke-Kissling P. (2004). Use of the BD Phoenix Automated Microbiology System for direct identification and susceptibility testing of gram-negative rods from positive blood cultures in a three-phase trial. Journal of Clinical Microbiology 42(4), 1466-1470.

Hameed S., Xie L., Ying Y. (2018). Conventional and emerging detection techniques for pathogenic bacteria in food science: A review. Trends in Food Science & Technology 81, 61-73.

Jansson E., Haenen O.L.M., Nonnemann B., Madsen L., van Gelderen E., Aspán A., Dalsgaard I. (2020). MALDI-TOF MS: a diagnostic tool for identification of bacterial fish pathogens. Bulletin of the European Association of Fish Pathologists 40(6), 240-248.

Laupland K.B., Valiquette L. (2013). The changing culture of the microbiology laboratory. Canadian Journal of Infectious Diseases and Medical Microbiology 24(3), 125-128.

Pečur Kazazić S., Topić Popović N., Strunjak-Perović I., Florio D., Fioravanti M., Babić S., Čož-Rakovac R. (2019). Fish photobacteriosis. The importance of rapid and accurate identification of *Photobacterium damselae* subsp. *piscicida.* Journal of Fish Diseases 42(8), 1201-1209.

Piamsomboon P., Jaresitthikunchai J., Hung T.Q., Roytrakul S., Wongtavatchai J. (2020). Identification of bacterial pathogens in cultured fish with a custom peptide database constructed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). BMC Veterinary Research 16(1), 52.

Saticioglu I.B., Isbilir F., Yavas O., Avci Z., Ozdemir B., Suzer B., Duman M. (2021). Description of lens atrophy caused by *Shewanella* sp. strain S-1, a putative novel species isolated from cultured rainbow trout. Aquaculture, 541, 736779.

Tanrıkul T.T., Gultepe N. (2011). Mix infections in rainbow trout (*Oncorhynchus mykiss*): *Lactococcus garvieae* and *Vibrio anguillarum* O1. Journal of Animal and Veterinary Advances 10(8), 1019-1023.

Teker T., Albayrak G., Akayli T., Urku C. (2019). Detection of haemolysin genes as genetic determinants of virulence in *Lactococcus garvieae*. Turkish Journal of Fisheries and Aquatic Sciences 19(7), 625-634.

Topić Popović N., Čož-Rakovac R., Strunjak-Perović I. (2007). Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria. Veterinarni Medicina, 52(2), 49-53.

Vendrell D., Balcázar J. L., Ruiz-Zarzuela I., De Blas I., Gironés O., Múzquiz J.L. (2006). *Lactococcus garvieae* in fish: A review. Comparative Immunology, Microbiology and Infectious Diseases 29(4), 177-198.

UTILIZING DRONES TO INVESTIGATE DIFFERENT WATER RESOURCES

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Abstract
Unmanned Aerial Vehicles (UAVs) are very powerful tools that have become necessary in many environmental applications focused on water resources. The capability of remotely recording airborne video and images have emerged their importance to be used for investigation scopes. This study presents three different examples of UAVs applications on three different water resources: i) a wetland, ii) a stream and iii) a coastal lagoon. The captured images were utilized in order to create the orthomosaic of each area while further analysis in ArcGIS was able to investigate these areas from an "airborne eye" and highlight different geomorphologic, engineering, environmental or ecological characteristics. These are just examples of UAVs applications and initial results of these investigations. Further analysis and frequent monitoring are to be scheduled in order to detect any changes during different periods e.g. dry *vs* wet period.
Keywords: *Orthomosaic, Water velocity, Water discharge, Geomorphology, Spatial analysis.*
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1. Introduction

Unmanned Aerial Vehicles (UAVs), or more commonly known as drones, are a new scientific tool that continue to increase in capabilities and easiness to use with many brands and different types of UAVs (micro, light-weighted, small-scale, rotorcrafts, flying-wing, etc.) in the global market (Cai *et al*. 2014). The UAV's systems performance has been growing geometrically in quality and quantity during the 21st century (Estrada & Ndoma 2019). In addition, the cost decreased although technological capabilities and application have increased (Zohdi 2020). UAVs proved to be critical in various environmental applications focused on water resources as described in Vélez-Nicolás *et al*. (2021). Video and time-averaged images have been used by image-based velocimetry methodologies in order to estimate the surface velocity on different water resources. Their applications by Tauro *et al*. (2016) and Koutalakis *et al*. (2019) are characteristic examples of such studies.

Additionally to surface velocity, the measurement of water level can result to the estimation of water discharge, especially in flood conditions (Perks *et al*. 2016; Ridolfi & Manciola 2018). Gkiatas *et al*. 2021) as well as Latsiou *et al*. (2021) have tested UAV-based images for environmental quality categorization of riparian areas, streams and rivers jointly with the use of environmental visual protocols. McDonald (2019) studied their application on urban stormwater management, Metni & Hamel (2007) performed bridge inspection with three-dimensional UAV-based datasets while Ridolfi *et al*. (2017) focused on dams. UAVs have also been used in various natural disasters events (Antoine *et al*. 2020). Hayashi & Schirling (2015) developed a visual tsunami warning system, Annis *et al*. (2020) assessed flood hazard, Koutalakis *et al*. (2020) monitored and analyzed geomorphologic changes along the stream banks and in riparian areas while, in another study, Koutalakis *et al*. (2021) utilized a UAV-based orthomosaic to map earth cracks and perform landslide morphometric measurements close to a stream.

Vousdoukas *et al*. (2011) employed UAV to generate high quality time-averaged images to provide information on the nearshore sandbar morphology and rip currents location, while Templin *et al*. (2018) focused on the investigation of inland lakes' shorelines. UAV-based LiDAR survey is also a recent and promising application based on the study of DeBell *et al*. (2015). Various cameras of different capabilities have been utilized including different bands of the spectrum and thermal data. Aicardi *et al*. (2017) used aerial thermal data to rapidly reveal and map the river point sources, while Handcock *et al*. (2012) used airborne thermal images to visualize the water temperature of streams and rivers. In addition, Kislik *et al*. (2018) used thermal cameras for water degradation and algae bloom mapping. Near-infrared cameras can be used for precision agriculture and indices can be used to map the water stress of specific vegetation species as described in the study by Radoglou-Grammatikis *et al*. (2020). UAVs are powerful tools for water resources degradation and monitoring of litter pollution as studied by Topouzelis *et al*. (2019).

Another good example of UAV application is the study of Lally *et al*. (2019) that presents the capability of UAVs to carry a variety of sensors for water sampling and analysis. This study presents the application of a UAV on different water resources to investigate and map geomorphologic dimensions and spatial changes. Generally, these are the primary results of the investigation, and additional field measurements are required along with frequent monitoring of these sites.

2. Materials and Methods

The DJI Mavic 2 Pro was utilized to capture the airborne images of three different water resources. This is a powerful tetra-copter which is easy to be transferred due to its small dimensions but also easy-to-use and navigated by any pilot according to Dorafshan *et al*. (2018). The images were inserted in the commercial Pix4D software to develop the orthomosaic of each area. The software is capable to combine and merge the images based on common points; a characteristic methodology for photogrammetric applications (Ruzgienė *et al*., 2015). Pix4D generates the point cloud, the mesh model, the texture, the orthomosaic, the 3D model (if images captured by different ankles) and the digital surface model (DSM). The generated orthomosaics were further investigated in ArcGIS 10.4 software (Palma *et al*., 2017). The areas selected for the needs of the study were different water systems: i) a wetland, ii) a stream and iii) a coastal lagoon.

3. Results & Discussion

The Agia Barbara Springs area is a recreational wetland (small lake) along with perennial streams located in the center of Drama City (EGSA87 X: 511700 and Y: 4555179). The UAV flight was programmed in May 6th 2021. The ground sampling distance of the orthomosaic was 1.16 cm. The wetland of Agia Barbara has a perimeter of 238.8 m and an area of 2309.7 m^2 according to the produced orthomosaic. The airborne product enables the identification of the underwater old ruins of the old church of Agia Barbara due to the clear water conditions. The ruins are highlighted inside the yellow squared area. Furthermore, an experienced “eye” can spot the presence of wildlife e.g. ducks (*Anas*) and swans (*Cygnus*) as indicated in white circle of the high resolution orthomosaic. If the flight conditions were done in a lower altitude, fish presence could be also visible due to the clear water visibility.

The second studied location is near Symboli village where there is a check dam that regulates the water flow at the confluence of Xiropotamos River and Aggitis River (EGSA87 X: 502704 and Y: 4541765). The studied stream is a tributary of the nearby Aggitis River. The flight occurred on April 8th 2021. The generated orthomosaic had a ground sampling distance of 1.16 cm. The environmental conditions of the stream are degraded in the right side of the existing bridge as the culvert under the bridge is filled with debris; thus an algae bloom was developed in front of the culvert. The algae bloom has intense odor, high turbidity and a huge population of frogs (*Anura*) and insects as validated by terrestrial field visit. A thermal camera would be very critical for this case study.

The third area is the old estuary of Strymonas River in the Strymonikos Gulf of the Aegean Sea (EGSA87 X: 488948 and Y: 4513889). This area is protected under the Natura 2000 Network as belongs to the site “EKVOLES POTAMOU STRYMONA” (Code: GR1260002). The drone’s flight took place on March 28th 2021. The developed orthomosaic had a ground sampling distance of 0.73 cm. The water flow can be regulated by a check gate. The day of the flight the gate was fully closed and the channel was filled with sand. This means that even if the gate is reopened there will be no channel for the water to flow to the sea. This is a recent coastal change as the lagoon area was connected to the Aegean Sea based on satellite images of May 2020. The UAV flight was also useful to map the area which is used for aquaculture purposes.

As a conclusion, drones can provide high resolution images and consequently orthomosaics that could be analyzed to measure dimensions of water resources e.g. algae bloom or other characteristics e.g. engineering constructions. They can be used in order to spot habitats or geomorphologic changes through different time periods. The airborne monitoring is necessary especially in places difficult to be visited by foot. As mentioned above, these are initial results of UAVs application on different water resources in order to investigate geomorphologic changes and environmental conditions. Frequent monitoring is required in order to record spatial changes over different time periods and sensors must be embedded in UAV platforms to study environmental conditions.

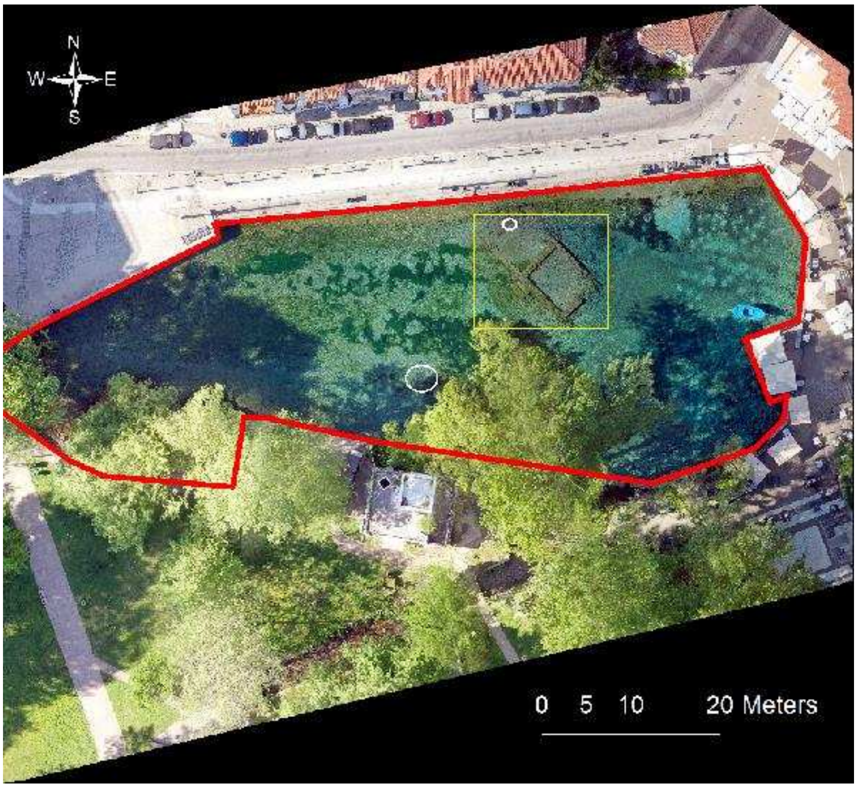

Figure 1. The recreational area and a small wetland (lake) in Agia Barbara. The red boundaries is the surface of the lake, the yellow square depicts infrastructure which is below water and the white circles indicate animals' presence.

Figure 2. A small tributary stream nearby Aggitis River. The culverts under bridge filled with debris from trees (yellow area) and an algae bloom was created (red area). The stream continues its flow at the left side of the bridge in the vegetated channel.

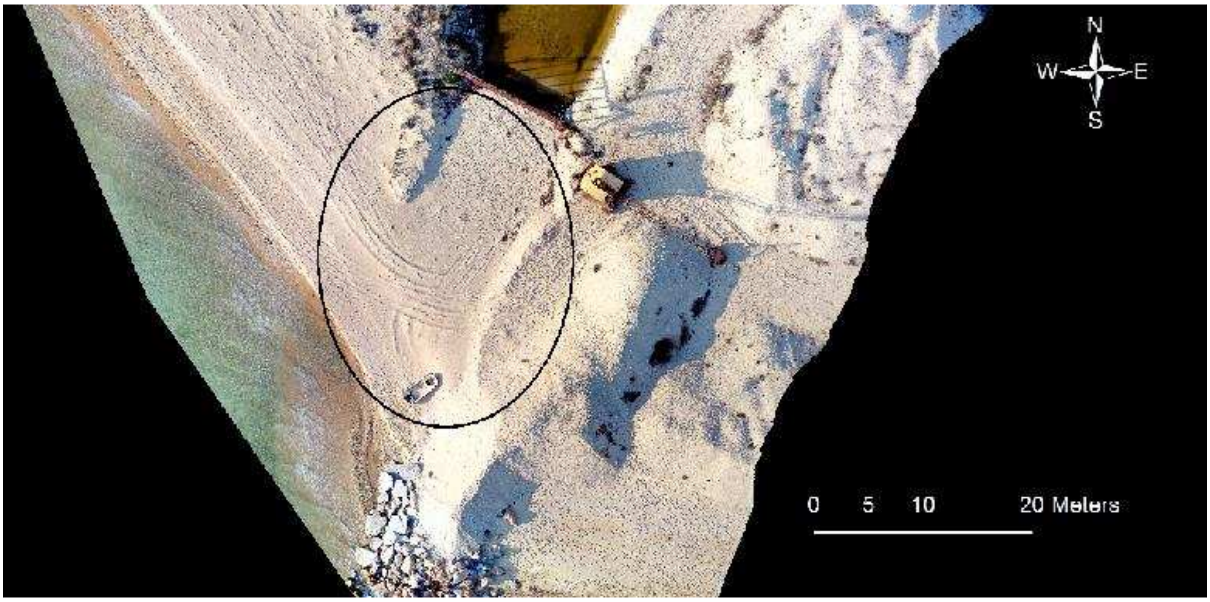

Figure 3. The lagoon area is the old estuary of Strymonas River in Strymonikos Gulf (Aegean Sea) which was block and recently filled by sand (black spotted area) for aquaculture scopes.

References

Aicardi I., Chiabrando F., Lingua A.M., Noardo F., Piras M., Vigna B. (2017). A methodology for acquisition and processing of thermal data acquired by UAVs: a test about subfluvial springs' investigations. Geomatics, Natural Hazards and Risk 8(1), 5-17.

Annis A., Nardi F., Petroselli A., Apollonio C., Arcangeletti E., Tauro F., Belli C., Bianconi R., Grimaldi S. (2020). UAV-DEMs for Small-Scale Flood Hazard Mapping. Water 12(6), 1717.

Antoine R., Lopez T., Tanguy M., Lissak C., Gailler L., Labazuy P., Fauchard C. (2020). Geoscientists in the Sky: Unmanned Aerial Vehicles Responding to Geohazards. Surveys in Geophysics 41(6), 1285-1321.

Cai G., Dias J., Seneviratne L. (2014). A survey of small-scale unmanned aerial vehicles: Recent advances and future development trends. Unmanned Systems 2(02), 175-199.

DeBell L., Anderson K., Brazier R.E., King N., Jones, L. (2015). Water resource management at catchment scales using lightweight UAVs: Current capabilities and future perspectives. Journal of Unmanned Vehicle Systems 4(1), 7-30.

Dorafshan S., Thomas, R.J., Maguire M. (2018). Fatigue crack detection using unmanned aerial systems in fracture critical inspection of steel bridges. Journal of Bridge Engineering 23(10), 04018078.

Estrada M.A.R., Ndoma A. (2019). The uses of unmanned aerial vehicles–UAV's-(or drones) in social logistic: Natural disasters response and humanitarian relief aid. Procedia Computer Science 149, 375-383.

Gkiatas G., Kasapidis I., Koutalakis P., Iakovoglou V., Savvopoulou A., Germantzidis I., Zaimes, G.N. (2021). Enhancing urban and sub-urban riparian areas through ecosystem services and ecotourism activities. Water Supply, ws2021114.

Handcock R.N., Torgersen C.E., Cherkauer K.A., Gillespie A.R., Tockner K., Faux R.N., Tan J. (2012). Thermal infrared remote sensing of water temperature in riverine landscapes. In: "Fluvial remote sensing for science and management", Carbonneau P.E., Piégay H. (eds), John Wiley & Sons Ltd, p. 85-113.

Hayashi M., Schirling P. (2015). Visual tsunami warning system. Proceedings of the 25th International Ocean and Polar Engineering Conference. International Society of Offshore and Polar Engineers, pp. 865.

Kislik C., Dronova I., Kelly M. (2018). UAVs in support of algal bloom research: a review of current applications and future opportunities. Drones 2(4), 35.

Koutalakis P., Tzoraki O., Zaimes G. (2019). UAVs for hydrologic scopes: application of a low-cost UAV to estimate surface water velocity by using three different image-based methods. Drones 3(1), 14.

Koutalakis P., Tzoraki O., Gkiatas G., Zaimes G.N. (2020). Using UAV to Capture and Record Torrent Bed and Banks, Flood Debris, and Riparian Areas. Drones 4(4), 77.

Koutalakis P.D., Tzoraki O.A., Prazioutis G.I., Gkiatas G.T., Zaimes G.N. (2021). Can Drones Map Earth Cracks? Landslide Measurements in North Greece Using UAV Photogrammetry for Nature-Based Solutions. Sustainability 13(9), 4697.

Lally H.T., O'Connor I., Jensen O.P., Graham C.T. (2019). Can drones be used to conduct water sampling in aquatic environments? A review. Science of the Total Environment 670, 569-575.

Latsiou A., Kouvarda T. Stefanidis K., Papaioannou G., Gritzalis K., Dimitriou E. (2021) Pressures and Status of the Riparian Vegetation in Greek Rivers: Overview and Preliminary Assessment. Hydrology 8, 55.

McDonald W. (2019). Drones in urban stormwater management: a review and future perspectives. Urban Water Journal 16(7), 505-518.

Metni N., Hamel T. (2007). A UAV for bridge inspection: Visual servoing control law with orientation limits. Automation in Construction 17(1), 3-10.

Palma M., Rivas Casado M., Pantaleo U., Cerrano C. (2017). High resolution orthomosaics of African coral reefs: A tool for wide-scale benthic monitoring. Remote Sensing 9(7), 705.

Perks M.T., Russell A.J., Large A.R. (2016). Advances in flash flood monitoring using unmanned aerial vehicles (UAVs). Hydrology and Earth System Sciences 20(10), 4005-4015.

Radoglou-Grammatikis P., Sarigiannidis P., Lagkas T., Moscholios I. (2020). A compilation of UAV applications for precision agriculture. Computer Networks 172, 107148.

Ridolfi E., Manciola P. (2018). Water level measurements from drones: A pilot case study at a dam site. Water 10(3), 297.

Ridolfi E., Buffi G., Venturi S., Manciola P. (2017). Accuracy analysis of a dam model from drone surveys. Sensors 17(8), 1777.

Ruzgienė B., Berteška T., Gečyte S., Jakubauskienė E., Aksamitauskas V.Č. (2015). The surface modelling based on UAV Photogrammetry and qualitative estimation. Measurement 73, 619-627.

Tauro F., Porfiri M., Grimaldi S. (2016). Surface flow measurements from drones. Journal of Hydrology 540, 240-245.

Templin T., Popielarczyk D., Kosecki R. (2018). Application of low-cost fixed-wing UAV for inland lakes shoreline investigation. Pure and Applied Geophysics 175(9), 3263-3283.

Topouzelis K., Papakonstantinou A., Garaba S.P. (2019). Detection of floating plastics from satellite and unmanned aerial systems (Plastic Litter Project 2018). International Journal of Applied Earth Observation and Geoinformation 79, 175-183.

Vélez-Nicolás M., García-López S., Barbero L., Ruiz-Ortiz V., Sánchez-Bellón Á. (2021). Applications of Unmanned Aerial Systems (UASs) in Hydrology: A Review. Remote Sensing 13(7), 1359.

Vousdoukas M.I., Pennucci G., Holman R.A., Conley D.C. (2011). A semi automatic technique for Rapid Environmental Assessment in the coastal zone using Small Unmanned Aerial Vehicles (SUAV). Journal of Coastal Research SI64, 1755-1759.

Zohdi T.I. (2020). The Game of Drones: rapid agent-based machine-learning models for multi-UAV path planning. Computational Mechanics 65(1), 217-228.

TRACE ELEMENTS, NITRATE, AND SALINITY IN SHALLOW AND DEEP AQUIFERS OF AN AGRICULTURAL BASIN (NORTH-EASTERN TUNISIA)

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Abstract
The growing use of pesticides in agriculture combined with the degraded and/or non-existent sanitation canals and misuse of wastes raise questions about Guenniche groundwater's quality (North-East Tunisia). The objectives of the current study are: (1) to assess the content of nutrients, salinity and trace elements (As, Cd, Mn, Fe, Cu, Hg, Zn, Cr, and Se) from the shallow and deep aquifers; and (2) to identify the affected areas using geospatial tools. The results show that the nitrate values in 60% of the samples from the shallow wells and in 30% from the deep wells exceed the national (NT) and international (WHO) standards. The nitrite overrides both standards in 20% of the shallow wells. The salinity values, measured as Total Dissolved Solids (TDS), exceed the WHO standard in 95% of the shallow wells' samples and 80% in the deep wells' samples. In a few examined shallow wells, Cd and Hg are found in concentrations slightly exceeding the limits. It is recommended not to utilize the aquifers' water from the El Alia region and the centre of Guenniche basin for drinking purposes due to its potential human health risk.
Key words: *Guenniche basin, Shallow and Deep Aquifers, Nitrate, Salinity, Trace elements.*
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1. Introduction

In Tunisia, as in Mediterranean countries, the groundwater are often the only source in many rural regions to cover the needs of drinking water, agriculture and industry (Zammouri *et al.* 2013). Several rural regions in the world witnessed during the last decades an availability limit and/or a degradability of groundwater quality caused by the pollution and the increasing demand (UN 2012). The important global groundwater pollutants are the presence of high nitrate and salinity that threaten water quality safety and its environment (Alfaifi *et al.* 2020). The high nitrate and salinity in groundwater are mostly resulting from the anthropogenic (agro-chemicals, the urban and industrial waste) and the natural factors (e.g. soil type, ions' dissolution) (Tlili-Zrelli *et al.* 2018, Hassen *et al.* 2016, Abboud 2018) indicated that the frequent irrigation and the climate conditions could negatively reflect on groundwater quality degradation and/or the depletion of its supplies. The soil type and the structural conditions are the most important natural conditions playing an important role in groundwater quality assessment (Hassen *et al.* 2016, Abboud 2018, Tlili-Zrelli *et al.* 2018). In addition to nitrate and salinity, in some parts of the world, the trace elements (TEs) with high levels in groundwater have become alarming to the safety, security, and future of its uses. TEs in water are typically caused by the weathering of bed-rocks or human activities (Egbueri 2020). The rural area of increasing urbanization, absence and/or erosion of sanitation canals, and excessive uses of agro-chemicals are more vulnerable to groundwater contaminations by TEs. In the rural area, knowing the levels of salinity, nitrate, and TEs have become a priority concern (Troudi *et al.* 2019; Singh *et al.* 2005), especially, when the groundwater is used for human needs. The use of water with high nitrate and TEs levels that are near or beyond the standard limits can strongly create health problems for the human body (Adimalla 2019; Egbueri 2020).

The Guenniche basin (GB) of North-Eastern Tunisia includes all the previously mentioned factors, which raise questions for its shallow and deep groundwater quality that are used for agricultural and human purposes. Providing an overview of both aquifers' nitrate, salinity, and TEs' levels with the zoning of the affected area is of the utmost importance to assist in identifying future decisions and recommendations. Therefore, the main objectives of the present study are (1) to analyse the nitrate, nitrite, salinity, and nine heavy metals of 20 shallow groundwater wells and 10 boreholes selected in the GB and compare them with the national and international standards, and (2) to identify the spatial distribution of the highest elements in both aquifers of the study region through GIS. The information presented in this paper will help the future planning, monitoring, and management of water resources within and around the study area.

2. Materials and Methods

2.1. Study site description

The GB (Figure 1) is a basin extending for over 130 km^2, has experienced a census rise of residents over the three past decades (from 59.000 to more than 74.000 inhabitants) (INS 2014). The study area's climate is typically Mediterranean, sub-humid to semi-arid, with seasonal variations in temperature and rainfall. Most of the rural communities are not connected and /or of obsolete sanitation canals use the ephemeral streams to dump wastewater, household waste and others solid and liquid scavenging (Troudi *et al.* 2019). GB is a farming region characterized by a high productivity of veggies' cultivation types with an annual production of more than 58000 tons (CRDA 2020), use very heavily agro-chemicals and of a frequent irrigation with the application of gravity-fed irrigation technique in most of the land (CRDA 2020). The region's population entirely relies on the shallow aquifer of Quaternary age which is exploited by low depth wells varying between 3 m and 30 m of depth, and on the deep aquifer of Mio-Pliocene age between 75 m to 300 m of depth, reaching Eocene levels in some places (Haj Ltaief 1995), to meet the demand from agriculture, human use, and industry. Rainfall is the source of the shallow and deep aquifer's recharge through infiltration across the mountains and hills bordering the basin's watershed. Both aquifers are separated by a clay substratum, semi-permeable in some regions, and are connected through the El Alia's fault (Haj Ltaief 1995).

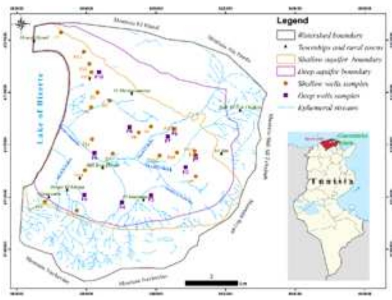

Figure 1. Map of the Guenniche basin area and distribution of shallow and deep wells' sampling points.

2.2. Methodology

At the end of the wet season (May 2016), a total of 30 samples were taken from shallow wells (20 wells) and deep wells (10 wells) (Figure 1). The standards, given by Rodier *et al*. (1996), are followed to collect all the groundwater samples. On the spot, (1) to avoid residual water's influence, each well and boreholes were pumped for more than 30 minutes, until steady state chemical conditions were obtained, (2) the physical parameters were measured (temperature (T), pH, and electrical conductivity (EC)) using hand-held analysing kits. Using the gravimetric method, the salinity presented by the Total Dissolved Solids (TDS) was determined. According to Rodier *et al.* (1996) method, the nitrate (using the salicylate of Sodium by molecular absorption method) and nitrite (using reactive Zambelli method) were measured using spectrometric method UV/VIS at wavelengths of 415nm and 435nm, respectively. The TEs' content in the water samples was analysed by atomic absorption spectrometry (Flame or Oven: AAS 240 FS Varian) with the three next methods: (1) Chromium detected by the third mode of Atomic Absorption Spectrometry; (2) Mercury and Arsenic achieved by the hydride mode of atomic absorption spectrometry; and (3) the Iron, Manganese, Copper, Zinc, Selenium, and Cadmium analysed by the Atomic Absorption Spectrometry flame mode. At a preliminary stage and for assessing the outcomes, all results were compared to the World Health Organization (WHO) and Tunisian National standards (NT) for drinking water. In the next step, the ArcGIS software developed by ESRI (Environmental Systems Research Institute) is used to visualize the spatial variation of the highest analysed data results from the groundwater samples.

3. Results

Descriptive statistical results of the physical parameters (T, pH, and EC), the TDS, NO_3^-, NO_2^-, and the nine TEs in the shallow and deep aquifers samples are listed in Table 1, accompanied by NT and WHO standards.

The pH data ranged from 6.8 to 7.72 and 6.97 to 7.72 for the shallow and deep samples, respectively. pH results show that all water samples are in desirable interval set by national (NT) and international (WHO) standards (Table 1), which is close to neutrality. The temperatures are characterized by close values varying from 19.1 to 21.8 °C and 19.3 to 21.5 °C for the shallow and deep samples, respectively. The temperature of water depends on the well's depth, with an average value of ≈20 °C for both aquifers' samples. For the shallow wells samples, the electrical conductivity (EC) values vary from 0.64 to 7.32 ms.cm^{-1} with a mean of 3,74 ms.cm^{-1}. Regarding to the deep wells' samples, the EC varies from 0.89 to 5.84 ms.cm^{-1} with a mean of 2.6 ms.cm^{-1}.

Table 1. Descriptive statistics of physical parameters, TDS, the nitrate, the nitrite, and heavy metals in the shallow wells and deep wells samples of the Guenniche basin.

Parameters		**T**	**pH**	**EC**	**TDS**	**NO_3^-**	**NO_2^-**	**Mn**	**Fe**	**Cu**	**Zn**	**Hg**	**As**	**Cr**	**Cd**	**Se**
Units		°C	_	ms.cm^{-1}	mg.l^{-1}	mg.l^{-1}	mg.l^{-1}	µg.l^{-1}	µg.l^{-1}	mg.l^{-1}	mg.l^{-1}	µg.l^{-1}	µg.l^{-1}	µg.l^{-1}	µg.l^{-1}	µg.l^{-1}
Standards	NT	-	6,5-8,5	1,5	1000	45	0,2	50	-	2	5	1	10	50	5	10
	WHO	-	6,5-9,5	2,5	2000	50	0,2	40	200	2	3	6	10	50	3	10
Shallow (20 samples)	Min	19,1	6,8	0,64	380	1,9	<MDL	0,12	10,2	0	0,01	0,04	0,05	0	0,06	0,31
	Max	21,9	7,72	7,32	5476	421,01	0,988	20,17	188,3	0,88	0,41	1,91	8,55	6,62	5,43	4,92
	Mean	20,9	7,21	3,74	2237,1	101,25	0,13	4,51	73,6	0,08	0,06	0,75	3,16	1,4	1,69	2,38
	SD	0.75	0.21	1.51	891.9	89.47	0.14	5.77	54.08	0.20	0.09	0.47	2.6	1.88	1.85	1.45
Deep (10 samples)	Min	19,3	6,97	0,89	510	8,2	0,01	4,32	28,89	0,001	0,003	0,032	0,8	0,002	0,03	0,48
	Max	21,5	7,72	5,84	3650	109.9	0,046	15,89	85,13	0,069	0,21	0,195	3,84	3,09	1,38	2,65
	Mean	20,5	7,17	2,63	1564,2	39.68	0,024	8,63	53,62	0,019	0,05	0,129	2,07	1,01	0,41	1,39
	SD	0.66	0.23	1.48	882.2	35.84	0.01	3.96	21.28	0.02	0.07	0.06	1.03	0.92	0.47	0.74

NT (2013): Tunisian National standards; WHO (2011): World Health Organization; SD: Standard deviation

The EC shallow wells' samples exceed the NT (2013) standard and the WHO (2011) standard at 95% and 65% of wells, and at 90% and 30% for the deep wells' samples, respectively. The EC results are related to the concentrations of ions in water with a positive correlation with the TDS. The TDS analysis results ranged between 380 and 5476 mg.l^{-1} in the shallow wells' samples and between 510 and 3650 mg.l^{-1} in the boreholes' samples, with the means of 2237.1 and 1564.2 mg.l^{-1}, respectively. Compared to both standards, 95% of the shallow wells override the international standard limit (WHO, TDS set at 1000 mg.l^{-1}) and 25% more than the national standard limit set at 2000 mg.l^{-1}; as for the deep aquifer, it turns out that 80% override the WHO limit and 20% exceed the NT limit. Nitrate (NO_3^-) should not exceed 50 mg.l^{-1} and 45 mg.l^{-1} in the water according to WHO and NT standards, respectively. In the GB, the nitrate interval results are between 1.9 and 421.01 mg.l^{-1} for the shallow wells' samples, and between 8.2 and 109.9 mg.l^{-1} for the boreholes' samples, with averages of 101.25 mg.l^{-1} and 39.68 mg.l^{-1}, respectively. The results indicate that 60% of the shallow wells' samples and 30% of the boreholes' samples exceed both NO_3^- limits. In the case of nitrite (NO_2^-), the WHO and NT standards set the limit at 0.2 mg.l^{-1}. In the examined shallow and deep aquifers, the NO_2^- results range from 0 to 0.988 mg.l^{-1}, and between 0.01 and 0.046 mg.l^{-1}, with an average of 0.13 mg.l^{-1} and 0.024 mg.l^{-1}, respectively. The results show that 20% of the analyzed shallow wells show values higher than both limits, as to the deep aquifer all the samples boreholes are lower than both limits.

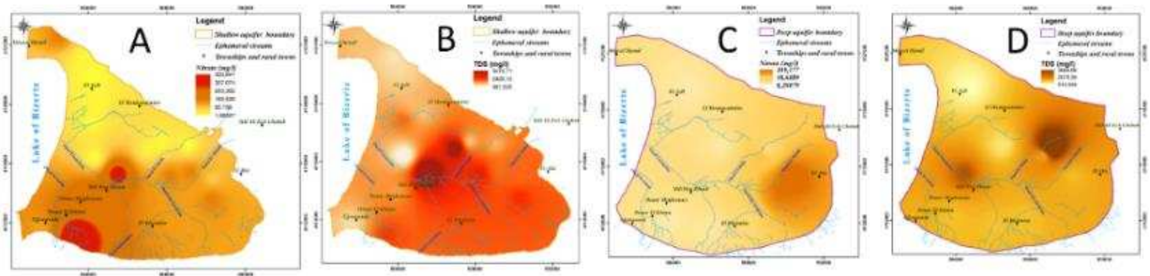

Figure 2. NO_3^- and TDS spatial distribution maps in shallow (A,B) and deep (C,D) water of the GB.

The shallow aquifer samples show that the Fe and As are close to the limits set by the international (WHO) and national (NT) standards in some wells ranged from 10.2 to 188.3 µg.l^{-1} with a mean of 73.6 µg.l^{-1}, and ranged from 0.05 to 8.55 µg.l^{-1} with a mean of 3.16 µg.l^{-1}, respectively. Whereby, two wells (P1 and PZ1) show Fe values more than 150 µg.l^{-1}, and two other wells (P1 and P4) present As values near both standard limits (WHO and TN) set at 10 µg.l^{-1}. These three wells exist at the borders of wadi Guenniche and wadi El Mallah. Hg and Cd concentration results varied from 0.04 to 1.91 µg.l^{-1} and from 0.06 to 5.43 µg.l^{-1} with averages of 0.75 µgL^{-1} and of 1.69 µgL^{-1}, respectively. Both elements exceed one or both standard limits in some shallow wells samples, in which 35% of samples surpass the Hg national standards limit set at 1µg.l^{-1}, whereas no wells exceed the Hg international standard set at 6µg.l^{-1}; regarding the Cd compared to the national (NT) standards (set at 5µg.l^{-1}) only 10% wells show a slightly exceeded but compared with the international standard 25% go beyond the limit set at 3 µg.l^{-1}. P1, P4, P10, PZ1, and PZ4 are the wells exceeding the Hg national limit, which were the same for the Cd besides two other wells P9 and P14, are in the border of the wadi Guenniche and El Mallah ephemeral streams.

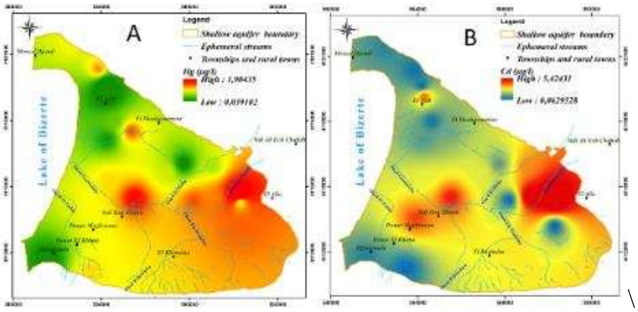

Figure 3. Hg (A) and Cd (B) spatial distribution maps the shallow aquifer water of the GB.

In comparison with international (WHO 2011) and national (NT 2013) standards, the deep aquifer results have shown that 100% of the boreholes' samples are within the limits of the standards for all nine studied TEs (Table 1) whereby the maximum of all results is less than the half of both standard limits. The spatial distribution maps of NO_3^- and TDS in both aquifers were achieved using the ordinary IDW method and all maps were depicted in Figure. 2. High levels of NO_3^- (Figure. 2 A and C) were found near El Alia region (the eastern part of Guenniche basin) in both aquifers, and in the shallow aquifer of the central and southern parts where five rural districts are located. According to the TDS distribution maps (Figure. 2 B and D) it was found that the entire shallow aquifer, with the exception of a single well located in the South of El Azib region (Figure. 2 B), and in the eastern and central parts for the deep aquifer, have high TDS content of more than 1500 $mg.l^{-1}$ (Figure. 2 D). Additionally, Hg and Cd were distributed only for the shallow aquifer's water (Figure 3A and B), which slightly exceed one/or both standards' limits. Figures 3A and B show that the Hg and Cd risk areas are located near to the ephemeral streams, exactly close to wadis El Mellah and Guenniche.

4. Discussion

The examined aquifers of GB face water degradation due to the high content of nitrate and salinity in both aquifers, plus the presence of a slight rise of NO_2^-, Hg and Cd in some shallow wells. NO_3^-, NO_2^-, Hg, and Cd in water may pose an adverse health risk through the drinking pathway and the dermal contact when the levels exceed the maximum permissible limit (Adimalla 2019; Jehan *et al*. 2020). The basic reason to override the limits of those elements in groundwater returns to the anthropogenic factors such as contamination of waterbodies (rivers and ephemeral streams) by human and livestock waste, wastewater effluents, industry-related pollution, and the overuse of chemical fertilizers in agricultural practices (Wolfe & Patz 2002). Moreover, the natural conditions of the aquifers (weathering and erosion of bedrocks, ore deposits, permeability type of aquifer) and the climate can contribute to the groundwater pollution and reinforce the presence of high NO_3^- and TDS in the groundwater (Tlili-Zrelli *et al.* 2018; Troudi *et al.* 2019) and the signs of existence for the TEs (Egbueri 2020). Similar results reported in different parts of world such as semi-arid region of Telangana, India (Adimalla 2019) northeastern Algeria (Zereg *et al.* 2018) and northeastern Tunisia (Tlili-Zrelli *et al.* 2018). They indicated that the anthropogenic and natural factors promote groundwater quality degradation that can lead to threaten their future safety and availability. Furthermore, they indicate that the pollution by nitrate and TDS occurred through animal waste and in agricultural fields, leakage from septic tanks, manures, wastewater disposal, and oxidation of nitrogenous human and animal excreta. Ahamad *et al* (2020) indicated high results of Cd and Hg in groundwater that are resulting from the uncontrolled disposal of industrial, agricultural, and urban wastes. In relation to the studied groundwater, the previously mentioned factors are the strong potential reasons behind the high TDS and NO_3^- in both of the aquifers and the high NO_2^-, Hg and Cd in few shallow wells of the study area. GB witnesses increasing of the population of 13% for the last 30 years, accompanied by the increasing demand from aquifers and the expanding irrigation areas, which this basin presents more than 11 shallow and deep wells in each of 1 km^2 (CRDA 2020). In addition, the discharge of untreated solid and liquid waste in the surface and /or in the ephemeral streams, and the irrigation water is enriched by chemical fertilizers $((NH_4)_3PO_4$ and $P_2O_5$with quantities exceeding 450 kg ha^{-1} $year^{-1}$ with more than 300 kg ha^{-1} $year^{-1}$ of DAP N-P2O3 (CTV 2015) are the reasons behind the groundwater contamination in this basin. Aquifers' type of layers and the climate contribute as natural factors in the groundwater pollutions (Tlili-Zrelli *et al*. 2018). The surface layer of GB is permeable in most of the area facilitating the infiltration of surface water (wastewater from urbanization, contaminated water from the ephemeral streams, and irrigation water) into the shallow aquifer. The fault of El Alia region and the semi-permeable substratum (in some substratum region) make both aquifers connected. Both these factors are the potential cause of high NO_3^- in some boreholes near these zones via the intrusion of shallow aquifer's water (that contained high level of NO_3^-) into the deep aquifer. The high TDS in groundwater is principally related to the natural conditions through the washing of salts from the topsoil by leaching processes, which are more influential in soils of semi-arid regions (Hassen *et al*. 2016). These processes are aggravated by the phenomenon of evaporation and excessive irrigation (Zereg *et al*. 2018). As is the case with GB which is located in a semi-arid region with the application of gravity-fed irrigation techniques. These conditions promote the weathering of layered-rocks and reflect on the high TDS and EC in groundwater (Hassen *et al*. 2016; Tlili-Zrelli *et al*. 2018).

5. Conclusions

The exceeding of recommended nitrate, salinity and TEs' limits in water can lead to dangerous diseases. To prevent health hazards, the wells and boreholes located in the Guenniche aquifers should not be used for drinking water (all shallow wells in exception of well P12, and boreholes F1, F2, and F7). Furthermore, given the high NO_3^- in both aquifers and the high Hg and Cd in the shallow aquifer, studying the potential human health, especially the children, is critically important to ensure the safe use of this groundwater.

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References

Abboud I.A. (2018). Geochemistry and quality of groundwater of the Yarmouk basin aquifer, north Jordan. Environmental Geochemistry and Health 40, 1405–1435.
Adimalla N. (2019). Spatial distribution, exposure, and potential health risk assessment from nitrate in drinking water from semi-arid region of South India, Human and Ecological Risk Assessment. Internat. Journal
Ahamad A., Raju N.J., Madhav S. et al. (2020). Trace elements contamination in groundwater and associated human health risk in the industrial region of S Sonbhadra, India. Envir. Geoch. Health 42, 3373–3391.
Alfaifi H.J., Kahal A.Y., Abdelrahman K. et al. (2020). Assessment of groundwater quality in S Saudi Arabia: case study of Najran area. Arab Journal Geosciences 13, 101.
CRDA-Bizerte (2020). Commissariat Régional de Développement Agricole Rpp. Int, 2007.
CTV (2015). Cellule Territoriale de Vulgarisation (Com. Régional de Développement Agricole) El Alia Bizerte.
Egbueri J.C. (2020). Heavy Metals Pollution Source Identification and Probabilistic Health Risk Assessment of Shallow Groundwater in Onitsha, Nigeria, Analytical Letters.
HajLtaief Z. (1995). Nappe de l'oued Guénniche: Evaluation des ressources et impact de la surexploitation. DEA, FS Tunis, Univ. Tunis II, 94 pp.
Hassen I., Hamzaoui-Azaza F., Bouhlila R. (2016). Application of multivariate analysis and hydrochemical and isotopic investigations for evaluation of groundwater quality and its suitability for drinking and agriculture purposes in Oum Ali-Thelepte aquifer, central Tunisia. Environ Monit. Asesment 188, 135.
INS (2014). Institut National de la Statistique, Recensementgénéral de la population et du logement.
Jehan S, Khattak SA, Muhammad S. et al. (2020) Human health risks by potentially toxic metals in drinking water along the Hattar Industrial Estate, Pakistan. Environment Sciences Pollution Res. 27, 2677–2690.
NT (2013). Tunisian National standards, Norme Tunisienne NT 09-14, Relative a la qualite des eaux de boisson.
Rodier J., Bazin C., Broutin J. P. et al. (1996). L'analyse de l'eau. 8éme édition. Paris: Dunod.
Singh U.K., Ramanathan A.L., Subramanian V. (2018). Groundwater chemistry and human health risk assessment in the mining region of East Singhbhum, Jharkhand, India. Chemosphere, 204, 501–513.
Tlili-Zrelli B., Gueddari M., Bouhlila R. (2018). Spatio-temporal variations of water quality of mateur aquifer in NE Tunisia: suitability for irrigation and drinking purposes. Hindawi J. of Chemistry, 2408632, 15 pp.
Troudi N., HamzaouiF., Zammouri M. et al. (2019). Distribution of trace elements in the shallow aquifer of Guenniche (North Tunisia). Advances in Science, Technology & Innovation, 113–116.
UN (2012). United Nations, World Water Development Report.
WHO (2011). Guidelines for Drinking-Water Quality. Health Organization, 4th ed., Geneva, 564pp.
Wolfe A. H., Patz J. A. (2002). Reactive nitrogen and human health: acute and long-term implications. Ambio, 31(2), 120–125.
Zammouri M., Jarraya-Horriche F., Odo B.O. et al. (2013) Assessment of the effect of a planned marina on groundwater quality in Enfida plain (Tunisia). Arab J Geoscience 7, 1187–1203.
Zereg S., Boudoukha A., Benaabidate L. (2018). Impacts of natural conditions and anthropogenic activities on groundwater quality in Tebessa plain, Algeria. Sustainable Environment Research, 28, 340–349.

HOW CLOSE TO *Posidonia oceanica* MEADOWS ARE THE GREEK FISH FARM PARKS?

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Abstract
The protected Mediterranean endemic seagrass *Posidonia oceanica* meadows growing at the coastal area are endangered by the operation of the nearby fish farm parks. This paper serves as a comprehensive record of the potential impact on the seagrass meadows which are affected by the far-reaching effects of the fish farm parks at predetermined distances. The Greek fish farms recorded in satellite images during the period 2016-2017 amount to 405. The affected seagrass meadows ranged from 12 (at ring distance i=0 m: 0m means the area under the cages) to 51 (at i=200-500m). Furthermore, the greater seagrass affected parts (with area>5% to total) consists of 64.10% of the total affected seagrass area. The total affected area of seagrass by farming activity (up to 500m around of farms) was found relatively low (7.15 km^2; ≈0.3% of total seagrass coverage). As national legislation prohibits not only a new establishment but also the renewal of the fish farm parks' license above the *Posidonia oceanica* meadows, the existing effects can be reduced or even averted.
Key words: *Seagrass meadows, Satelite image, Fish farms, Environmental impacts*
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1. Introduction

As Greek aquaculture has been exponentially developed since 1980 (Theodorou *et al*. 2015), an investigation for its impacts on the environment derived from the waste was released during farming (Karakassis *et al.* 2000; Apostolaki *et al.* 2007; Kalantzi *et al.* 2021). The impacts on the endemic Mediterranean seagrass *Posidonia oceanica* (Linnaeus) Delile meadows have been studied in particular for their major ecological, sedimentary and economic role in coastal ecosystems. Therefore, many Mediterranean countries have legally protected the meadows as a priority habitat under the European Union's Habitat Directive (92/43/CEE) (Pergent-Martini *et al.* 2006; Diaz-Almela *et al.* 2008; Ruiz *et al.* 2010; Apostolaki *et al.* 2007, 2011).

The density of the meadow, the foliage, the rhizome as well as the accumulation of the epiphytes are the main impacts mentioned in most studies (Pergent-Martini *et al.* 2006; Holmer *et al.* 2008, Apostolaki *et al.* 2011), while the affected distance from the cages has also been recorded. Thus, the overloaded sediment under the fish cages causes the death of the *P. oceanica* meadow, while the leaf-rhizome production is significantly decreased up to 25 meters away (Pergent-Martini *et al*. 2006). In addition, the size of the shoots and the growth of the leaves are significantly reduced at 50 meters resulting in dead seagrass meadow in ten years (Ruiz *et al*. 2001; Pergent-Martini *et al*. 2006). At a distance of 100 meters, the biomass of the epiphytes is significantly increased, while leaf - rhizome production is decreased, leading to rapid seagrass decline due to reduced recruitment (Pergent-Martini *et al.* 2006; Apostolaki *et al.* 2007).

Seagrass shoot half-life is significantly increased up to 200 meters away from farms, resulting to exponentially decreased seagrass mortality and/or decreased recruitment according to distance from farm, which leads to degraded or dead seagrass meadow with visible dead *Posidonia* rhizomes in a period of ten years (Pergent-Martini *et al.* 2006; Diaz-Almela *et al.* 2008; Holmer *et al*. 2008). The impacts on *P. oceanica* vertical growth are detected up to 400 m distance from the cages (Marbá *et al*. 2006), while the regression curve of shoot mortality does not reach global shoot recruitment rates up to 500 meters away (Holmer *et al*. 2008), resulting to the instability of the meadow in this distance. However, no significant impacts are detected to *P. oceanica* meadow at distances 500 to 1000 meters away from the fish farm parks (Ruiz *et al*. 2010). This study provides an overview of the possible overlapping of the seagrass meadows (*P. oceanica*) by fish farms in Greece and identify the risk of potential impact on their conservation status.

2. Materials and Methods

The Greek coastline was scanned by means of Google Earth satellite images for the period June 2016-May 2017 where fish farm cages were recorded. The cages, where the distance among them was lower than 20m, composed of a cages array (thereafter *l*). With a polygon around the *l* outside of edges that include the cages, the *l* was georeferenced, while the included area on polygon is defined as area of *l* (Ar_l). The georeferenced data was mapped using GIS software (QGIS ver. 3.16.2).

Using the QGIS commands: Dissolve buffer and buffer layer difference from land layer, for each *l* the potential environmental Impact marine Zones (IMZ_i) at distances i: 0, 25, 50, 100, 200 and 500 m were fixed on map. The command dissolve buffer returns the area j (cAreai, j) created by one or more cages arrays for each category i ignoring the overlapping individually areas which originated by each cages array. The difference from higher to smaller impact zone created a layer of rings (Rlayer) of i': 0, 0-25,25-50,50-100,100-200 & 200-500m.

Using the data of spatial distribution of seagrass in Greek waters (Sgrlayer), estimated by Topouzelis *et al.* (2018), the seagrass rings, i': 0, 0-25, 25-50, 50-100,100-200 & 200-500m, were retrieved by clipped of *Rlayer* on *Sgrlayer* (clip command; QGIS) and their area was calculated. Furthermore, the number of impacted seagrass patches and the number of implicated cages arrays were identified and charted. Map on the coordination reference system ETRS89LAEA - ETRS89 Lambert Azimutal Equal Area, was also projected.

3. Results

In total, 405 fish farm cages arrays were recorded in the Greek coastline during the period 2016-2017. As per data retrieved from Topouzelis (2017) a total of 4,598 patches of seagrass meadows ranging from 0.0011-125 km^2 (0.569±3.31 km^2) cover 2,619.25 km^2 of Greek coastal zone.

The number of affected seagrass patches range from 12 (at i'=0 m) to 51 (at i'=200-500m), while the number of impacted parts of affected seagrass patches ranges from 30 (at i'=0 m) to 44 (at i'=200-500m). The implicated arrays range from 30 (at i'=0 m) to 57 (at i'=200-500m). The mean area of affected seagrass patches is 1.28±2.61 km^2, while the mean area of impacted parts of affected seagrass patches is 0.041±0.089 km^2. The total area of impacted parts of seagrass, are 0.26, 0.25, 0.27, 0.58, 1.20 and 4.58 km^2 at i': 0, 0-25, 25-50, 50-100, 100-200 and 200-500m, respectively (Table 1).

Table 1. Descriptive statistic of impacted seagrass meadows (SG) by fish farms. SD: standard deviation

	i' zones						
	0	0-25	25-50	50-100	100-200	200-500	total
Total impacted SG (km^2)	0.26	0.25	0.27	0.58	1.12	4.58	7.15
Mean impacted SG (km^2)	0.009	0.010	0.010	0.019	0.034	0.104	0.041
SD of impacted SG (km^2)	0.012	0.007	0.009	0.016	0.033	0.152	0.09
Number of implicated fish farm cages arrays	30	26	29	31	39	57	
Number of parts of affected SG	30	26	28	30	35	44	
Number of affected SG Patches	12	13	17	19	26	51	
Mean of SG affected SG patches (km^2)	1.77	1.68	1.30	1.22	0.95	1.23	1.29
SD of affected SG patches (km^2)	3.24	3.12	2.79	2.65	2.29	3.93	2.61
Total area of affected SG patches (km^2)	21.26	21.78	22.18	23.26	24.81	62.96	176.25

The greater seagrass affected parts (with area>5% to total) consists of 64.10% of the total affected seagrass area, belonging in seven seagrass patches (Seagrass IDs in figure 1: 4448, 4487; total impacted seagrass area: 0.96 by 11 cages arrays and 0.56 km^2 by 5 cages arrays, respectively), Seagrass IDs in figure 1: 3487 (total impacted seagrass area: 0.63 km^2, by two cages arrays), Seagrass IDs in figure 1: 2747 (total impacted area: 0.46 km^2, by one cages arrays), Seagrass IDs in figure 1: 2291 (total impacted seagrass area: 1.11 km^2, by two cages arrays), Seagrass IDs in figure 1: 462 (total impacted seagrass area: 0.91 km^2, by two cages arrays) and Seagrass IDs in figure 1: 834 (total impacted seagrass area: 0.92 km^2, by seven cages arrays) (Figure 1).

4. Discussion

In the present study, the spatial distribution of the impact zones of the Greek fish farms was estimated and mapped in relation to their impact on the protected seagrass meadows.

Our results indicated that the total affected area of *P. oceanica* by fish farming activity (up to 500m around of farms) was relatively low (7.15 km^2; ≈0.3% of total seagrass coverage) and given that it refers to a low number of sites that the impacts could be averted. The national legislation has already posed prohibitions with regard to the new establishment and license for further renewal of fish farms above the seagrass meadows.

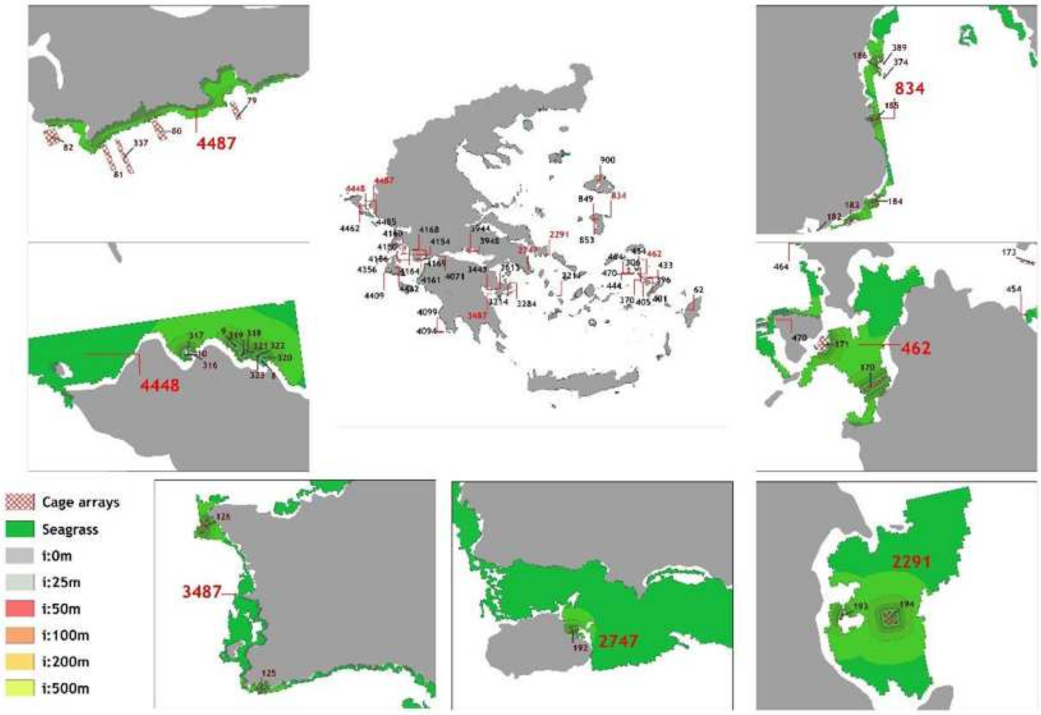

Figure 1. Impact zones per distance category (i), seagrass meadows, and affected cage arrays. By red color number the ID of seagrass patch, by black color number the ID of cage array.

However, several factors control the accuracy of the shape and size of impact zones: a) The hydrodynamic conditions of each location play key role to the dispersion of waste (Modica *et al.* 2006; Sarà, 2006; Sarà *et al.* 2006; Macleod *et al.* 2007; Borja *et al.* 2009), b) In the present study the impact zones are estimated as a projection on the sea surface. Thus, the actual impacted area of bottom is expected to be greater than estimated due the deformations of bottom terrain while small variations in bathymetry can result in significant changes in sedimentation pattern (Jusup *et al.* 2007), c) Changes of cages array location (relocation, add or remove) can change the impact zones sizes and d) the relative low accuracy of seagrass mapping (mean accuracy of 76.3% of the seagrass mapping; Toupouzelis *et al.* 2018).

Due to the fact that the estimated impacted areas of seagrass meadows are small (0.041±0.089 km^2) in combination to the relative low accuracy of seagrass mapping (as well as of the other limitations that control the estimation accuracy of impacted areas), is expected to have a significant error on the above estimations. Although this leads to the need for each site of cages array to be examined in situ, the findings of the present study provide a prioritization of sites evaluation of their contribution on seagrass meadows impact.

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References

Apostolaki E.T., Tsagaraki T., Tsapakis M., Karakassis I. (2007). Fish farming impact on sediments and macrofauna associated with seagrass meadows in the Mediterranean. Estuarine, Coastal and Shelf Science 75(3), 408-416.

Apostolaki E.T., Holmer M., Marbà N., Karakassis I. (2011). Epiphyte dynamics and carbon metabolism in a nutrient enriched Mediterranean seagrass (*Posidonia oceanica*) ecosystem. Journal of Sea Research 66(2), 135-142.

Borja Á., Rodríguez J.G., Black K., Bodoy A., Emblow C., Fernandes T.F., Forte J., Karakassis I., Muxika I., Nickell T.D., Papageorgiou N., Pravoni F., Sebastou K., Tomassetti P., Angel D. (2009). Assessing the suitability of a range of benthic indices in the evaluation of environmental impact of fin and shellfish aquaculture located in sites across Europe. Aquaculture 293(3-4), 231-240.

Diaz-Almela E., Marbà N., Álvarez E., Santiago R., Holmer M., Grau A., Mirto S., Danovaro R., Petrou A., Argyrou M., Karakassis I., Duarte C. M. (2008). Benthic input rates predict seagrass (*Posidonia oceanica*) fish farm-induced decline. Marine Pollution Bulletin 56(7), 1332-1342.

Holmer M., Argyrou M., Dalsgaard T., Danovaro R., Diaz-Almela E., Duarte C.M., Frederiksen M., Grau A., Karakassis I., Marbà N., Mirto S., Pérez M., Pusceddu A., Tsapakis M. (2008). Effects of fish farm waste on *Posidonia oceanica* meadows: synthesis and provision of monitoring and management tools. Marine Pollution Bulletin 56(9), 1618-1629.

Jusup M, Geček S, Legović T. (2007). Impact of aquacultures on the marine ecosystem: modelling benthic carbon loading over variable depth. Ecological Modelling 200: 459–466.

Kalantzi I., Rico A., Mylona K., Pergantis S.A., Tsapakis M. (2021). Fish farming, metals and antibiotics in the eastern Mediterranean Sea: Is there a threat to sediment wildlife? Science of The Total Environment 764, 142843.

Karakassis I., Tsapakis M., Hatziyanni E., Papadopoulou K.N., Plaiti W. (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES Journal of Marine Science 57(5), 1462-1471.

Macleod C.K., Moltschaniwskyj N.A., Crawford C.M., Forbes S.E. (2007). Biological recovery from organic enrichment: some systems cope better than others. Marine Ecology Progress Series 342, 41-53.

Marbà, N., Santiago, R., Díaz-Almela, E., Álvarez, E., & Duarte, C. M. (2006). Seagrass (*Posidonia oceanica*) vertical growth as an early indicator of fish farm-derived stress. Estuarine, Coastal and Shelf Science 67(3), 475-483.

Modica A., Scilipoti D., La Torre R., Manganaro A., Sara G. (2006). The effect of mariculture facilities on biochemical features of suspended organic matter (southern Tyrrhenian, Mediterranean). Estuarine, Coastal and Shelf Science 66(1-2), 177-184.

Pergent-Martini C., Boudouresque C.F., Pasqualini V., Pergent, G. (2006). Impact of fish farming facilities on *Posidonia oceanica* meadows: a review. Marine Ecology 27(4), 310-319.

Ruiz J. M., Pérez M., Romero J. (2001). Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. Marine Pollution Bulletin 42(9), 749-760.

Ruiz J.M., Marco-Méndez C., Sánchez-Lizaso J.L. (2010). Remote influence of off-shore fish farm waste on Mediterranean seagrass (*Posidonia oceanica*) meadows. Marine Environmental Research 69(3), 118-126.

Sarà G. (2006). Hydrodynamic effects on the origin and quality of organic matter for bivalves: an integrated isotopic, biochemical and transplant study. Marine Ecology Progress Series 328, 65-73.

Sarà G., Scilipoti D., Milazzo M., Modica A. (2006). Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics. Marine Ecology Progress Series 313, 261-270.

Theodorou J.A., Perdikaris C., Filippopoulos N.G. (2015). Evolution through innovation in aquaculture: a critical review of the Greek mariculture industry. Journal of Applied Aquaculture 27(2), 160-181.

Topouzelis K., (2017). Supplementary files of paper titled "Seagrass mapping in Greek territorial waters using Landat-8 satellite images" [Data set]. In International Journal of Applied Earth Observation and Geoinformation (#1). Zenodo. https://zenodo.org/record/1120338#.YQO3yb0zaUk (accessed: 23-11-2020).

Topouzelis K., Makri D., Stoupas N., Papakonstantinou A., Katsanevakis S. (2018). Seagrass mapping in Greek territorial waters using Landsat-8 satellite images. International Journal of Applied Earth Observation and Geoinformation 67, 98-113.

MAPPING OF POTENTIAL IMPACTS OF MARINE FISH FARMS IN CENTRAL IONIAN SEA (W. GREECE)

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Abstract
In the present study the spatial distribution of the possible impact zones in the central Ionian Sea coastline of fish farms, were estimated and mapped. The studied coastline was scanned via Google Earth satellite images for the period June 2016 to May 2017 with 72 fish farms cages arrays, being detected. For each cage array the zones at difference distances corresponding on various impacts were sketched and the area was estimated using GIS technologies. Although, the spatial pattern of the impact zones is the result of the legal frame of aquaculture, it seems that the long distance impacts (up to 3 km) revealed possible conflicts by small scale fishery as well as negative effects on functions of protected habitat as lagoons.
Keywords. *Fish farm, Satelite image, Aquaculture waste, Environmental impacts*
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1. Introduction

The increased demand of fishery products with high nutritional value and in line with the decline of fishery production currently being available at higher costs has led to the rapid growth of fish farming in the world (Steiner-Asiedu *et al.* 1991, Agren & Hanninen 1993, Hanson *et al.* 1994, Lall 1995). However, as aquaculture activity worldwide is increased, much criticism arose in relation to the environmental impact of the effluents on the receiving ecosystems and alteration of coastal habitats and wildlife disturbance (Savini *et al*. 2010, Grigorakis & Rigos 2011, Martinez-Porchas *et al.* 2012). The impacts of marine fish farms refer to the total change of the benthic community and nutrients enhancement of water column under and near the cages with reduced footprints according the distance, being traceable at distances up to 500-1000m (Borja *et al*. 2009; Karakassis *et al.* 2013, Tomassetti *et al.* 2016, Karakassis *et al*. 2000, Smith *et al.* 2005, Papageorgiou *et al.* 2010, Kalantzi *et al*. 2013).

Similarly, in a distance not far from the farms aggregations of wild fishes were recorded while the effect of farms on the wild fish demography is traceable more specifically at distances up to 3-6km (Dempster *et al.* 2009, Uglem *et al.* 2009, Arechavala-Lopez *et al.* 2010, 2011, 2015, Giannoulaki *et al.* 2005). Satellite images and geospatial information systems have been widely used for mapping aquaculture activities (Trujillo *et al.* 2012, Telesca *et al.* 2015, Ottinger *et al.* 2018). A typical example is the widespread use of the Google Earth Engine platform as it can be easily used and assist researchers to capture spatio-temporal alterations (Prasad *et al.* 2019, Xia *et al.* 2020, Fu *et al.* 2021). This study provides a mapping of the environmental impacts of fish farm in western Greece in order to highlight the potentially affected areas.

2. Materials and Methods

The central Ionian Sea Greek coastline was scanned via Google Earth satellite images for the period 2016-2017 (June 2016-May 2017) with fish farms cages arrays, being detected. The cages, where the distance among them was lower than 20m, composed of a cages array (thereafter *l*). With a polygon around the l outside of edges that include the cages, the l was georeferenced, while the included area on polygon is defined as area of l (Ar$_l$). The georeferenced data by a GIS software (QGIS ver. 3.16.2), was mapped. Using the QGIS commands: dissolve buffer and buffer layer difference from land layer, for each *l* the potential environmental Impact marine Zones (IMZ$_i$) at distances i: 0, 25, 100, 200, 500, 1000 and 3000 m were also fixed on map. The command "*dissolve buffer*" returns the area j (cAreai,j) that is created by one or more cages arrays for each category i, ignoring the overlapping individually areas which originated by each cages array. The *l*'s that create the common cArea$_{3000,j}$ are the member of the spatial cluster j, while the maximum distance between *l*'s for membership is 6km.

In each *l*, the number, type (circular: NC and square: NS) and the dimensions (diameter and side for circular and square cages, respectively), were recorded.

For each *l*, the farming volume (V) was estimated as:

$V_l = \sum_{k=0}^{x} a_k^2 + \sum_{k1=0}^{x1} \pi \left(\frac{d_{k1}}{2}\right)^2 * dp$, where k is the square cages, k1 is the circular cages, x and x1 the number of square and circular cages, respectively, *a* the side of square and d the diameter of circular cage, respectively. *dp* is the cage's functional depth for farming, which defined as 10 m.

Mapping on the coordination reference system ETRS89LAEA - ETRS89 Lambert Azimutal Equal Area, was projected.

3. Results

In total, 72 fish farm cages arrays were recorded in the Greek coastline of central Ionian Sea for the period 2016-2017 (Figure 1). The mean (± SD) farming volume of array was 108.48 ± 90.80 x 10^3 m^3, the mean number of squared cages and circular cages were 7.16 ± 16.3 and 15.88 ± 11.99 cages, respectively. The total farming volume of arrays was 7,810.16 x 10^3 m^3, while the total number of cages was 1,671 (527 squared and 1,144 circular cages). The mean distance from coastal line was 212.3 ± 136 m. In total, 308 patches were recorded in eight spatial clusters. The number of patches according to i, ranged from 8 to 72 (at i: 3000 and 0, respectively). The total impacted area per i ranged from 1.19 km^2 at i=0 to 354.7 km^2 at i=3000 while in spatial cluster #15 recorded the greater impacted areas (0.80 km^2 at i=0 to 198.8 km^2 at i=3000), the number cages arrays (47 arrays, 65% of the study area), and farming volume 5,628.8 x10^3 m^3 (72% of the study area). The spatial cluster #6 and #30 with impacted areas ranged from 0.15 km^2 at i=0 to 44.5 km^2 at i=3000 and from 0.06 km^2 at i=0 to 29.4 km^2 at i=3000 with 11 and 8 cages arrays and farming volume of 748.2 x10^3 m^3 and 532.2 x10^3 m^3, respectively. The impacted areas of the remaining spatial clusters ranged between 0.01-0.04km^2 at i=0 to 12.7-21.3 km^2 at i=3000, embodied 1 to 2 cages arrays, with farming volume ranged from 114.9 x10^3 m^3 (#46) to 244.2 x10^3 m^3 (#63) (Table 1).

Table 1. Area and number of patches (in parenthesis) per spatial cluster of cages arrays (IDG). sV: total functional volume of IDG, Scg/Ccg: number of squared cages/cyclical cages, nAr: number of cages arrays, patches: number of patches.

	Area(patches) (km^2)											
IDG	**i:0**	**i:25**	**i:50**	**i:100**	**i:200**	**i:500**	**i:1000**	**i:3000**	**sV(x10^3 m^3)**	**Scg/Ccg**	**nAr**	**patches**
6	0.15(11)	0.33(8)	0.51(6)	0.88(6)	1.70(6)	4.62(4)	11.21(1)	44.49(1)	748.19	39/145	11	43
15	0.80(47)	1.60(40)	2.48(33)	4.41(26)	8.46(21)	21.2(15)	50.14(7)	198.8(1)	5628.76	230/768	47	190
30	0.06(8)	0.17(8)	0.30(7)	0.57(3)	1.14(3)	3.00(2)	7.408(2)	29.23(1)	531.96	66/94	8	34
33	0.03(1)	0.05(1)	0.08(1)	0.14(1)	0.23(1)	0.72(1)	1.828(1)	12.73(1)	127.42	0/24	1	8
37	0.04(1)	0.06(1)	0.09(1)	0.14(1)	0.24(1)	0.65(1)	1.748(1)	14.33(1)	197.99	101/20	1	8
45	0.04(1)	0.07(1)	0.09(1)	0.16(1)	0.30(1)	0.91(1)	2.605(1)	17.22(1)	217.53	60/27	1	8
46	0.01(2)	0.03(1)	0.05(1)	0.10(1)	0.22(1)	0.79(1)	2.548(1)	21.39(1)	114.90	31/20	2	9
63	0.03(1)	0.06(1)	0.09(1)	0.16(1)	0.31(1)	0.75(1)	2.182(1)	16.39(1)	244.23	0/46	1	8
Total	1.19(72)	2.4(61)	3.73(51)	6.59(40)	12.6(35)	32.7(26)	79.68(15)	354.7(8)	7810.98	527/1144	72	308

4. Discussion

In the present study the spatial distribution of the possible impact zones in the Greek central Ionian Sea coastline of fish farms, were estimated and mapped. It is obvious that this is very useful for the understanding of spatial distribution of farming impacts, the revealed spatial interactions for land usage, as well as the spatial planning of aquaculture activity.

The possible impacted areas from fish farming activity ranged from 1.19 km^2 (under the cages arrays: important impact on benthic communities: Borja *et al.* 2009; Karakassis *et al*. 2013; Tomassetti *et al.* 2016; Karakassis *et al.* 2000; Smith *et al*. 2005; Papageorgiou *et al.* 2010; Kalantzi *et al.* 2013) to 354.7 km^2 (zone 3000m around the arrays: impact on nekton-communities demography: Dempster *et al*. 2009; Uglem *et al.* 2009; Arechavala-Lopez *et al.* 2010, 2011, 2015; Gianoulaki *et al.* 2005) that created 8 spatial clusters (Table 1).

The spatial pattern of impacts could be attributed to the legal frame of aquaculture. The Greek legislation (Decision 31722/4−11−2011, OGG 2505/B/2011) has given detailed definition as to the fish farm unit (owning entity), the park (leased marine area about 10-100 x10^4 m^2 in which installed cages) and the arrays of cages. The distance among the parks of a unit can be between 100 – 250 m and among the units higher than 500 m. Moreover, the allowing carrying capacity (related by functional volume) of a specific location (in practice the leased area) is controlled by several parameters (i.e currents' velocity, geomorphology due to openness/exposure of location to open sea, bathymetry and distance of farm from shoreline) (121570/1866/12-6-2009 common newsletter of Ministry Environmental, spatial planning and Ministry Rural development and foods of Greece; Karakassis *et al.* 2013). The above legal guidelines have lead to a spatial pattern of cages arrays establishment where the short-distance zones (i<50) is impacted absolutely by one cages array, while the mid-distance (i:50-500) and long-distance zones (i>500) are impacted by cages array of parks of same unit and by different units, respectively.

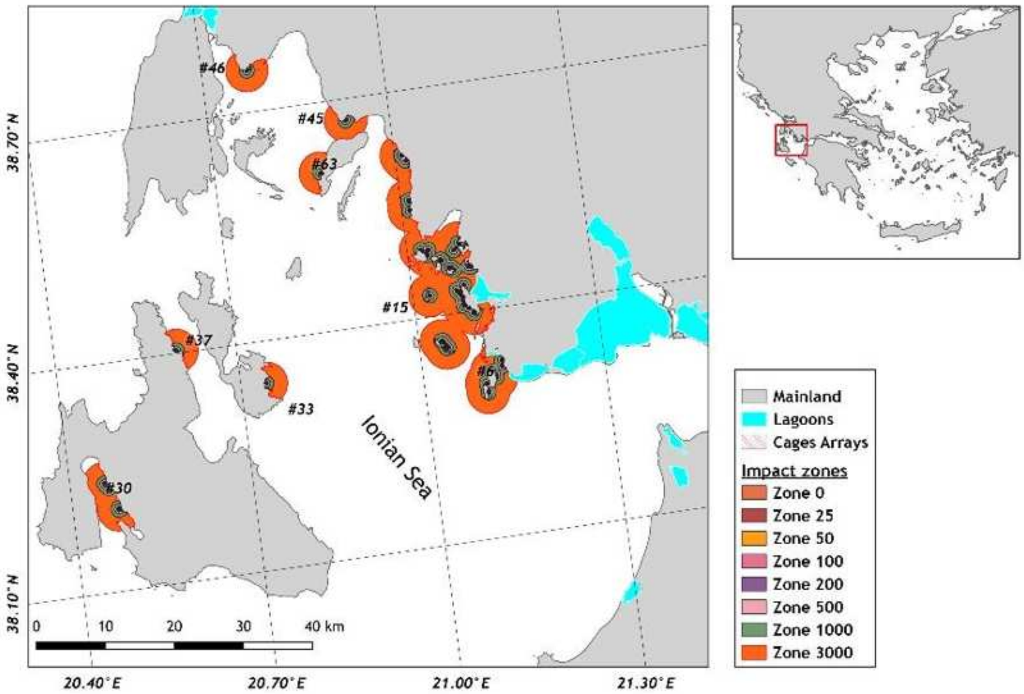

Figure 1. Spatial distribution of estimated impact zones of fish farm cages arrays in central Ionian Sea.

The longest-distance zone (i=3000m) seems that it consists of a concentration site for wild fishes (Machias *et al.* 2004). It is characterized, by the sites in proximity of cages arrays, as abundance hot spot of wild fish (Dempster *et al.* 2002) while the distances between cages arrays of zone offer a connectivity among arrays (Dempster *et al.* 2002, Giannoulaki *et al.* 2005). Locations with high clustering of farms showed notable increase of small-scale fishery production (Machias *et al.* 2006). The fact that these zones belong to the coastal zone of regions with small scale fishery (Tzanatos *et al.* 2005) supports a possible important conflict among these two activities. Finally, the presence of extensive fish farming activity (spatial groups 6# & #15) in the vicinity of Mesolonghi Etoliko lagoon (protected habitat: Natura 2000) has played a crucial part not only in lagoonal ichthyofauna biodiversity changes (Dimitriou *et al.* 2007) but also in the reduction of fishery production of lagoon (Katselis *et al.* 2010, 2013), thus, affecting the functions of the lagoon habitat.

However, a number of factors control the accuracy of the shape and size of impact zones: a) The hydrodynamic conditions of each location play key role to the dispersion of waste (Modica *et al.* 2006, Sarà 2006; Sarà *et al.* 2006, Macleod *et al.* 2007, Borja *et al.* 2009), b) In the present study the impact zones are estimated as a projection on the sea surface so the actual impacted area (especially that referred on the substrate: i=0 up i=500m) of the bottom is expected to be greater than estimated due to the deformations of bottom terrain with small variations in bathymetry resulting in significant changes in sedimentation pattern (Jusup *et al.* 2007), c) Changes of cages array location (relocation, add or remove) can change the impact zones sizes.

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References

Ågren J.J., Hänninen O. (1993). Effects of cooking on the fatty acids of three freshwater fish species. *Food Chemistry*, *46*(4), 377-382.

Arechavala-Lopez P., Uglem I., Sanchez-Jerez P., Fernandez-Jover D., Bayle-Sempere J.T., Nilsen R. (2010). Movements of grey mullet *Liza aurata* and *Chelon labrosus* associated with coastal fish farms in the western Mediterranean Sea. *Aquaculture Environment Interactions*, *1*(2), 127-136.

Arechavala-Lopez P., Sanchez-Jerez P., Bayle-Sempere J., Fernandez-Jover D., Martinez-Rubio L., Lopez-Jimenez J.A., Martinez-Lopez, F. J. (2011). Direct interaction between wild fish aggregations at fish farms and fisheries activity at fishing grounds: a case study with *Boops boops*. *Aquaculture Research*, *42*(7), 996-1010.

Arechavala-Lopez P., Izquierdo-Gomez D., Uglem I., Sanchez-Jerez P. (2015). Aggregations of bluefish *Pomatomus saltatrix* (L.) at Mediterranean coastal fish farms: seasonal presence, daily patterns and influence of farming activity. *Environmental Biology of Fishes*, *98*(2), 499-510.

Borja Á., Rodríguez J.G., Black K., Bodoy A., Emblow C., Fernandes T.F., Forte J., Karakassis I., Muxika I., Nickell T.D., Papageorgiou N., Pravoni F., Sebastou K., Tomassetti P., Angel D. (2009). Assessing the suitability of a range of benthic indices in the evaluation of environmental impact of fin and shellfish aquaculture located in sites across Europe. *Aquaculture*, *293*(3-4), 231-240.

Dempster T., Sanchez-Jerez P., Bayle-Sempere J.T., Giménez-Casalduero F., Valle C. (2002). Attraction of wild fish to sea-cage fish farms in the south-western Mediterranean Sea: spatial and short-term temporal variability. *Marine Ecology Progress Series*, *242*, 237-252.

Dempster T., Uglem I., Sanchez-Jerez P., Fernandez-Jover D., Bayle-Sempere J., Nilsen R., Bjørn P.A. (2009). Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. *Marine Ecology Progress Series*, *385*, 1-14.

Dimitriou E., Katselis G., Moutopoulos D., Akovitiotis C., Koutsikopoulos C. (2007). Possible influence of reared gilthead sea bream (*Sparus aurata,* L.) on wild stocks in the area of the Messolonghi lagoon (Ionian Sea, Greece). *Aquaculture Research,* 38:398-408.

Fu Y., Deng J., Wang H., Comber A., Yang W., Wu W., You S., Lin Y., Wang K. (2021). A new satellite-derived dataset for marine aquaculture areas in China's coastal region. *Earth System Science Data*, *13*(5), 1829-1842.

Giannoulaki M., Machias A., Somarakis S., Karakassis I. (2005). Wild fish spatial structure in response to presence of fish farms. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom*, *85*(5), 1271.

Grigorakis K., Rigos G. (2011). Aquaculture effects on environmental and public welfare–the case of Mediterranean mariculture. *Chemosphere*, *85*(6), 899-919.

Hanson G.D., Rauniyar G.P., Herrmann R.O. (1994). Using consumer profiles to increase the US market for seafood: implications for aquaculture. *Aquaculture*, *127*(4), 303-316.

Jusup M, Geček S, Legović T. (2007). Impact of aquacultures on the marine ecosystem: modelling benthic carbon loading over variable depth. Ecological Modelling 200: 459–466.

Kalantzi I., Shimmield T.M., Pergantis S.A., Papageorgiou N., Black K.D., Karakassis I. (2013). Heavy metals, trace elements and sediment geochemistry at four Mediterranean fish farms. *Science of the Total Environment*, *444*, 128-137.

Karakassis I., Tsapakis M., Hatziyanni E., Papadopoulou K.N., Plaiti W. (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES Journal of Marine Science*, *57*(5), 1462-1471.

Karakassis I., Papageorgiou N., Kalantzi I., Sevastou K., Koutsikopoulos C. (2013). Adaptation of fish farming production to the environmental characteristics of the receiving marine ecosystems: A proxy to carrying capacity. Aquaculture,408–409, 184-190.

Katselis G., Koukou K., Moutopoulos D. (2010). Yield per recruit and spawning stock biomass models for the management of four Mugilidae species in Mesolonghi–Aitoliko lagoon (W. Greece). *International Aquatic Research*, *2*(3), 155-162.

Katselis G.N., Moutopoulos D.K., Dimitriou E.N., Koutsikopoulos C. (2013). Long-term changes of fisheries landings in enclosed gulf lagoons (Amvrakikos gulf, W Greece): Influences of fishing and other human impacts. *Estuarine, Coastal and Shelf Science*, *131*, 31-40.

Lall S.P. (1995). Macro and trace elements in fish and shellfish. *Fish and fishery products*, 187-213.

Machias A., Karakassis I., Labropoulou M., Somarakis S., Papadopoulou K.N., Papaconstantinou C. (2004). Changes in wild fish assemblages after the establishment of a fish farming zone in an oligotrophic marine ecosystem. *Estuarine, Coastal and Shelf Science*, *60*(4), 771-779.

Machias A., Giannoulaki M., Somarakis S., Maravelias C.D., Neofitou C., Koutsoubas D., Papadopoulou K.N., Karakassis I. (2006). Fish farming effects on local fisheries landings in oligotrophic seas. *Aquaculture*, *261*(2), 809-816.

Macleod C.K., Moltschaniwskyj N.A., Crawford C.M., Forbes S.E. (2007). Biological recovery from organic enrichment: some systems cope better than others. *Marine Ecology Progress Series*, *342*, 41-53.

Martinez-Porchas M., Martinez-Cordova L.R. (2012). World aquaculture: environmental impacts and troubleshooting alternatives. *The Scientific World Journal*, *2012*.

Modica A., Scilipoti D., La Torre R., Manganaro A., Sara G. (2006). The effect of mariculture facilities on biochemical features of suspended organic matter (southern Tyrrhenian, Mediterranean). *Estuarine, Coastal and Shelf Science*, *66*(1-2), 177-184.

Ottinger M., Clauss K., Kuenzer C. (2018). Opportunities and challenges for the estimation of aquaculture production based on earth observation data. *Remote Sensing*, *10*(7), 1076.

Papageorgiou N., Kalantzi I., Karakassis I. (2010). Effects of fish farming on the biological and geochemical properties of muddy and sandy sediments in the Mediterranean Sea. *Marine environmental research*, *69*(5), 326-336.

Prasad K.A., Ottinger M., Wei C., Leinenkugel P. (2019). Assessment of coastal aquaculture for India from Sentinel-1 SAR time series. *Remote Sensing*, *11*(3), 357.

Sarà G. (2006). Hydrodynamic effects on the origin and quality of organic matter for bivalves: an integrated isotopic, biochemical and transplant study. *Marine ecology progress series*, *328*, 65-73.

Sarà G., Scilipoti D., Milazzo M., Modica A. (2006). Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics. *Marine Ecology Progress Series*, *313*, 261-270.

Savini D., Occhipinti–Ambrogi A., Marchini A., Tricarico E., Gherardi F., Olenin S., Gollasch S. (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, *26*, 1-7.

Smith J.N., Yeats P.A., Milligan, T.G. (2005). Sediment geochronologies for fish farm contaminants in Lime Kiln Bay, Bay of Fundy. In *Environmental effects of marine finfish aquaculture* (pp. 221-238). Springer, Berlin, Heidelberg.

Steiner-Asiedu M., Julshamn K., Øyvind L. (1991). Effect of Local Processing Methods (cooking, drying and smoking) on Three Fish Species from Ghana: Part I. Proximate Composition, Fatty Acids, Minerals, Trace Elements and Vitamins. *Food Chemistry*, 40, 309-321.

Telesca L., Belluscio A., Criscoli A., Ardizzone G., Apostolaki E.T., Fraschetti S., Gristina M., Knittweis L., Martin C.S., Pergent G., Alagna A., Badalamenti F., Garofalo G., Gerakaris V., Pace M.L., Pergent-Martini C., Salomidi M. (2015). Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. *Scientific Reports*, *5*(1), 1-14.

Tomassetti P., Gennaro P., Lattanzi L., Mercatali I., Persia E., Vani D., Porrello S. (2016). Benthic community response to sediment organic enrichment by Mediterranean fish farms: Case studies. *Aquaculture*, *450*, 262-272.

Trujillo P., Piroddi C., Jacquet J. (2012). Fish farms at sea: the ground truth from Google Earth. *PLoS One*, *7*(2), e30546.

Tzanatos E., Dimitriou E., Katselis G., Georgiadis M., Koutsikopoulos C. (2005). Composition, temporal dynamics and regional characteristics of small-scale fisheries in Greece. *Fisheries Research*, *73*(1-2), 147-158.

Uglem I., Dempster T., Bjørn P.A., Sanchez-Jerez P., Økland, F. (2009). High connectivity of salmon farms revealed by aggregation, residence and repeated movements of wild fish among farms. *Marine Ecology Progress Series*, *384*, 251-260.

Xia Z., Guo X., Chen R. (2020). Automatic extraction of aquaculture ponds based on Google Earth Engine. *Ocean & Coastal Management*, *198*, 105348.

USE OF FUZZY COGNITIVE MAPS AS A DECISION-MAKING TOOL TO FISHERIES MANAGEMENT

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Abstract
Fisheries management in relation to modifying or adopting new policies requires an understanding of all factors affecting and integrating fishers' expertise as well as regulating the social ecosystem in which all above act. Blending the opinion of experts, competent authorities, scientists, and industry in a participatory-modeling approach can be utilized by efficient soft computing methodologies in order to provide managers with the means to perform scenario analysis and decision making. In this paper we demonstrate the innovative utilization of Fuzzy Cognitive Maps (FCM) in evaluating the factors that affect the fisheries freight outcome in relation to catchment volumes, vessel count and common European policies. The scenario analysis experimental verification of the proposed model is based on European based bibliographic data for the Greek fisheries and for the years 2001-2009. Our novel modeling approach allows open-ended improving assessments to fit in other similar decision-making problems.
Keywords: *Fuzzy Cognitive Maps, Fisheries, Scenario Analysis, Decision Making*
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1. Introduction

For the management of fishing policies for all European countries, EU's common fisheries policy (CFP) focuses on: (a) preservation and sustainable management of aquaculture, (b) regulation of each country fishing fleet capacity, (c) structuring of the markets whose aim is to ensure the adequacy of supply and demand for the benefit of producers and consumers of aquatic products and (d) support of fisheries and aquaculture industries in the process of adapting equipment and organizing it to the requirements of today's European market (Eur-Lex 2021). At the same time, local authorities are allowed to implement and alter these policies to adopt to the idiosyncrasies of specific regions (Moutopoulos & Koutsikopoulos 2014). According to these authors, local restrictive measures, and other dynamic factors (fuel price, environmental and climate change, spatiotemporal structure of fisheries fleet, etc.) must be encountered on a dynamic basis scenario analysis to determine policy making. Furthermore, the great Greek economic crisis of the previous decade has deeply affected the fishery sector in conjunction with the large number of small enterprises and artisanal vessels as well as the unreported income or landings of the fishing industry (Machias *et al*., 2016).

Greece experienced overfishing, thus drastic measures have been taken to diminish this ecosystem pressure. In consequence the number of fishing fleet was drastically reduced with the near extinction of wooden vessels. The same affect was experienced with the freight counts of almost all major fishing Greek sites and this phenomenon was similar in most countries of the Mediterranean European countries. To understand all the factors that lead to the sustainability of aquaculture and establish the interlinking and the interrelations of these factors we need a common framework (methodology) that organizes this type of interoperability and deduces results of improvement or deterioration of concepts of interest (Ostrom, 2009). We propose a model called Fuzzy Cognitive Map (FCM), that captures the dynamics of processes and factors that influence various ecosystem services and interventions thus, providing the modeler or policy maker with a valuable tool to address problems of such nature (Collins *et al*., 2011). Addressing the fishing industry in Greece we are looking on how the freight volumes are affected by: (a) the technical measures aimed at modifying fishing gear, (b) the control of fishing effort and capacity through competent authority intervention in the fishing fleet (quantitative and qualitative), and (c) the intervention in spatial and temporal access within fisheries.

We create an FCM model that blends the aforementioned factors/concepts with other socio-economic factors imposed by field experts and utilizes bibliographic data of the Greek fishing industry to make a decision support system for policy making. Such models have been proven successful in other research disciplines that attempt to simulate social-ecological ecosystems in a holistic approach (Kokkinos & Karayannis 2020; Kokkinos *et al*. 2019). In the following sections we provide the materials and methods analyzed, a thorough description of the FCM model and its structural characteristics, the construction of the FCM for the problem at hand using the MentalModeller simulation software (MentalModeller 2021) and the scenario analysis performed using the bibliographic data on the fishing freight counts.

2.Materials and Methods

2.1 FCM Model

FCMs are neural network type graphs (Kosko 1986, 1987) with nodes C_i of the graph representing concepts and edges representing concept bidirectional weighted interrelations (causalities). When linguistic variables are used to determine concept causalities, proper defuzzification converts them into a normalized numbers within the range of [-1..1] to evaluate their degree. When activation/transformation functions are imposed on the FCM, it also affects the concepts' representation as a value A_i. FCMs can be represented as weighted adjacency matrices which allow inference processing similar to neural nets. Causalities in the range (0, 1] for concepts C_i, C_j indicate an increase of concept C_j when C_i increases and as a decrease of concept C_j when C_i decreases respectively. On the other hand, causalities the range [-1, 0) indicate an increase of concept C_j when C_i decreases and as a decrease of concept C_j when C_i increases respectively. When inference is actuated, the A_i of each C_i is computed in each inference step counting in the overall influence of all concepts C_j to C_i. Most popular inference rules are the following:

Kosko's inference $$\boldsymbol{A_i(k+1) = f\left(\sum_{j=1, j\neq i}^{N} w_{ji} \times A_j(k)\right)} \quad (1)$$

Modified Kosko's inference $$A_i(k+1) = f\left(A_i(k) + \sum_{j=1, j\neq i}^{N} w_{ji} \times A_j(k)\right) \quad (2)$$

Rescale inference $$A_i(k+1) = f\left((2 \times A_i(k) - 1) + \sum_{j=1, j\neq i}^{N} w_{ji} \times (2 \times A_j(k) - 1)\right) \quad (3)$$

Inference with lag $$A_i(k+1) = f\left(A_i(k) + \sum_{j=1, j\neq i}^{N} w_{ji} \times A_j(k - lag_{i,j})\right) \quad (4)$$

Furthermore, the threshold function $f(.)$ is used for the output of the inference for each concept in each step and can be bivalent (Eq. 5), trivalent (Eq. 6), sigmoid (Eq. 7), or hyperbolic (Eq. 8).

$$\boldsymbol{f(x) = \begin{cases} 1 & x > 0 \\ 0 & x \leq 0 \end{cases}} \quad (5)$$

$$\boldsymbol{f(x) = \begin{cases} 1 & x > 0 \\ 0 & x = 0 \\ -1 & x < 0 \end{cases}} \quad (6)$$

$$\boldsymbol{f(x) = \frac{1}{1+e^{-\lambda x}}} \quad (7)$$

$$\boldsymbol{f(x) = tanh(\lambda \times x)} \quad (8)$$

For the sigmoid threshold we must make sure that $A_i \in [0, 1]$ for each concept. For that reason, we can use the hyperbolic tangent function if the values of the concepts correspond to negative causalities.

2.2 Greek Aquaculture and Shipping Fleet Use Case

The FCM concepts that participated on the following FCM model were derived both from a literature search on fisheries from different perspectives (e.g., Machias et al., 2016; Kapantagakis et al., 2016), as well as from statistics directly related to statistical representation of fisheries (source Hellenic Statistical Service).
The data relate to the application of additional policies and measures at the EC level to deal with the overfishing problem thus to alleviate the fishing pressure to the affected stocks. Therefore, we investigate how policy making affects the loss of fishing fleet capacity and finally the freight count and the total trading value. All FCM concepts are

mentioned and analyzed in (Kapantagakis et al., 2016). Two experts were involved to provide insights in constructing interrelations between concepts and give initial causality measures. Network characteristics are tabulated in Table 1.

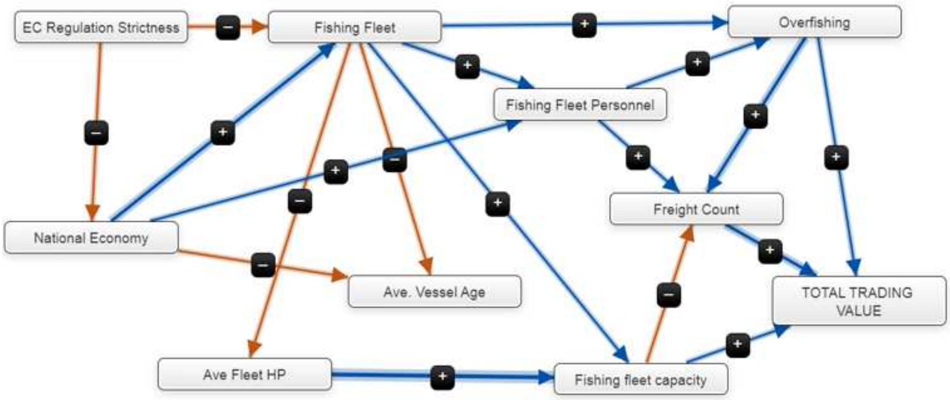

Figure 1. FCM Modeling the effect of EC policies to the Fishing Fleet Capacity, the freight count and the total trading value.

Table 1. FCM Model Characteristics

Component	Indegree	Outdegree	Centrality	Type
Fishing Fleet	1.03	1.59	2.53	ordinary
Fishing Fleet Personnel	0.81	0.56	1.37	ordinary
Ave. Vessel Age	0.59	0.12	0.59	receiver
Ave Fleet HP	0.42	0.87	1.29	ordinary
Fishing fleet capacity	1.16	0.71	1.81	ordinary
Freight Count	1.22	0.68	1.93	ordinary
TOTAL TRADING VALUE	1.27	0.34	1.27	receiver
Overfishing	0.63	0.92	1.55	ordinary
National Economy	0.34	1.37	1.71	ordinary
EC Regulation strictness	0.17	0.82	0.86	driver

3. Results

For the resulted FCM we run several simulations to explore the nature of the concept inter-causalities. We focused on the FCM convergence using the clamping process (Kosko, 1986). Special attention is how driver concepts affect the receiver concepts. We first consider the steady state of the FCM as the starting point and then we run the best and the worst scenario compared to the steady state. For the worst scenario all driver concepts get the value of 0.1and oppositely in the best scenario the value of 1. Figure 2 shows the worst-case scenario results with a decrease of 5% in total trading value receiver while fishing fleet and freight count get a significant decrease of 7% each.
In relation to the best-case scenario, we set the driver concepts to value 1 and we see a significant increase on the total trading value by 5% while at the same time we se also increase on the fishing fleet personnel, overfishing, fishing fleet capacity and freight count by 5%, 3%, 2% and 1% respectively. Furthermore, we experience no observed fishing fleet increase. However, this can be explained due to the fact that the EC policy in the last decade drove to the tremendous decrease of small family-business vessels.

At the same time, we observe drastic increase on the fishing personnel indicating that either we had a simultaneous increase of large-scale vessels or, the remained vessels overexploited the Greek seas using more personnel and performing overfishing. This resulted into the trading value increase. Figure 3 shows the best-case concept inter-causalities to support this evidence. Note that the FCM results are in accordance with the real statistics referred in (Kapantagakis et al., 2016) thus, verifying the value of the FCM models a decision support tools to use in policy making for aquaculture.

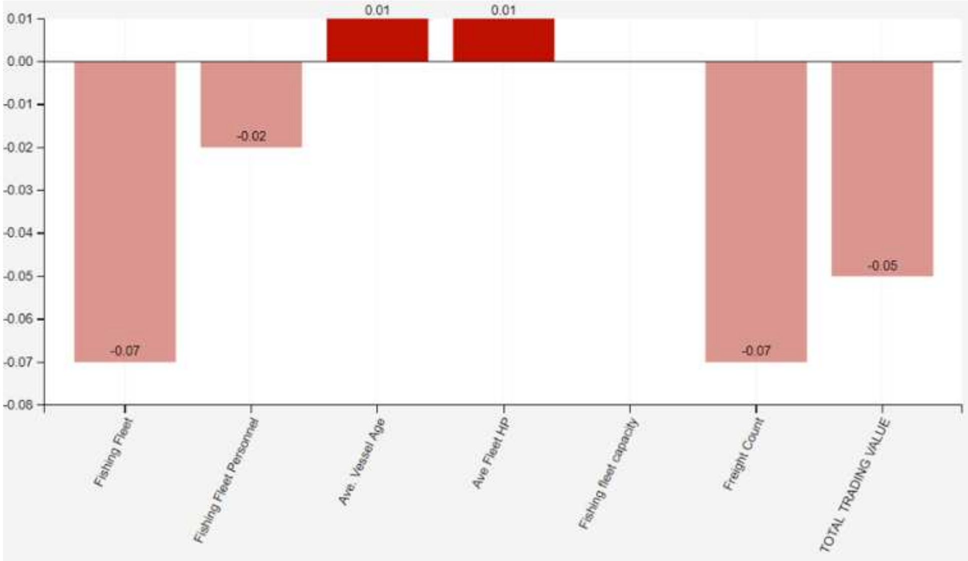

Figure 2 Worst case scenario analysis of the FCM.

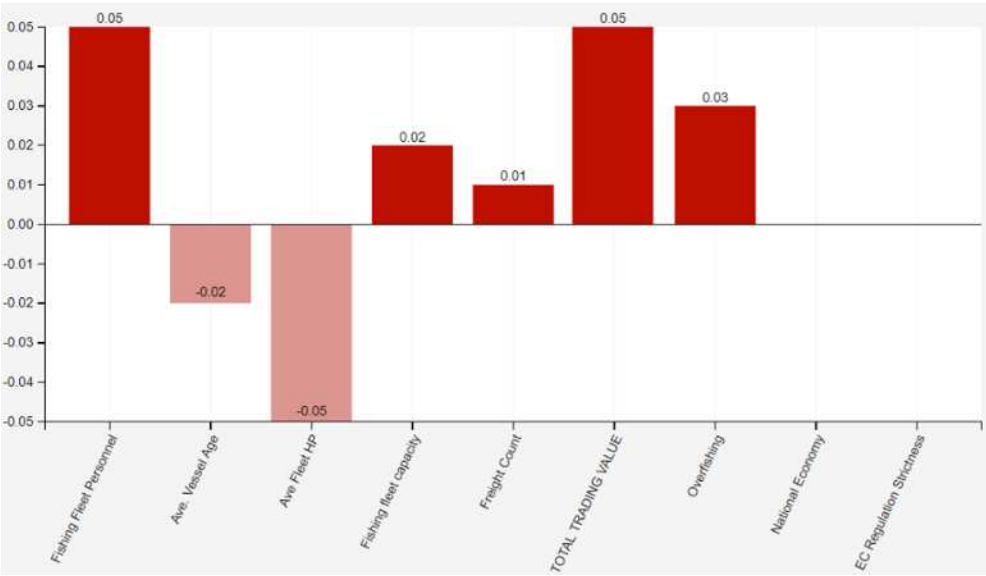

Figure 3 Best case scenario analysis of the FCM.

4. Discussion and Conclusions

In this paper we use a fuzzy model of soft computing to create a decision support system for the policy making regarding Greek aquaculture. Points of attention are the effects of EC regulations to the fishing fleet count; freight counts and total trading value. The construction of the FCM model was based on academia experts that indicated the type of inter-causalities between the concepts involved. Concepts as well as the inter-causality initial normalized values within the range of [-1..1] have been derived by bibliographic data related to the consequences of the politics and authority policies to the withdrawal of fishing vessels.

The model was evaluated compared to its steady state of inference with the worst and best-case scenario of the relation between the driver and the receiver concepts. At the same time (especially for the best-case analysis) intermediate concept trends were studied to explain the hidden effect of the EC policies to the fishing fleet counts. Such intermediate concepts included the fishing fleet personnel and the average fleet capacity. The analysis results are in accordance to real statistics for the Greek case making the model an effective decision support tool for decision making in aquaculture related policies.

References

Collins S.L., Carpenter S.R., Swinton S.M., Orenstein D.E., Childers D.L., Gragson T.L., Grimm N.B., Grove J.M., Harlan S.L., Kaye J.P., Knapp A.K. (2011). An integrated conceptual framework for long-term social–ecological research. Frontiers in Ecology and the Environment, 9(6), 351-357.

Eur-Lex (2021.) Summaries of EU Legislation: Common Fisheries Policy. https://eur-lex.europa.eu/summary/glossary/fisheries.html [Last accessed May 21st, 2021].

Kapantagakis A., Laurijsen J., Palikara E., Lioudakis L. (2016). Consequences of the political withdrawal of fishing vessels. Institute of Marine Biological Resources - Hellenic Centre for Marine Research presented in the Conference Coastal cultures in times of change, Copenhagen Denmark 2016.

Kokkinos K., Karayannis V. (2020). Supportiveness of low-carbon energy technology policy using fuzzy multicriteria decision-making methodologies. Mathematics-MDPI, 8(7), p.1178.

Kokkinos K., Karayannis V., Lakioti E., Moustakas K. (2019). Exploring social determinants of municipal solid waste management: survey processing with fuzzy logic and self-organized maps. Environmental Science and Pollution Research, 26(35), 35288-35304.

Kosko B. (1986). Fuzzy cognitive maps. International Journal of Man-Machine Studies, 24(1), 65-75.

Kosko B. (1987). Adaptive inference in fuzzy knowledge networks. Proceedings of the First IEEE International Conference on Neural Networks, vol. II, San Diego, California, 261-268.

Machias A., Tsagarakis K., Matsaganis M. (2016). Greek fisheries and the economic crisis: structural analogies. Ethics in Science and Environmental Politics, 16 (1), 19-23.

Mental Modeler Software, 2021. www.mentamodeler.org, Last accessed 17th of May, 2021.

Moutopoulos D.K., Koutsikopoulos C. (2014). Fishing strange data in national fisheries statistics of Greece. Marine Policy, 48, 114-122.

Ostrom E. (2009). A general framework for analyzing sustainability of social-ecological systems. Science, 325(5939), 419-422.

SALMON MATURITY CLASSIFICATION BASED ON BAYESIAN NETWORKS AND SUPPORT VECTOR MACHINES METHODOLOGIES

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Abstract
In recent years, Machine Learning (ML) methodologies are extensively applied in aquaculture and have proved to be a versatile modeling approach to realize digital fish farming. In this paper we illustrate a comparative analysis of two ML methodologies (Bayesian Networks and Support Vector Machines) on classifying maturity status of salmon species. Both methodologies have been applied to the same datasets which span to a variety of locations in the north Atlantic regions and to a variety of species (Chinook Chum, Coho Pink, and Sockeye). Apart from the location (longitude, latitude) data also date, species, length in frozen state, length in wet state, weight in wet state, and number of parasitic sea lice found on fish body are used as independent dimensions to determine fish maturity in a binary classifier methodology. Both methods reach adequate performance levels in general, however, Bayesian Networks consistently outperform Support Vector Machines in all datasets used indicating preference in usage.
Keywords: *Salmon, Classification, Maturity, Bayesian Networks, Support Vector Machines*
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1. Introduction

In recent years, Data Science (DS) research in conjunction with Data Mining (DM) and Machine Learning (ML) has advanced and applied in interdisciplinary thematic areas aiming to provide automatic precise clustering and classification methodologies. This can replace man hours with automation on the aforementioned applications reaching accuracy rates on the 95% which is acceptable by most appearances of human visual estimation procedures. Currently, salmon maturity is determined indirectly by first providing an identification of its sex. However, such a procedure is actuated mechanically by human visual examination. This is meticulous work, and rapid and exact determination necessitates the use of skilled employees.

To address this issue, we try to provide an automated salmon maturity categorization system (Mature/Adult: A, Juvenile: J) based on two soft computing approaches namely Bayesian Networks, (BN) and Support Vector Machines (SVM) (Masoum *et al*. 2007; Xu *et al*. 2017). Bayesian classifiers are easy to construct probabilistic classifiers based on evidence and assumptions of independent variables/dimensions and the application of the Bayes theorem. Using also SVM, we identify significant variables (dimensions) to be incorporated in a multivariate model fitting approach (Hosmer & Lemeshow 2000). The fitting is achieved via a forward model selection approach, constantly updating an initially null model by adding dimensions which have been discovered in the previous fitting step. This system starts with a constant probability of point occupancy and using the Akaike's information criterion (AIC) score and the maximum rescaled R^2 criteria succeeds goodness-of-fit statistics (Hellmair *et al*. 2018).

We treat the problem as a two-class classic multi-variate categorization problem affected by several parameters such as: (a) the catching location, (longitude, latitude), (b) species, (c) weight at wet and frozen state, (d) length at wet and frozen state, and (e) number of parasitic sea lice found on fish body. Note that the set of parameters used is totally different than the shape of the head, the adipose fin and the caudal fin which is usually chosen to determine the sex and the maturity level of the fish by visual inspection. This antithesis along with the promising results of our methodologies when compared with the real (visual inspection evaluations) makes this research unique and worth to further investigate.

In the following sections we provide the materials and methods analyzed, a thorough description of the data and the catching region, results and performances of the two classification methodologies as well as Confusion Matrix (CM) and Root Mean Square Error (RMSE) statistics on these performance evaluations.

2. Materials and Methods

2.1. Data Acquisition

Under the consideration that salmon survival and growth are linked to oceanic variability, for our research we used publicly available National Marine Fisheries Service studies as they appear in (Wertheimer *et al*. 2009).

This data was intended to be used for a retrospective analysis of capture per unit effort vs oceanographic and prey variables in order to identify and indicate what factors influence the distribution of chinook, pink, chum, coho, and sockeye salmon in the region of the Gulf of Alaska that is bounded by Lat. (54.29 – 57.95) and Lon (-151.62 – -157.43). The sampling was performed during Oct. 19th, 2004 and Nov. 8th, 2004 and refers to the aforementioned salmon species. More specifically the data refers to 31 catching locations spanning on the virtual rectangle bounded by the longitude and latitude values previously. Out of the 11659 fish items in the data set there were integrated data values only for only 2109 fish items with the rest to have missing data. Furthermore, data were split into different files for each catching area with each table including values for: (a) length of the fish at frozen state and wet state, (b) weight of the fish at frozen and wet state, (c) sex (d) maturity indication (e) sea lice count found. All fishes were sorted by species and counted. Data was collected from the BCO-DHO project repository as the following Figure 1 shows:

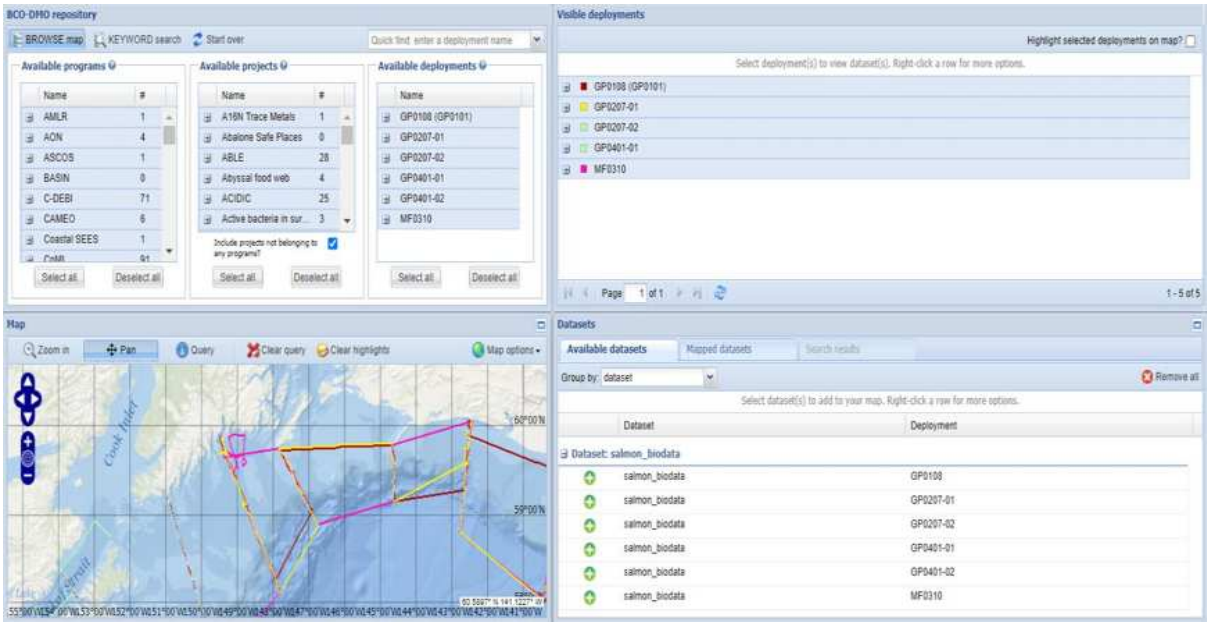

Figure 1. Form of data collected from the BCO-DHO project repository.

2.2. Bayesian Networks

For the Bayesian classifier we used multiple growth model fitting to the data and compared the different fitting methods with the AIC as in (Katsanevakis & Maravelias 2008). More specifically we used the von Bertalanffy growth model (Smart *et al*. 2016):

$$L_a = L_\infty - (L_\infty - L_0)e^{-ka} \quad (1)$$

the Logistic growth model (Smart et al., 2016):

$$L_a = (L_\infty L_0 e^{ka})/(L_\infty + L_0(e^{ka} - 1)) \quad (2)$$

and the Gompertz growth model:

$$L_a = L_0 e^{(\log(\frac{L_\infty}{L_0})(1-e^{-ka})} \quad (3)$$

where L_a is the fish length at age-a, L_∞ is the asymptotic length, L_0 the fish length and k is the growth completion parameter. The main results of this approach are posterior distributions for each one of the parameters used.

As the model grows starting using the fish lengths, the fish weights and the parasitic sea lice we reach the likelihood distribution using the Bayes' theorem:

$$\Pr(\theta|Y) = \frac{\Pr(Y|\theta)\Pr(\theta)}{\int \Pr(Y|\theta)\Pr(\theta)\,d(\theta)} \tag{4}$$

with Pr denoting the probability, Y is the fish length at certain age and θ the synthetic function of the rest of the aforementioned parameters.

2.3. Support Vector Machines

The SVM methodology separates the variable/dimension hypersurface by applying the following steps: (a) using nonlinear isomorphisms it transforms the problem into a one of higher dimensionality feature space having a priori (kernel) and (b) it makes a marginal hyperplane based on the hyperspace of step (a) while maximizing the distance between the closest feature vectors that correspond to the different classes in the classification scheme.

The method aims to maximize the margin M between the proposed classes i.e., making clear their distinction. This can be achieved if $M = 2/\|w\|$ where w is the weight vector which optimizes (minimizes) the classification error by solving the following optimization problem

$$\min\left\{0.5\|w\|^2 + C\sum_{i=1}^{N}\delta_i\right\} \tag{5}$$

subject to the constraints

$$y_i(< w^T.x > +b) \geq 1 - \delta_i \quad \text{for each class} \quad y_i = \pm 1, \text{ and } \delta_i > 0\ \forall\ i \tag{6}$$

3. Results

To appraise the performance of the proposed ML methodologies we used a dataset of salmon metrics of 2109 fish items as discussed in the Data Acquisition section. This dataset was pre-processed to make sure that no null-values and no outliers exist by just eliminating such cases. 70% of the total data was used for training and the rest of 30% for testing and evaluation of the algorithm correction. For the testing data, we subtracted the maturity values and we let the two algorithms project the fish maturity status.

In this multi-variate system additional variables such as the average fish weight and length and the average sea lice count participated in the process along with the corresponding fish weight and length in the frozen state and the sex of the fish. For each of the five salmon species both algorithms performed satisfactorily with precision rating between 81% to ~92%. Results for the Bayesian case and the SVM case are presented in Tables 1 and 2 respectively.

Table 1. Bayesian Classifier Performance.

	Chinook	**Chum**	**Pink**	**Coho**	**Sockeye**
Ave. Length (mm)	572.1	439.3	612.4	393.8	458.9
Ave. Weight (gr)	283.3	221.7	375.2	198.2	239.1
Ave. Lice Count	2.671	2.131	2.031	1.937	1.241
Accuracy	0.874	0.835	0.931	0.912	0.937
Precision	0.862	0.811	0.910	0.894	0.918
False Positive Rate	8.21%	8.09%	7.23%	8.03%	7.02%
F1-Score	0.824	0.789	0.892	0.862	0.897
MSRE	0.883	0.862	0.925	0.946	0.931

For both cases the accuracy, the precision, the false positive rate in the confusion matrix, the F1-Score and finally the RMSE was calculated. As shown in the tables there are three major findings explained further: (a) both methodologies performed with adequate and sufficient accuracies ranging in the area of 0.831- 0.922 for the SVM of 0.831- 0.922 for the SVM and 0.835 – 0.937 for the Bayesian case respectively.

3

Table 2. SVM Classifier Performance.

	Chinook	Chum	Pink	Coho	Sockeye
Ave. Length (mm)	572.1	439.3	612.4	393.8	458.9
Ave. Weight (gr)	283.3	221.7	375.2	198.2	239.1
Ave. Lice Count	2.671	2.131	2.031	1.937	1.241
Accuracy	0.863	0.831	0.907	0.922	0.892
Precision	0.856	0.828	0.915	0.848	0.882
False Positive Rate	9.36%	8.78%	7.44%	9.36%	10.12%
F1-Score	0.784	0.769	0.713	0.881	0.812
MSRE	0.793	0.819	0.895	0.906	0.883

Note also that same pattern holds for the precision, the false positive rate and the F1-Score between the two methods indicating that, the Bayesian approach constantly performed better than the SVM in almost all salmon species tests. (b) Few performance exceptions in favor of the SVM case can be explained due to the inability of the von Bertalanffy and the Gompertz growth model to capture and render the association between the fish length, fish weight and maturity. (c) the results in relation to the RMSE ranging from 0.793 to 0.946 are satisfactory when compared with similar categorical data forecasting methods in similar forecasting problems.

4. Discussion and Conclusions

This paper contributed to research by formulating a bifold ML classification methodology using a Bayesian network and a SVM to recognize maturity status of salmon species based on fish size and sea lice count data. For this purpose, five features; namely frozen and wet state weight, frozen and wet state length and sea lice count were extracted from 2109 salmon species fish items caught in the Gulf of Alaska. Data were split into a training and a testing set using the 70%-30% splitting rule to form the basis for the classification process. The evaluation results exhibited the potential of both algorithms for reliable forecasting of the fish maturity status avoiding thus man-kind visual inspection. Both algorithms performed adequately with the Bayesian approach to slightly outmatch the SVM methods in almost all individual species datasets. However, the obtained recognition accuracy of around 80% in the worst cases reveals that there is still potential for improvement. Future research therefore will have as a primary aim to improve the aforementioned accuracy rates. This can be done possibly by providing an ensemble ML framework that will include a broader repertoire of classification algorithms accompanied with a primitive expert system to automatically perform decision making in relation to selecting the appropriate algorithm for specific datasets. We believe that such an approach may be proven valuable and cost effective in the automatic recognition of all fish species maturity status.

References

Hellmair M., Peterson M., Mulvey B., Young K., Montgomery J., Fuller, A. (2018). Physical characteristics influencing nearshore habitat use by juvenile Chinook salmon in the Sacramento River, California. North American Journal of Fisheries Management, 38(4), 959-970.

Hosmer D. W. Lemeshow Jr. S. (2000). Applied logistic regression. Wiley, New York.

Katsanevakis S, Maravelias CD. (2008). Modelling fish growth: multi-model inference as a better alternative to a priori using von Bertalanffy equation. Fish and Fisheries, 9(2):178–87.

Masoum S., Malabat C., Jalali-Heravi M., Guillou C., Rezzi S., Rutledge D.N. (2007). Application of support vector machines to 1 H NMR data of fish oils: methodology for the confirmation of wild and farmed salmon and their origins. Analytical and Bioanalytical Chemistry, 387(4), 1499-1510.

Smart J.J., Chin A., Tobin A.J., Simpfendorfer C.A. (2016). Multimodel approaches in shark and ray growth studies: strengths, weaknesses and the future. Fish and Fisheries, https://doi.org/10.1111/faf.12154

Wertheimer A., Cokelet E., Farley E., Moss J. (2009). Salmon length, weight, sex, stomach data from F/V Great Pacific, R/V Miller Freeman multiple cruises in the Coastal Gulf of Alaska, NE Pacific from 2001-2004 (NEP project). Biological and Chemical Oceanography Data Management Office (BCO-DMO). doi:10.1575/1912/bco-dmo.3106.

Xu J.L., Riccioli C., Sun, D.W. (2017). Comparison of hyperspectral imaging and computer vision for automatic differentiation of organically and conventionally farmed salmon. Journal of Food Engineering, 196, 170-182.

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THE NUTRITIONAL VALUE OF THE CAPRELLID *Caprella scaura* FROM FISH FARM CAGES: A POTENTIAL SOURCE OF MARINE ANIMAL PROTEINS AND LIPIDS IN FISH NUTRITION?

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Abstract
The total lipid and protein content of the invasive caprellid amphipod *Caprella scaura,* from fish farm cages in the Pagasitikos Gulf were measured. Proteins were the most abundant component (48-49%). Lipid content was relatively high and showed a seasonal fluctuation with much higher values in Spring and lower in Autumn. The presence of high levels of EPA and DHA fatty acids, together with the high lipid content, makes the species a potential candidate for use of these organisms in aquaculture.
Keywords: *fatty acids, integrated multi-trophic aquaculture, Kjeldahl, Gas chromatography*
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1. Introduction

The aquaculture sector is still on the search for suitable feedstuff alternatives to fishmeals and fish oils in aquafeeds. Although the search is focusing on feedstuffs from terrestrial sources, such as land animal proteins (Psofakis et al. 2020), insect proteins (Henry *et al.* 2015) and plant meals and oils (Bell & Waagbø 2008), still the marine aquatic environment could possibly provide credible and sustainable solutions for fishmeal and fishoil replacement. In this context, a promising alternative could be proved to be the marine amphipods of the Caprellidae Family (Woods 2009), such as *Caprella scaura*. This caprellid species is invasive in the Mediterranean Sea and is commonly found as biofouling in huge populations in the nets of farmed fish cages. Although its nutritional value has not been studied extensively, the species is known for its high protein content and richness in omega-3 fatty acids (Guerra-García *et al.* 2016). The aim of the present study was to determine the nutritional content, and its seasonal fluctuation, of the *Caprella scaura* collected from fish farm cages.

2. Material and Methods

Samples of *C. scaura* were collected in March and October 2019, from the bryozoan *Bugula neritina* attached to mooring lines, ropes and nets from an off-coast fish farm in Pagasitikos Gulf (39° 7’ 6” N, 22° 57’ 28” E). After the primary preliminary sorting conducted in situ, sample material was transported to the laboratory in plastic containers and immediately frozen and stored at −40 °C for later analysis. Three replicate samples from each season (Spring – Autumn) were analyzed. Crude protein content was determined by Kjeldahl method, using a conversion factor of 6.25. Total lipid was extracted by chloroform:methanol (2:1). Fatty Acid (FA) determination analysis was conducted by preparing Fatty acid methyl esters (FAME) by acid catalyzed transesterification, while separation and quantification of FAMEs was conducted by gas-liquid chromatography. Individual fatty acids were expressed as the percentage by weight of the total fatty acids characterized.

3. Results and Discussion

Results from the nutritional analyses are presented in Table 1. In general, the composition of *C. scaura* was characterized by high levels of protein and high levels of lipid contents, similar to other studies on amphipods (Baeza-Rojano *et al.* 2014). The higher levels of lipid content in Spring, contrary to the lower values in Autumn can be attributed to the behavioral trait of amphipods in general which tend to store fat during winter (Guerra-García *et al.* 2004). The relative high levels of EPA and DHA, two very important fatty acids for the nutritional value of farmed fish, makes *C. scaura* as a potential candidate species as a marine natural resource for feedstuff or as a novel aquatic organism utilized as live feed in aquaculture, within the framework of the ‘Integrated Multi-Trophic Aquaculture’ (IMTA) concept (Guerra-García *et al.* 2016). In order for aquaculture to sustainably use this species as a resource, further effort should be focused on developing massive rearing protocols to establish a proper availability in the fish hatchery or in the market and thus not to rely on natural stocks.

Table 1. Protein and lipid contents (% of dry weight) as well as fatty acid profile (% of total fatty acids) of *Caprella scaura.* (Presented values are average ± standard deviation)

	Crude Proteins (%)	Crude Lipids (%)	SFA (%)	MUFA (%)	PUFA n-6 (%)	PUFA n-3 (%)	EPA 20:5 (n-3) (%)	DHA 22:6 (n-3) (%)
Spring	48.9 ± 0.4	34.0 ± 4.0	34.4 ± 0.7	30.0 ± 1.1	18.7 ± 1.2	17.0 ± 0.9	8.3 ± 0.7	8.3 ± 0.3
Autumn	49.3 ± 0.3	6.7 ± 3.1	34.9 ± 0.4	31.6 ± 0.9	16.6 ± 1.4	16.9 ± 0.7	6.8 ± 0.4	6.6 ± 0.3

(SFA: Saturated Fatty Acids, MUFA: Mono Unsaturated Fatty Acids, PUFA: Poly Unsaturated Fatty Acids, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid)

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References

Baeza-Rojano E., Hachero-Cruzado I., Guerra-García, J.M. (2014). Nutritional analysis of freshwater and marine amphipods from the Strait of Gibraltar and potential aquaculture applications. Journal of Sea Research 85, 29-36, doi:10.1016/j.seares.2013.09.007.

Bell J.G., Waagbø, R. (2008). Safe and Nutritious Aquaculture Produce: Benefits and Risks of Alternative Sustainable Aquafeeds. In M. Holmer, K. Black, C. M. Duarte, N. Marbà, & I. Karakassis (Eds.), Aquaculture in the Ecosystem (pp. 185-225). Dordrecht: Springer Netherlands.

Guerra-García J.M., Hachero-Cruzado I., González-Romero P., Jiménez-Prada P., Cassell C., Ros M. (2016). Towards Integrated Multi-Trophic Aquaculture: Lessons from Caprellids (Crustacea: Amphipoda). PLOS ONE, 11(4), e0154776, doi:10.1371/journal.pone.0154776.

Guerra-García J.M., Martínez-Pita I., Pita, M.L. (2004). Fatty acid composition of the Caprellidea (Crustacea: Amphipoda) from the Strait of Gibraltar. Scientia Marina, 68(4), 501-510, doi:10.3989/scimar.2004.68n4501.

Henry M., Gasco L., Piccolo G., Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. Animal Feed Science and Technology, 203, 1-22, doi:10.1016/j.anifeedsci.2015.03.001.

Psofakis P., Karapanagiotidis I.T., Malandrakis E.E., Golomazou E., Exadactylos A., Mente, E. (2020). Effect of fishmeal replacement by hydrolyzed feather meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and growth-related gene expression of gilthead seabream (Sparus aurata). Aquaculture, 521, 735006, doi:10.1016/j.aquaculture.2020.735006.

Woods C.M.C. (2009). Caprellid amphipods: An overlooked marine finfish aquaculture resource? Aquaculture, 289(3–4), 199-211, doi:10.1016/j.aquaculture.2009.01.018.

ANTI-PROLIFERATIVE ACTIVITIES OF THE HOLOTHURIAN *Holothuria tubulosa* (ECHINODERMATA) EXTRACT ON HUMAN CANCER CELLS UNDER HYPOXIA

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Abstract
Marine organisms are known as a potential source of natural products, which contain bioactive substances with therapeutic properties. Sea cucumbers are prominent among marine organisms because they offer an important source of therapeutic and medically valuable metabolites with anticancer, anti-microbial and anti-oxidant activities. A common feature of most tumors is a low level of oxygen, called hypoxia, due to increased cell proliferation and limited blood supply. The aim of this study was to evaluate the effect of Sea Cucumber extract (*Holothuria tubulosa*) on the proliferation of cervical cancer-derived HeLa cells and hepatocarcinoma-derived Huh7 cells under hypoxia (1% O_2). Sea cucumber extract was assessed for its anti-proliferative activities in HeLa and Huh7 cells by MTT assay in hypoxia and in physiological levels of oxygen. Under hypoxic conditions, Holothuria extract treatment at concentrations of 20, 50 and 100 μg/ml decreased cellular proliferation in both cell lines after 24 hours compared to control, followed by altered cellular morphology. These data reveal promising anti-proliferative activities in Holothuria extracts in cancer cell models under hypoxia, a condition which, it is well known, contributes to therapy resistance by inducing cell quiescence.
Key words: *sea cucumber, hypoxia, cancer, proliferation.*
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1. Introduction

Loss of oxygen homeostasis, called hypoxia, is a phenomenon common in a majority of malignant tumors. Tumor-hypoxia leads to advanced but dysfunctional vascularization resulting in cell mobility and metastasis (Semenza 2012). Adaptation of tumor cells to the hypoxic conditions depends on the hypoxia-inducible factors (HIF). Over-expression of its regulated HIF-α subunit, an important target of anti-cancer therapy, is observed in many cancers including Hepatocellular carcinoma (HCC) and is associated with severity of tumor growth and poor patient prognosis (Befani & Liakos 2018).

The marine environment harbors a wealth of organisms that produce a wide variety of primary and secondary metabolites with demonstrated significant biological activities. Sea cucumbers are an abundant group of marine invertebrates classified as echinoderms belonging to the class Holothuroidea with a total of about 1250 existing species identified so far (Feidantsis *et al.* (2021). Besides their nourishing value, sea cucumbers offer an important source of therapeutic and medically valuable metabolites with anticancer activities. The aim of this study was to evaluate the effect of Sea Cucumber extract (*Holothuria tubulosa*) on the proliferation of cervical cancer-derived HeLa cells and hepatocarcinoma-derived Huh7 cells under hypoxia.

2. Material and Methods

Preparation of sea cucumber extract: Sea cucumber samples were extracted with ethanol. The extracted materials were evaporated in vacuum and then, were defatted with dichloromethane/water. The water layer was extracted with n-butanol and the organic layer was evaporated in vacuum to get the n-butanol extract. The n-butanol extract were concentrated and dissolved in water. This fraction of *Holothuria tubulosa* Extract (HE) was used in MTT assay.

Cell culture: Huh7 and HeLa cells were cultured normal atmospheric air containing 5% CO2 (denoted as 21% O_2) in a standard humidified incubator. For hypoxic treatment, cells were exposed for 24 h to a gas mixture containing 1% O_2, 94% N_2 and 5% CO_2 (denoted as 1% O_2) in an IN VIVO2 200 hypoxia workstation.

MTT assay: Cell proliferation was monitored using the colorimetric MTT assay at 540 nm. Cells treated with or without HE for 30 min followed by hypoxia for 24 h. Cell viability was expressed as percentage of control from three independent experiments performed in triplicate

3. Results and Discussion

HeLa cell survival in the presence of HE was significantly inhibited in concentrations of 20 to 100 μg/ml in 24 hr under normoxia or hypoxia relative to the untreated groups (Figure 1A). Huh7 cell proliferation was significantly suppressed in the presence of HE in concentrations of 5 to 100 μg/ml in 24 hr under both normoxia and hypoxia compared to the control groups (Figure 1B). In thus, the detailed analysis of the results clearly suggested that HE caused significant inhibition of cancer cell viability in dose dependent manner and Huh7 cells were more sensitive to HE than HeLa cells.

The morphological analysis of Huh7 cells was performed using inverted microscope. Treated cells showed significant morphological changes in comparison with untreated cells, including cell shrinkage and reduction in size, volume and density of the cells, cytoplasmic membrane blebbing and lose contact with neighboring cells (Figure 2)

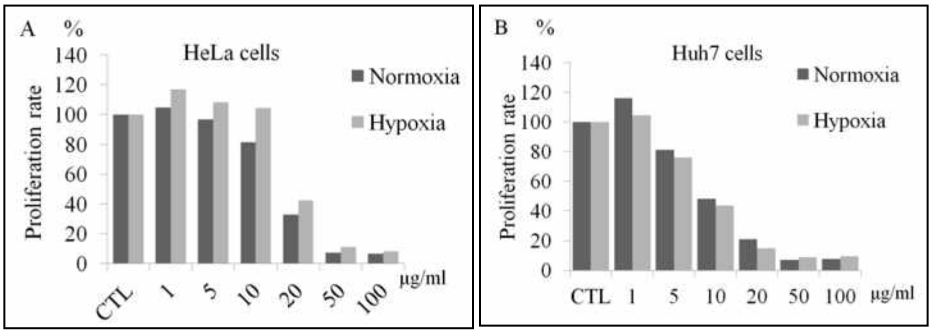

Figure 1. Effect of *Holothuria tubulosa* extracts on cell proliferation of (A) HeLa cells, (B) Huh7 cells under normoxia and hypoxia for 24 h.

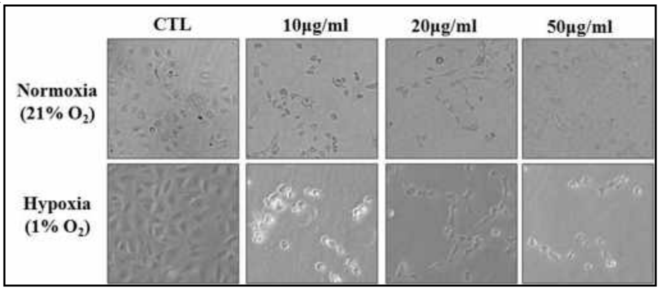

Figure 2. Effect of *Holothuria tubulosa* extracts on cell morphology in Huh7 cells under normoxia and hypoxia for 24 h.

Additionally, the mechanisms leading to decreased cell proliferation under hypoxia and the *Holothuria tubulosa* extract effect on the hypoxia-inducible factors will be examined.

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References

Semenza G.L, (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy, Trends in Pharmacology Science 33, 207-214

Befani C., Liakos P. (2018). The role of hypoxia-inducible factor-2 alpha in angiogenesis, Journal of Cellular Physiology 233. 9087-9098.

Feidantsis K., Michaelidis B., Raitsos. D, Vafidis D. (2021). Seasonal metabolic and oxidative stress responses of commercially important invertebrate species. Marine Ecology Progress Series 658, 27–46.

GROWTH RESPONSES OF F3 GROWTH-SELECTED AND NON-SELECTED BROODSTOCKS OF *Sparus aurata* TO FASTING AND RE-FEEDING IN RAS

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Abstract
The effects of fasting and re-feeding on three F3 growth-selected and one non-selected broodstocks were evaluated under two thermal regimes (controlled and natural) and two salinities (10 and 37 ppt) in recirculation systems (RAS). A longitudinal approach using internal tagging was used. Growth-selected broodstocks showed the highest loss and gain of weight as determined by individual growth rates. Such values were correlated with expected breeding values indicating selected genetic backgrounds were well-adapted to RAS. Moreover, a clear effect of salinity and thermal regime was observed with the highest loss and gain of weight under a controlled temperature and at low salinity. However, a significant interaction between these two factors was found both in fasting and re-feeding periods. All these data are relevant for developing new aquaculture models for *S. aurata* under eco-intensive RAS.
Keywords: *RAS, Sparus aurata, re-feeding, fasting, salinity, genetic background, thermal regime*
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1. Introduction

Gilthead seabream (*Sparus aurata*) is one the main species produced in the Mediterranean aquaculture that overall accounts for more than 250,000 metric tons. This species is euryhaline and it has been successfully cultivated in brackish (2.5-12 ppt) and seawater (39 ppt) (Laiz-Carrión *et al.* 2005). Due to its high adaptability to environmental conditions, this species is produced under different systems such as inshore and off-shore cages, estuarine ponds and flow-through land facilities. However, new attempts to cultivate under eco-intensive recirculating systems (RAS) to align with EU policies for an environmental-friendly aquaculture are ongoing.

Genetic breeding programs that are still at the beginning in this species could be an excellent tool to select the better adapted fish. In Spain, PROGENSA is a genetic breeding program that evaluate fish under different production models to quantify the interaction genotype×environment. In this way, a wide range of growth-, morphology-, disease- and flesh quality-related traits have been genetically evaluated (Lee-Montero *et al.* 2015; Carballo *et al.* 2020). Currently, this program is on the third selected fish generation (F3) for growth performance.

However, a step forward toward evaluation of broodstock on sustainable production models such us RAS needs to be done. The aim of the study is to evaluate the growth performance of a non-selected and three F3 growth-selected and broodstocks under a RAS production model. Growth responses after a fasting and re-feeding periods were evaluated under different salinities (37 and 10 ppt) and thermal regimes (natural thermal oscillations and controlled at 20ºC).

2. Material and Methods

Three broodstocks (referred to as TE5, TP6 and TP7) selected for weight at harvest were selected for evaluation. The TE5 (n=60; 2017-year class) had an average estimated breeding value (ABV) of 16.2%. TP6 contained a mix of breeders from year classes 2016 (n=31) and 2017 (n=40) with ABV of 20.9 and 12.6%, respectively. The TP7 (n=48; 2016-year class) had an ABV +6%. A non-selected broodstock used as a control (n=38; 2016-year class) with ABV -8.3%. Average weight at harvest for year classes 2016 and 2017 was 471.0 and 540.6, respectively. Broodstock were induced to spawning by photoperiod control and the eggs from four consecutive days were mixed. Larval rearing was as previously described (Carballo et al. 2020). Animals at 210 days (>4 g) were intraperitoneally tagged with Passive Integrated Transponder. A total of 25 fish were tagged from each broodstock and mixed in the 12 tanks attached to four RAS modules (three tanks per module). Before the trial commencement, the animals were cultivated for 212 days (d) and fed ~1.2% biomass (Biomar EFICO). At the beginning of the trial, all animals were weighted. Two RAS modules were under controlled temperature and two modules were under natural thermal oscillations. Moreover, one module of each thermal regime was shifted for salinity from 37 to 10 ppt establishing four treatments: Controlled T 10 ppt, Controlled 37 ppt, Natural T 1 0ppt and Natural T 37ppt. Animals were fasted for 19 d and then re-fed for 13 d supplying as average 1.1% biomass with the same feed as indicated above. Trial was carried between april and may 2021 in South Spain (Cadiz) and average temperatures in the fasting period were 21.4 ± 0.6 and 20.0 ± 0.1 for Controlled T and Natural T groups, respectively. In the refeeding period these values were 23.0 ± 1.3 and 20.2 ± 0.4. Individual growth rates (IGR) were calculated as follows: IGR=[Ln(W2)-Ln(W1)]/t, where W1 and W2 stand for the value of weight at the end and at the beginning of the period analyzed respectively, and t stands for the total of days of such period. Statistical analysis was carried using a General Linear Model (GLM) analysis including the thermal regime (TR), salinity (SAL) and genetic batch (GB) as fixed factors and the tank as a nested one. A longitudinal analysis was also carried for the whole period using a repeated-measures ANOVA with the same fixed factors as indicated above. All statistical analyses were carried out using SPSS v25.0

3. Results and Discussion

Significant differences in weight were found between genetic batches at the beginning of the trial after cultivating for 212 d in the same tanks. Control fish was smaller (147.3±2.5g) than F3 growth-selected fish (156.7g±2.6 TE5, 184.0±2.5g TP6, 168.6±2.4g TP7). During fasting, significant differences in IGR associated with GB, TR, Sal and a significant interaction TR×Sal were detected. Reduction in weight was bigger in selected fish from TE5 and TP6 than TP7 and control. These differences in selected fish from TP7 with respect to TE5/TP6 suggest a genotype×fasting interaction that needs to be investigated by elucidating family structure. Moreover, weight loss was also greater under controlled temperature than under natural thermal oscillations and at 10 ppt than 37 ppt, however such differences between salinities were more evident under natural thermal oscillations.
When the effect of re-feeding was evaluated, growth-selected fish gained weight faster than controls and IGR was highest for TE5. These animals showed a 22.9% higher IGR than control during the re-feeding period. The other two broodstocks had a 12.0-14.9% higher IGR than control demonstrating that genetic selection can also benefit the seabream production in RAS. Moreover, IGR was also significant higher at 10 than 37 ppt and under controlled temperature than natural thermocycles. As in fasting period, a significant interaction TR×Sal was found with a higher gain at 10 ppt under controlled temperature regime. The mortality by treatment in the fasting or re-feeding periods was lower than 3.4% with a maximal cumulative mortality of 5.4% without significant differences between treatments. The lineal approach using repeated-measures ANOVA for weight from the beginning, during fasting and after re-feeding indicated that the weight loss and gain in control group was significantly different from selected groups. Moreover, significant differences associate with TR and Sal were found with a more accentuated weight gain than loss in the controlled thermal regime and a more pronounced loss of weight during fasting than gain during re-feeding at 10 ppt.

The data presented in this work demonstrate that selected broodstocks for growth in PROGENSA (Carballo *et al.* 2020) are also suitable for production under RAS models. The IGRs during the fasting and re-feeding reached maximum values in the TE5 broodstock with the highest ABV indicating that energy demand is crucial in this fast-growing families. We also demonstrated that a salinity of 10 ppt determine a higher loss or gain of weight that could related with optimal metabolism rates and the proper management and energy allocation that is expected in animals when surrounding salinity is close to the isosmotic point. The observation of higher IGR under controlled temperature (close to 20ºC) than under natural thermal oscillations (average ranging between 21.4-23.0) highlight the importance of a stable system to promote growth. Moreover, the interaction between TR×Sal in fasting and re-feeding demonstrates that a tight control of both factors is important since the effects of low salinity on growth are more evident under controlled temperature.

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References

Carballo C., Shin H.S., Berbel C., Zamorano M.J., Borrego J.J., Armero E., Afonso J.M., Manchado M. (2020) Heritability estimates and genetic correlation for growth traits and LCDV susceptibility in gilthead sea bream (Sparus aurata). Fishes 5, 2.

Laiz-Carrión R., Sangiao-Alvarellos S., Guzmán J.M., Martín del Río M.P., Soengas J.L., Mancera J.M. (2005) Growth performance of gilthead sea bream Sparus aurata in different osmotic conditions: Implications for osmoregulation and energy metabolism. Aquaculture 250, 849-61.

Lee-Montero I., Navarro A., Negrin-Baez D., Zamorano M.J., Borrell Pichs Y.J., Berbel C., Sanchez J.A., Garcia-Celdran M., Manchado M., Estevez A., Armero E., Afonso J.M. (2015) Genetic parameters and genotype-environment interactions for skeleton deformities and growth traits at different ages on gilthead seabream (Sparus aurata L.) in four Spanish regions. Animal Genetics 46, 164-74.

EFFECT OF FEED DEPRIVATION ON GROWTH PERFORMANCE AND PROXIMATE COMPOSITION IN GILTHEAD SEABREAM

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Abstract
A feed-deprivation study was conducted for 6 weeks with two feeding regimes, in order to estimate growth and proximate composition of gilthead seabream *Sparus aurata.* For this purpose, a total of 137 juveniles were acclimatized in a recirculating aquaculture system (RAS) and were randomly divided into six aquaria. The two feeding regimes included a continuously fed (control) group and a group that was twice deprived for 2 weeks and refed for 1 week. At the end of the experiment, both fish groups exhibited weight gain, however, either complete or partial growth compensation was not achieved in the treatment group. The final body weight and the weight gain rate, in the control group were higher than in the treatment one, which means that the last group did not catch up the expected body weight. The proximate composition of the carcass was unaffected by the feeding trial.
Keywords: *gilthead seabream, feed deprivation, refeeding, proximate composition*.
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1. Introduction

Providing suitable feeding strategies in aquaculture is crucial since the major costs of intensive aquaculture systems are fishfeeds (40–50%). Determination of the optimum feeding scheme for both maximum growth and profit is of paramount importance for aquaculture. As both underfeeding and overfeeding have negative concequences on aquaculture (Yilmaz & Eroldogan 2011), successful aquaculture, mainly depends on feed management (Chatzifotis *et al*. 2011). Feeding strategies including satiation feeding, restriction feeding, starvation, and re-feeding are effective procedures for employing the best feed management (Lovell 1998). The present study investigated whether a specific feeding strategy can affect growth and proximate composition of sea bream *Sparus aurata.*

2. Material and Methods

The study was conducted with 137 juveniles of gilthead seabream, which had a mean total weight and length of 25.95±0.63g and 12,15±0,09mm, respectively. Fish were randomly distributed into two groups with three replicate aquaria per group in a recirculating aquaculture system (RAS). Fish were acclimatized to laboratory conditions for a month, and then were weighed and measured to record its initial size. Throughout the experiment, the water temperature was stable at 21±1°C, photoperiod was adjusted to 12h light:12h dark and the water quality parameters were monitored on a daily basis. The control group was fed daily with commercial pellets (BIOMAR, Greece) and the second group was feed deprived for 2 weeks and was re-fed for 1week. This sequence was replicated. Whole carcasses (n=36 in total) were stored at −20 °C for proximate composition analysis and the following performance parameters were evaluated: weight gain rate: WGR (%) = 100 × [final weight (g) –initial weight (g)]/ initial body weight; specific growth rate: SGR (%) = 100 × [ln final weight (g) –ln initial weight (g)]/ experimental period; Fulton's condition factor: K = [weight/(total length)^3]; and feed conversion ratio: FCR = food supplied (g)/weight gain (g).

In addition, at the end of the experiment, body composition such as protein, moisture, lipid, ash and energy were determined for the two groups. Fish were sacrificed by phenoxyethanol overdose. Fish samples were freeze-dried for constant weight and homogenized for the analysis. Protein content was analyzed using the Kjeldahl method (N × 6.25; Behr Labor-Technik, Germany). Moisture content was determined after drying the fish in a 105 °C dry oven for 24 h. Lipid content was measured using the Soxhlet method and ash content was obtained after combustion at 600 °C for 5 h. Energy content was determined using an IKA oxygen bomb calorimeter (C5000, IKA Werke, Staufen, Germany). All data were expressed as the mean±standard deviation and analyzed using paired t-test.

3. Results

All fish adapted well to the feeding regimes, and no mortalities were recorded throughout the experimental period. As shown in Table 1, in both groups at the end of the experiment, an increase in their weight was observed. The control group had significantly higher final body weight and final length. Fish subjected to restricted feeding exhibited the lowest FCR, SGR and K. No significant differences were observed between the groups for moisture, dry matter, proteins, lipid content, ash and gross energy.

Table 1. Growth parameters, whole body proximate composition (% on dry weight base) and energy content (MJ/kg) of different *S. aurata* groups. Values represent mean ± standard deviation. Values within each row with different superscript letters differ significantly (P<0.05).

	CONTROL	TREATMENT
Initial Weight (gr)	26.41±5.72	25.37±8.74
Final Weight (gr)	48.38±10.72^{a}	30.71±9.27^{b}
Initial Length (cm)	12.3±0.90	12.01±1.18
Final Length (cm)	15.05±1.04^{a}	13.10±1.16^{b}
WGR (%)	83.19	21.01
SGR (%)	1.32	0.42
K	1.41	1.37
FCR	1.37	1.10
Moisture %	67.05±1.68	68.14±2.30
Dry matter %	32.95±1.68	31.86±2.30
Proteins %	52.45±3.06	52.80±1.98
Lipid %	32.00±5.07	30.97±2.75
Ash %	11.73±0.78	12.08±0.98
Gross energy (MJ/kg)	24.08±1.31	23.88±0.78

4. Discussion

Suitable feeding strategy during production has always been an important issue. Recently, there has also been an increased awareness on the suitable feeding scheme to reduce production costs together with growth enhancement. Results of the present experiment showed that the growth performance of gilthead seabream was significantly affected when subjected to the specific feed deprivation protocol. The final weight was significantly different between the control and the treatment group, indicating that no partial or total compensation was achieved. Likewise, Yılmaz & Eroldogan (2011) didn't notice full compensation of seabream populations in all feeding protocols used, although control fish control fish consumed less feed than the starved groups. This result is not in accordance with another study, that concluded that seabream fully compensates body weight but not length after a deprivation period (Bavcevic *et al.* 2010). No significant difference was observed at any parameter tested between the two groups, concerning the whole-body proximate composition. On the contrary Yılmaz & Eroldogan (2011) measured higher protein content in fish from the control group. In conclusion, the current fasting-feeding protocol does not seem to provide compensation and therefore more research is needed, as feeding protocols vary depending on the conditional factors.

References

Bavcevic L., Klanjscek T., Karamarko V., Anicic I., Legovic T. (2010). Compensatory growth in gilthead sea bream (Sparus aurata) compensates weight, but not length, Aquaculture 301, 57-63

Chatzifotis S., Papadaki M., Despoti S., Roufidou C., Antonopoulou E. (2011). Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (Dicentrarchus labrax). Aquaculture 316, 53–59.

Yılmaz H.A., Eroldogan O.T. (2011). Combined effects of cycled starvation and feeding frequency on growth and oxygen consumption of gilthead sea bream, Sparus aurata. Journal of the World Aquaculture Society 42, 522–529.

CULTIVATION OF *Tetraselmis striata* UNDER OPTIMIZED GROWTH CONDITIONS AND BIOMASS QUALITY EVALUATION FOR FISH FEED PRODUCTION

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Abstract
The marine microalga *Tetraselmis striata* was cultivated in drilling waters obtained from the commercial fish farm Plagton S.A. (western Greece). The waters were supplemented with the fertilizer Nutri-Leef 30-10-10 of which the optimum growth quantity was found to be 0.2 g L^{-1}. The effect of different pH and temperature values on biomass and lipid yields was examined. The optimum growth conditions were found to be pH 8 and temperature 25 ºC and lead to maximum biomass productivity of 79.8 mg L^{-1} d^{-1} and a specific growth rate of 0.16 d^{-1}. The biomass of *Tetraselmis* produced in the optimum growth conditions was rich in bioactive compounds and especially crude protein (51.3%), while analysis of the fatty acids revealed high percentages (up to 14%) of eicosapentaenoic acid (EPA).
Keywords: *substrate optimization, high value products*
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1. Introduction

Aquaculture is one of the most fast growing food industries worldwide and faces significant challenges including the high costs of fishmeal and fish oil for inclusion in the diets of farmed fish. Microalgae are a proven source of protein, lipids, carbohydrates and vitamins, and can improve fish growth and fillet quality. The nutritional value of microalgae is determined mainly by their protein and polyunsaturated fatty acid (PUFA) content (e.g., EPA, arachidonic acid (ARA), and docosahexaenoic acid (DHA)). Strategies such as light intensity, composition of growth media, pH and temperature manipulation, permit both lipid composition modulation and optimization of overall lipid yield.

In this study, the microalga *Tetraselmis striata* was cultivated in drilling waters obtained from the commercial fish farm Plagton S.A.. The drilling waters had natural salinity of 29 ‰ and no nutrient load, thus mineral supplements are essential for microalgal growth. Aiming to minimize production costs that could enable full-scale cultivation, the commercial fertilizer Nutri-Leef (30% -TN, 10% -P, 10% -K) together with an additional inorganic carbon source ($NaHCO_3$) was employed as growth medium for *Tetraselmis*. The quantity of fertilizer required to enrich the growth medium was recorded, as well as effects of pH and temperature on the specific growth rate and the ability of the strain to biosynthesize bioactive metabolic products.

2. Material and Methods

Samples of 50 mL were taken every 48 hours to determine growth and intracellular products. Biomass productivity, expressed in mg $L^{-1}d^{-1}$ and maximum specific growth rate (d^{-1}) estimated according to Tsolcha *et al.* (2017). Intracellular carbohydrates were determined by the method of Dubois (1956), while lipids were extracted from biomass following Folch's method (1957). Crude protein content was determined with Kjeldahl method following AOAC (1995) and amino acid composition of the lyophilized biomass was carried out according to Kotzamanis *et al*. (2018). Finally, pigment concentration in the wet biomass was estimated applying the equations given by Lichtenthaler & Buschmann (2005), using acetone with 20% (v/v) water as the solvent.

3. Results and Discussion

Initially, the optimal quantity of fertilizer for the highest specific growth rate and biomass productivity of *T. striata* was determined. Quantities of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 g Nutri-Leef per litre were tested at 25±1ºC ºC, under uncontrolled pH, while 0.18 g L^{-1} of $NaHCO_3$ was added into all experimental sets. The results showed that high biomass productivities were achieved for both 0.1 and 0.2 g L^{-1} of fertilizer (106.8 and 134.6 mg $L^{-1}d^{-1}$) corresponding to specific growth rates of 0.15 and 0.24 d^{-1} respectively. However, for fertilizer quantities over 0.4 g L^{-1}, biomass yields gradually reduced, and showed productivities ranging from 60.8 to 92.3 mg $L^{-1}d^{-1}$ and specific growth rates of between 0.13 d^{-1} and 0.21 d^{-1}. A similar pattern was also seen for the lipid yield results, indicating that as the fertilizer increased in quantity, the lipid content decreased from 16.1 to 9.8% d/w. Thus the optimum fertilizer quantity was found to be 0.2 g L^{-1} which led to the highest biomass productivity and enhanced lipid content (16.3%). Using the optimum fertilizer quantity two different pH values of 7 and 8 were then tested and pH 8 was found to present significant biomass productivity of 79.8 mg L^{-1} d^{-1} with a specific growth rate of 0.16 d^{-1} and maximum oil content of 26.4%. The effect of temperature (at 19 ±1 and 25±1°C) was then examined under the optimized pH conditions. The microalga grew well at both temperatures although the highest biomass yield recorded at 25±1°C, while lipid content slightly enhanced at 19±1°C (Table 1). Finally, the biomass of *T. striata* produced under the optimum growth conditions (pH=8, T=25°C) was rich in lipids, carbohydrates, proteins and pigments (27.3, 18.3%, 51.3% and 3.6%, respectively). The optimized biomass presented adequate amino acid profile with high essential amino content (22.7%) and high essential to non-essential amino acid ratio (EAA/NEAA) of 0.96, which are considered suitable aquafeed ingredients. Among them the most abundant amino acids was Leucine (4.7%) and Lysine (3.4 %). In conclusion, fatty acid analysis revealed that the biomass contained 10-14% EPA, indicating its high value for incorporation into conventional fish feed.

Table 1. Effect of pH and temperature on *T. striata* biomass productivity, specific growth rate and lipid content.

Growth condition	Biomass productivity mg $L^{-1}d^{-1}$	Specific growth rate d^{-1}	Lipid content % dw	Lipid productivity mg $L^{-1}d^{-1}$
pH =7, T= 25± 1 °C	60.1	0.14	25.0	19.1
pH= 8, T= 25± 1 °C	79.8	0.16	26.4	17.1
pH= 8, T= 19± 1 °C	60.0	0.190	27.0	17.8

4. Conclusions

According to the experimental results, under the optimized growth conditions the microalga *Tetraselmis striata* was able to produce high quality biomass rich in nutrients and EPA important for fish growth.

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References

AOAC (1995) Official Methods of Analysis of AOAC International. Vol. I. Agriculture Chemicals; Contaminants, Drugs. 16th edn. AOAC International, Arlington, VA.

DuBois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. (1956). Colorimetric method for determination of sugars and related substances. Analyical Chemistry 28, 350–356.

Folch J., Lees M., Sloane-Stanley G.A. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497–509.

Kotzamanis Y., Kouroupakis E., Ilia V., Haralabous J., Papaioannou N., Papanna K., Richards R., Gisbert E. (2018). Effects of high-level fishmeal replacement by plant proteins supplemented with different levels of lysine on growth performance and incidence of systemic noninfectious granulomatosis in meagre (Argyrosomus regius). Aquaculture Nutrition 24, 1738-1751.

Lichtenthaler H.K. & Buschmann C. (2005) Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Handbook of Food Analytical Chemistry 2, 171–178.

Lowry O.H, Rosebrough N.J, Farr A.L., Randall R.J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265–275.

Tsolcha O.N., Tekerlekopoulou A.G., Akratos C.S., Aggelis G., Genitsaris S., Moustaka-Gouni M., Vayenas D.V. (2017). Biotreatment of raisin and winery wastewaters and simultaneous biodiesel production using a *Leptolyngbya*-based microbial consortium. Journal of Cleaner Production 148, 185–193.

A NOVEL METHOD FOR DETECTION OF AMOEBA (*Endolimax sp*) IN WATER FROM CULTURE TANKS AND APPLICATION TO THE EVALUATION OF METRONIDAZOLE TREATMENTS IN SENEGALESE SOLE

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Abstract
Endolimax sp. is a protozoal genus causative of granulomatous lesions in internal organs of Senegalese sole. Although lethality associated with this disease is low, infected animals highly delay growth. In this study, we evaluate a novel detection method of carriers to detect the amoeba from the water tank by filtering through different mesh sizes (1-500 µm). Amoebic DNA was quantified by a new qPCR assay. Endolimax DNA was mainly detected in the 500µm mesh during a fasting period indicating that amoeba cells are trapped into the debris size facilitating the detection from production tanks. This method was used to evaluate the usefulness of two consecutive freshwater baths (5 min) and/or metronidazole (30 mg kg^{-1}) treatments to control amoebic infections. Majority of positive animals to amoeba infection before treatments were negative after metronidazole administration. This removal of amoeba from the gut coincided with an increase of amoebic DNA in the water tank. All these results indicate the usefulness of our approach to detect carriers in production tanks and identify the metronidazole as a target drug to control this parasite infection in Senegalese sole aquaculture.
Keywords: *Senegalese sole, Endolimax, diagnosis, metronidazole*
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1. Introduction

The recirculation systems (RAS) are technologically high demanding and require strict control measures to monitor and manage health status. This is the case of the flatfish Senegalese sole aquaculture that is almost exclusively produced in RAS and has to deal with some parasitic diseases that hamper growth and represent a real challenge that needs to be solved. Protozoa from Endolimax genus have been described as the causative agent of a granulomatous disease found in this species produced in RAS. Size of this amoeba ranges between 5 and 30µm and it localizes mainly in the intestine although it can disseminate and reach internal organs causing granulomatous lesions. Although these lesions are not lethal, they impose an important delay in growth and, in some cases, make fish not suitable for commercialization. Hence, feasible methodologies for detection and effective treatments need to be explored (Carballo *et al.* 2020; Constenla *et al.* 2014; 2018). Previously, Carballo *et al.* (2020) described a non-lethal diagnostic methodology to identify fish carriers based on an intestinal washing procedure using a flexible polypropylene catheter. Nevertheless, industry demands new methods for a wide scale monitoring of this parasite in RAS facilities at population level.

The aim of the study is to evaluate a novel methodology for the detection of Endolimax in water tanks as a monitoring tool to identify infected production units. Moreover, this methodology was applied to evaluate the effects of freshwater treatments and metronidazole, an anti-protozoal drug in humans and animals, to control the infection in fish. The results are of high impact for sole aquaculture and open new chemotherapeutic window to amoebic infections in RAS.

2. Material and Methods

2.1. Detection of Endolimax in water samples

A homemade filtration system for high volumes of water was tested in a Senegalese sole culture tank (2 m^3) containing 70 animals with a previous history of infection with Endolimax. The filtration system was tested using different size nylon mesh pore sieves (500 µm and 40µm) and bag filters (5 µm and 1 µm). Tank water was filtered from the outlet under a flow-through circuit (~20ºC, 37 ppt) for 5 days (d) with a normal feeding protocol followed by fasting for 2 d. Samples taken were 6 and 5 for feeding and fasting period, respectively. To validate our results those animals were later distributed in total 8 tanks (0.4 m^3; n = 8 per tank). In each tank, at least three animals were detected as positive by oral or anal intubation. Water samples were taken in fasted fish corresponding to a volume 360 L (2h) and 4300 L (24 h) using a filtration system composed by 2 circular nylon mesh of 500 µm and 40µm situated in the top of a 200 mm PVC pipe with a bag filter of 1µm inside situated in the tank outlets.

Samples from the filters were washed with sterile seawater and later centrifuged at 11 000×g for 15 min and supernatant removed. The pellet was used for DNA purification using NucleoSpin Tissue mini kit for DNA

(Macherey-Nagel). The detection of amoeba DNA in the samples was evaluated by PCR (Carballo *et al.* 2020) and by qPCR using specific primers designed in this study (F, 5'-ACAAAAGCGAAAGCATTCCACA-3'; R, 5'-ATCATCTTCGATCCCCCAACT-3'), that amplified an amplicon of 70 bp from 16S rDNA.

2.2 Fish trial for to evaluate effects of metronidazole

Four experimental groups (total n = 64) were evaluated in duplicate (n =8 per tank) using two RAS systems. Fish were those previously used in the trial reported above. Group 1 was orally administrated with metronidazole (30 mg kg^{-1}), group 2 was treated with a freshwater bath for 5 min, group 3 was treated with a freshwater bath as in group 2 and 24 h later orally with metronidazole as in group 1 and the group 4 remained as untreated although managed in parallel with other groups and administrated PBS (carrier for metronidazole) or a seawater treatment. The freshwater bath was applied directly within the RAS tanks without any manipulation of fish substituting progressively the water in the tank until reach a salinity of 0.9 g/l, keeping the treatment for 5 min and, thereafter replacing progressively by seawater. Treatments were repeated at 48 h. Amoeba was detected just 1h after second treatment.

At the beginning of the trial and after treatment, animals were sampled individually to validate the infection using the methodology described in Carballo *et al.* (2020). Briefly, 3ml of sterile seawater dyed with food colouring were inoculated by oral and anal routes to collect the tube digestive content using a flexible polypropylene catheter. One ml of recovered content was centrifuged and DNA isolated as indicated above to evaluate the presence of amoeba DNA by PCR/qPCR. Moreover, amoeba was detected in the water tank using the filtration system as indicated above.

3. Results & Discussion

In order to detect the amoeba in water tank, a different combination of mesh sizes was tested under a flow-through system. The amoeba DNA was detected in one out of 6 sampling times during the feeding period and three out of 5 sampling times in the fasting. All PCR positives samplings were detected in the 500 µm mesh. Only 1 positive sample was detected in 40 µm mesh during fasting and no positive samples were detected in 5 and 1µm filters. In the second trial, all samples collected in the 500 and 40 µm amplified positively by qPCR. Only six out the 8 samples at 2h and five out the 8 samples at 24h were positive in 1µm filters. These results demonstrate that amoeba can be detected in a feasible way in water. Moreover, the lower positive rates in the 1µm filter compared to 40 and 500 µm indicate that amoeba cells adhere to debris and become trapped in filters with larger pore sizes facilitating the detection in production tanks.

To evaluate the effects of metronidazole and freshwater bath treatments to control amoeba infections, fish were evaluated before and after treatment by oral and anal routes. In the group treated with metronidazole, five positive animals by PCR before treatment (n=6) were negative after metronidazole administration (group 1). When fish were previously challenged with a freshwater bath (group 3), all 4 positive fish were later negative after treatment with metronidazole and, in the group 2 without metronidazole, five out of 6 fish remained as positive after treatment. In contrast, seven out of 8 fish in the control group were positive in both sampling points (group 4). Also, amoeba DNA was detected before and after treatments in all tanks. Interestingly, DNA copies detected in water after treatment were higher in metronidazole treated groups (group 1 and 3) than the groups 2 and 4. All these results suggest that metronidazole is a promising treatment to eliminate this amoeba from gut although further experiments are required to elucidate the optimal doses and times for dietary therapies.

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References

Carballo C., Berbel M.C., Zerolo R, Manchado M. (2020). Metodología de detección de portadores de un nuevo protozoo patógeno en lenguado senegalés. 3er Coloquio de Nutrigenómica y Biotecnología Acuícola (CONYBA). Nayarit, Mexico.

Constenla M., Padrós F., Palenzuela O. (2014). Endolimax piscium sp. nov. (Amoebozoa), causative agent of systemic granulomatous disease of cultured sole, Solea senegalensis Kaup. Journal of Fish Diseases 37(3), 229-240.

Constenla M., Padrós F., Villanueva-González A., Del Pozo R., Palenzuela O. (2018) Horizontal transmission of Endolimax piscium, causative agent of systemic amoebiasis in Senegalese sole Solea senegalensis. Disease of Aquatic Organisms 27(3), 235-240.

GENE EXPRESSION PATTERNS ASSOCIATED WITH FAST- AND SLOW-GROWING FAMILIES AND GENDER IN SENEGALESE SOLE, *Solea senegalensis*

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Abstract
Senegalese sole is an important species of flatfish in aquaculture. However, knowledge about the molecular mechanisms that govern growth associated with genetic families and gender is still limited. In this study, gene expression patterns in muscle of three fast (FG) and two slow growing (SG) genetic families cultivated under the same conditions and between males and females were investigated. RNA-seq analysis identified 81-99 differentially expressed transcripts (DETs) between FG and SG families and between 5 743-5 805 DETs between sexes. Only 26.4% of DETs were shared in both comparisons. A functional analysis of DETs between FG and SG families detected some amino acid metabolism process pathways as significantly enriched. Cell cycle, gamete generation and methylation related pathways appeared mainly enriched between sexes. Some genes involved in energy metabolism and appetite control were shared in both comparisons. All this information highlights the importance of energy provision in muscle as key regulators of growth performance that is useful for genetic breeding programs in sole.
Keywords: *Senegalese sole, growth, RNA-seq, selective breeding*
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1.Introduction

Senegalese sole is an important flatfish species whose aquaculture is rapidly growing in Southern Europe. Due to interest for culturing under intensive recirculation systems (RAS), genetic breeding programs have been initiated. Some growth-related traits such as weight at harvest and weight before entering the on-growing period in RAS were reported to have a moderate-high heritability indicating the high potential of genetic selection schemes to improve growth-related traits. Moreover, a high effect of gender on growth was also demonstrated with females being 8.0-22.7% heavier than males (Guerrero-Cozar *et al*. 2021a). Since growth traits are highly influenced both by environmental (temperature, fish density among others) and genetic factors, it is important to elucidate the main molecular mechanisms associated with growth performance and gender.

The aim of the study is to decipher gene expression patterns associated with growth and gender in sole families according to their estimated breeding values using muscle as the target tissue. Three families with high (referred to as FG) and two with low (SG) breeding values for weight at harvest were selected. Expression profiles were determined by RNA-seq deep sequencing and main differentially expressed pathways were identified. The data obtained are high relevant for a better understanding of *loci* on selection in genetic breeding programs.

2. Material and Methods

To select families, animals from the breeding program currently ongoing in collaboration with the company CUPIMAR (San Fernando, Spain) were evaluated (n = 2 691) for weight at harvest (800 d). Animals were genotyped and pedigree reconstructed by using a SSR Multiplex PCR and breeding values (BV) were calculated using mixed models as implemented in WOMBAT according to Guerrero-Cozar *et al*. (2021a). BV for the three selected FG families were +94 g, +169 g and +160 g and for the two SG families -124 g and -158g. For muscle sampling, animals of each family were randomly selected and euthanized using a phenoxyethanol overdose. Gender was registered and a piece of muscle was fixed in liquid nitrogen and stored at -80ºC until use.

RNA purification and Illumina sequencing were carried out as described in Córdoba-Caballero *et al*. (2020). For sequencing, fish samples (between 8-12 individuals per family) were pooled by family and gender (from 3 to 5 per family). RNA-seq differential expression analyses for growth (12 FG *vs* 7 LG libraries) and gender (12 Females *vs* 7 Males libraries) were carried out using edgeR and DESeq with a false discovery rate (FDR) cut-off of 0.05 as implemented in the DEgenesHunter package. Two transcriptomes, for male (GM) and female (GF), were used as reference for read mapping (Guerrero-Cozar *et al*. (2021b). Functional analysis was also carried out with this package using the RefSeq information from orthologous zebrafish (*Danio rerio*) genes. Enrichment results were considered significant at FDR $p< 0.05$.

3. Results and Discussion

Five full-sib families (three FG and two SG) were selected according to estimated BV. Average weight at sampling was 1 243 ± 37.6 g for FG and 355.4 ± 28.1 g for SG families. Moreover, females were heavier than males (1 054 ± 73.6 g *vs* 783 ± 121.9 g) what agrees with previously data reported in soles at harvest (Guerrero-Cozar *et al.* 2021a). The estimated familial BV as inferred by mixed models from the whole testing population confirmed the differences in size and the robustness of our prediction for family selection.

To investigate expression profiles of FG and SG families, a total of 19 Illumina libraries were sequenced (average 31.6-33.5$\times10^6$ paired-reads per sample). Library cleaning indicated more than 94% of useful reads as average with mapping rates higher than 89% in both the GF and GM transcriptomes. Statistical analysis identified 99 and 81 differentially expressed transcripts (DETs) using GF and GM, respectively. A total of 61.5% DETs were up-regulated in the SG with respect to FG families. With respect to gender, a total of 5 743 and 5 805 DETs were identified using GF and GM, respectively. The 81.3 % of DETs were up-regulated in females with respect to males. Only 26.4% of DETs in the growth comparison were also differentially expressed between sexes. All these data demonstrate that gender is a major factor controlling regulatory expression pathways in the muscle although only a small subset of transcripts seems to be specifically related to growth performance.

To understand molecular pathways associated with FG and SG families, a functional enrichment analysis was carried and identified the tRNA aminoacylation for protein translation, cellular amino acid biosynthetic and amino acid betaine metabolic processes, neuron migration, hematopoietic stem cell differentiation and female gamete generation as the most relevant. These data suggest major changes in amino acid-related pathways for energy provision. Recently, a QTL for growth performance within the *mtfp1* gene involved in mitochondrial physiology was reported in sole (Guerrero-Cozar *et al*. 2021a) demonstrating the relevance of energy-related pathways on fish growth. In the case of gender, pathways related to cell cycle, gamete generation, macromolecule methylation, DNA replication and nucleobase-containing compounds metabolic process represented >75% of the differentially enriched routes. These data demonstrate that endocrine regulation, environmental signals and muscle reprogramming play a key role to define muscle trajectories (Carballo *et al.* 2018). Shared DETs between genetic families and gender were the cocaine- and amphetamine-regulated transcript, early growth response 3 and NACHT, LRR and PYD domains-containing protein 12 and others involved in energy metabolism were differentially expressed both for gender and genetic family indicating that appetite signals and energy balance are key factors controlling growth in sole.

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References

Córdoba-Caballero J., Seoane P., Jabato F.M., Perkins J.R., Manchado M., Claros M.G. (2020) An improved *de novo* assembling and polishing of *Solea senegalensis* transcriptome shed light on retinoic acid signalling in larvae. Scientific Reports 10, 20654.

Carballo C., Firmino J., Anjos L., Santos S., Power D.M., Manchado M. (2018) Short- and long-term effects on growth and expression patterns in response to incubation temperatures in Senegalese sole. Aquaculture 495, 222-31.

Guerrero-Cozar I., Jimenez-Fernandez E., Berbel C., Córdoba-Caballero J., Claros M.G., Zerolo R., Manchado M. (2021a) Genetic parameter estimates and identification of SNPs associated with growth traits in Senegalese sole. Aquaculture 539, 736665.

Guerrero-Cozar I., Gomez-Garrido J., Berbel C., Martinez-Blanch J.F., Alioto T., Claros M.G., Gagnaire P.A. & Manchado M. (2021b) Chromosome anchoring in Senegalese sole (*Solea senegalensis*) reveals sex-associated markers and genome rearrangements in flatfish. Scientific Reports 11, 13460.

GENETIC ASSOCIATION STUDY REVEALS NEW SHAPE QUALITY-RELATED MARKERS IN SENEGALESE SOLE (*Solea senegalensis*)

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Abstract
Senegalese Sole is a high economically important flatfish. Due to the importance of morphology on sole commercialization, it is important to establish the genetic estimates and identify molecular markers associated to shape quality. In this study, genetic estimates for two traits associated with the caudal fin (length (CFL) and width (CFW)) were determined. A total of 1 840 animals were evaluated at harvest (800 d). Heritability for CFL was 0.29±0.07 and for CFW 0.32±0.08. Genetic correlation between both traits was 0.85±0.06. Genetic correlation with other three morphological traits (body height at the insertion of the pectoral fin (BHP), body maximum height (BMH) and caudal peduncle height (CPH)) and weight ranged between 0.78 and 0.85 and for ellipticity -0.57 and -0.63 for CFL and CFW, respectively. Genetic association analysis using a low-density array identified four significant SNP markers for CFW and two shape related traits (BHP and CPH).
Keywords: *molecular markers, shape quality, Senegalese sole*
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1- Introduction

Senegalese sole (*Solea senegalensis*) is a flatfish species whose aquaculture is rapidly growing in Southern Europe. This species is characterized by large morphological and behavioral changes during metamorphosis from a pelagic larva to a benthic juvenile stage. During its life cycle, this sole tends to accumulate skeletal malformations that modifies its typical elliptical body shape (Losada *et al.* 2014) with a great effect on market value and consumer acceptance, hence major efforts to obtain high-quality shaped fish are ongoing. A previous study of our group demonstrated a high heritability for some morphological traits such as ellipticity and growth (Guerrero-Cozar *et al.* 2021a).

However, morphological traits related to caudal fin morphology are less studied in spite of that have impact on consumer perception of marketed product and in fish health. While genetic estimates are very important for genetic selection schemes, genome-wide association studies (GWAS) using SNPs are gaining popularity to improve the precision of genetic estimates Hence, the aim of this study is to obtain genetic estimates for two traits associated with caudal fin (length and width) and carried out a GWAS analysis to identify SNP markers associated with four shape-related traits (ellipticity, body height at the insertion of the pectoral fin, body maximum height and caudal peduncle height) and the two caudal fin traits. The information is relevant for genetic breeding programs

2- Materials and methods

A total of 1 840 animals (1 007 males and 833 females) were evaluated at harvest (800 d) for caudal fin length (CFL) and width (CFW) using ImageJ software. Genetic estimates were determined as previously indicated in Guerrero-Cozar *et al.* (2021a) including the evaluation batch, the sex and amoebic disease as fixed factors. Statistical ANOVA analysis for phenotypic data were carried out using SPSS v25 and for BLUP analysis with WOMBAT. Moreover, GWAS analysis was carried out using a low-density array (Guerrero-Cozar *et al.* 2021b) for the two caudal fin traits investigated in this study and four shape-related traits (ellipticity, body height at the insertion of the pectoral fin (BHP), body maximum height (BMH) and caudal peduncle height (CPH)) whose genetic estimates were reported in Guerrero-Cozar *et al.* (2021a). For this purpose, four genetic families (n = 279) were selected according to weight at harvest and ellipticity(E). Slow-growing (SG) families had an average weight (W) and E of 262.8 g and 0.416 for SG1 and 229.5g and 0.444 for SG2, respectively. W and E values for fast-growing (FG) families were 442.8g and 0.420 for FG1 and 382.0g and 0.405, respectively. DNA was isolated from blood or caudal fin samples using Qiagen DNA kit according to the manufacturer's recommendation. Quality and quantity of DNA were measured using a Nanodrop ND-8000 spectrophotometer and after electrophoresis in agarose gels. DNA arrays were loaded with an

Openarray AccuFill ™ System and amplified in a real-time PCR (QuantStudio12KFlex model) according to the manufacturer's instructions. GWASpoly was used for GWAS analysis.

3- Results and discussion

Mean values for CFL and CFW were 2.75±0.97 and 3.27±1.11 cm, respectively. Statistical ANOVA analysis showed statistically significant differences associated with the evaluation batch and amoebic disease in both traits, while only CFW showed significant differences associated with gender (3.37±1.05 and 3.12±1.01 cm for females and males, respectively). About genetic estimates for caudal fin traits, a low-moderate heritability was observed for CFL (0.29±0.07) and CFW (0.32±0.08). Heritability for CFL was lower than that observed in the flatfish Japanese flounder (0.6) (Liu et al. 2014) or in Atlantic cod (0.38-055) (Gjerde *et al.* 2004) that could explained by the bottom-dwelling and swimming behavior of sole more prone to traumatic damages in caudal fin. Genetic correlation between CFL and CFW was 0.85±0.06. These two traits were high positively correlated with BHP, BMH, CPH and weight (ranging from 0.78-0.85), and negative with ellipticity (-0.57±0.13 and -0.63±0.11 for CFL and CFW, respectively). These data indicate that selection for bigger fish will also produce fish with longer caudal fin improving lanceolate perception of soles (Guerrero-Cozar *et al.* 2021a).

Association analysis using a mixed linear model approach and a general model revealed four significant markers: Sse_0569, located in linkage group (LG) 2, was significant for CPH and CFW and it was associated with gem-associated protein 8 (*gemin8*); Sse_0034, located in LG5, was significant for CPH and it was associated with reticulon-4 receptor (*rtn4r*); Sse_0590 located in LG19, that was significant for BHP and it was associated to inactive rhomboid protein 1 (*rhbdf1*); Sse_1980, located in LG21 was significant for CFW and it was associated with general transcription factor 3C polypeptide 4 (*gtf3c4*) . No significant association in the panel evaluated for E, BMH and CFL traits was found. In rat, *gtf3c4* is located within a region where localizes a QTL rat associated with body weight (Casiro *et al.* 2017). In mouse, *rhbdf1* is located in a QTL for skeletal muscle weight QT Overall, these results demonstrate that caudal fin morphological traits are selectable and can therefore be included in a genetic breeding program for the improvement of Senegalese sole production. Moreover, the DNA mid-density chip revealed as a very useful for selection programs in aquaculture.

Acknowledgment

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References

Casiro S., Velez-Irizarry D., Ernst C.W., Raney N.E., Bates R.O., Charles M.G., Steibel J.P. (2017). Genome-wide association study in an F2 Duroc x Pietrain resource population for economically important meat quality and carcass traits. Journal of Animal Science 95, 545–558.

Gjerde B., Terjesen B.F., Barr Y., Lein I., Thorland I. (2004) Genetic variation for juvenile growth and survival in Atlantic cod (Gadus morhua). Aquaculture 236, 167-77.

Guerrero-Cózar I., Jimenez-Fernandez E., Berbel C., Espinosa E., Claros MG., Zerolo R., Manchado M. (2021a) Genetic estimates for growth and shape-related Traits in the flatfish Senegalese Sole. Animals 11, 1206.

Guerrero-Cózar I., Jimenez-Fernandez E., Berbel C., Córdoba-Caballero J., Claros MG., Zerolo R., Manchado M. (2021b). Genetic parameter estimates and identification of SNPs associated with growth traits in Senegalese sole. Aquaculture 539, 736-765.

Liu Y., Jiang L., Liu H., Yang R. (2014). Phenotypic and genetic parameter estimation of morphological traits related to axial body growth in Japanese flounder. Fisheries Science, 80, 317-321.

Lionikas A., Meharg C., Derry J.M., Ratkevicius A., Carroll A.M., Vandenbergh D.J., Blizard D.A. (2012). Resolving candidate genes of mouse skeletal muscle QTL via RNA-Seq and expression network analyses. BMC Genomics 13, 592.

Losada A.P., de Azevedo A.M, Barreiro A., Barreiro J.D., Ferreiro I., Riaza A., Quiroga M.I., Vázquez S. (2014). Skeletal malformations in Senegalese sole (Solea senegalensis Kaup, 1858): gross morphology and radiographic correlation. Journal of Applied Ichthyology 30, 804-808.

MULTITROPHIC-AQUAPONIC SYSTEM FOR SUSTAINABLE FOOD PRODUCTION

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Abstract
The aim of this study was the growth performance of two different fish species (*Dicentrarchus labrax*, and Oreochromis sp) and the lettuce plants (*Lactuca sativa* var. Green Towers) in three indoor small scale aquaponic systems. NO_3 concentration played the most important role as it was essential for the growth of the plants. After the trial period, fish had a statistical significant specific growth performance but a non-significant feed conversion ratio. In addition, for the plants the most effective treatment was the one with the highest initial biomass, which gave 1.26 kg/m^2 lettuce production.
Keywords: *Aquaponics, Dicentrarchus labrax, Oreochromis sp, Lactuca sativa*
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1. Introduction

Aquaponics combine the culture of fish and plants in closed recirculating water systems (Rakocy *et al.* 2004) and it contributes to water resource management, biodiversity conservation and energy savings (Buzby & Lin 2014). The present study was conducted to determine the production of *D. labrax* and red tilapia in an aquaponic system with the lettuce (*Lactuca sativa* var. Green Towers).

2. Materials and Methods

The experiment was conducted in three indoor small scale aquaponic systems for 45 days. Each system had three fish tanks, mechanical and biomechanical filter, water pump, one grow-bed filled with clay, air pumps to oxygenate the water and one HPS light 400 W over each grow-bed (12L:12D). *Dicentrarchus labrax* and red tilapias with initial biomass of 1246.24g, 758.13 g and 925.90 g, respectively were used combined with eight lettuce plants (*Lactuca sativa* var. Green Towers) placed in each grow-bed in each aquaponic system. pH, temperature, and O_2, were recorded and it was 6.70 ± 0.10, 21.73 ± 0.33 and 8.10 ± 0.13, respectively, and twice week measurements of nitrate (NO-3) were taken. SPSS version 26 was used for analyzing the data with $p>0.05$.

3. Results

No significant differences were found among the treatments in NO_3 concentrations. The higher concentration of NO_3 was in aquaponic system 1 (Table 1). The absorption of NO_3 I from the plants in all aquaponic systems was 12.82 ± 0.6.

Table 1. Nitrate (NO_3) concentrations

mg l^{-1}	Aquaponic system 1	Aquaponic system 2	Aquaponic system 3
NO_3 (GBin)	171.15 ± 10.53	140.38 ± 15.38	163.46 ± 9.65
NO_3 (GBout)	157.69 ± 9.55	128.85 ± 13.82	150 ± 9.81

There were no significant differences ($p>0.05$) between weight gain and feed conversion ratio (FCR%) of *D. labrax* while specific growth rate (SGR%) was statistically significant ($p<0.05$) different between aquaponic systems 1 and 2 (Table 2). The weight gain of *D. labrax* was higher in aquaponic system 3 2 and lower in aquaponic system 2 while the weight gain of Oreochromis sp. was higher in aquaponic system 1 and lower in aquaponic system 2.

Table 2. Weight gain, FCR, SGR values.

	Aquaponic system 1	Aquaponic system 2	Aquaponic system 3
Weight gain (gr)	48.82 ± 0.07	38.70 ± 7.35	46.84 ± 2.82
FCR (%)	1.46 ± 0.07	1.67 ± 0.53	1.24 ± 0.09
SGR (%/d)	0.97 ± 0.02^{a}	0.83 ± 0.1^{b}	1.19 ± 0.05^{a}

In addition, the higher FCR observed in aquaponic system 2 while the lower in aquaponic system 3. The best biomass production of lettuce was in aquaponic system 1 (1.26 kg/m^2) followed by aquaponic system 3 (1.11 kg/m^2) and aquaponic system 2 (0.52 kg/m^2). The stem height at aquaponic system 1 and 2 was not statistically significantly different ($p>0.05$) while aquaponic system 3 showed differences ($p<0.05$) compared with the other two aquaponic systems.

4. Discussion

Rakocy *et al.* (2006) found that the recommended NO_3 concentrations on lettuce in aquaponic systems are between the range of 116.42 mg L^{-1} to 185.93 mg L^{-1}. In the present study, NO_3 concentrations were similar to the above accepted range values and the plant intake was similar in all the aquaponic systems (12.82 ± 0.6 mg L^{-1}). Plants, in large NO_3 concentrations did not utilize the available higher amounts but absorb only the required quantity for their growth (Vandam *et al.* 2017). Even though the availability of NO_3 was sufficient in every aquaponic system and the assimilation was the same from the plants, every aquaponic system had different plant growth performance. In aquaponic system 2, FCR was the lowest and SGR was the higher. Ani *et al.* (2021) found that less fish in fish tank give better growth rate. Many studies (Palm *et al.* 2014; Greenfeld *et al.* 2018; Maucieri *et al.* 2019) agree with this result. The results of this study showed that the number of fish in the tanks depends on the design of the aquaponics system and there is a minimum and a maximum fish biomass that supports the best plant growth performance.

References

Ani J.S., Manyala J.O., Masese F.O., Fitzsimmons K. (2021) Effect of stocking density on growth performance of monosex Nile Tilapia (Oreochromis niloticus) in the aquaponic system integrated with lettuce (*Lactuca sativa*). Aquaculture and Fisheries. https://doi.org/10.1016/j.aaf.2021.03.002

Buzby MK, Lin LS. (2014). Scaling aquaponic systems: balancing plant uptake with fish output. Aquacultural Engineering 63:39–44 DOI 10.1016/j.aquaeng.2014.09.002.

Greenfeld A., Becker N., Mcilwain J., Fotedar R., Bornman J. F. (2018). Economically viable aquaponics? Identifying the gap between potential and current uncertainties. Reviews in Aquaculture, 1–15. https://doi.org/10.1111/raq.12269

Maucieri C., Nicoletto C., Zanin G., Birolo M., Trocino A., Sambo P. (2019). Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. PloS One 14(5), https://doi.org/10.1371/journal.pone.0217561

Palm H.W., Bissa K., Knaus U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part II: Fish and plant growth. Aquaculture, Aquarium, Conservation & Legislation *7*(3), 162–175.

Rakocy J.E, Shultz R.C, Bailey D.S., Thoman E.S. (2004) Aquaponic Production of Tilapia and Basil: Comparing a Batch and Staggered Cropping System. Acta Horticulturae 648(648), 63-69. DOI: 10.17660/ActaHortic.2004.648.8

Vandam D.A., Anderson T.S., De Villiers D., Timmons M.B. (2017) Growth and Tissue Elemental Composition Response of Spinach (Spinacia oleracea) to Hydroponic and Aquaponic Water Quality Conditions. Horticulturae 3, 32.

GIS IN SAMPLING STRATEGY OF AQUACULTURE ENVIRONMENTAL IMPACTS ASSESSMENT

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Abstract

For the environmental impacts assessment of a marine aquaculture, a systematic sampling is required. In this study, a sampling strategy based on Geographical Information Systems (GIS) was developed, in order to organize point sampling for *in situ* measurements. The results will be used for spatial modelling and will be combined with satellite data.

Keywords: *Aquaculture, GIS, sampling method, satellite data*

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1. Introduction

Floating fish farming is a human activity that mainly takes place into the sea. As a result, through its operation affects the environment where is located. Several studies have shown that affects the water column (Neofitou & Klaoudatos 2008) and the biochemical parameters of the sediment (Neofitou *et al.* 2010).

However, there is a need to assess and monitor the aquaculture environment based on an organized sampling method (Neofitou *et al.* 2019). In order to achieve this, it is necessary to organize a study, which will be based on point sampling in order to investigate and monitor the environmental impact of a fish farming. This methodological approach was developed in a Geographic Information System (GIS) and will be applied for *in situ* measurements.

The sampling method selected was the Spatial Sampling and especially the Point Systematic Sampling. The method of Spatial Sampling is often used in environmental studies and utilizes GIS technologies to conduct spatial sampling, GPS to locate sampling points on the ground and remote sensing for cartographic backgrounds (Iliopoulou 2017).

2. Material and Methods

The present study is carried out at the facilities of a fish farming, which is located in Limnionas bay, in Milina Magnesias on the southeastern coast of Pagasitikos Gulf (Figure 1).

Figure 1. Location of the study area

Initially, the study area was delimited by a polygon (Figure 2a) digitized along the coastline. The same process was applied to define the area around the cages. Buffer analysis was used in order to determine a thirty (30) meter zone around coastline (Figure 2b) and cages (Figure 2c). The buffer zone was altered according to the spatial resolution of the satellite imagery. Then, the cages' buffers were removed (Figure 2d) from the initial study area.

Systematic point sampling requires a grid. The intersections of the grid lines are the sampling points. The dimension of the grid cellis is calculated according to the number of points of the sample. A grid should be created including as many points as required in the sampling. However, systematic sampling could be implemented with a step that seems suitable on the map (Iliopoulou 2017). Point Systematic Sampling was chosen for sampling planning.

The selected sampling stations consists of points on the map which were defined by X and Y coordinates and were chosen on regular basis and in a stable and predetermined way. Specifically, each of them was located in equal distance from each other, along the X and Y axes (Koutsopoulos 2009). If some points are difficult to be accessed or do not have the expected properties, they have to be replaced (Iliopoulou 2017). A 150×150m grid was created. The grid was adjusted (rotation and movement) in parallel to the cages in order to cover the study area and include the predefined number of points. These points could be used for interpolation in order to predict the values at the points where there are no measurements or to be related with satellite data.

3. Results

In total, twenty-seven (27) sampling stations were selected (Figure 2e). Among them, three (3) were located inside the floating fish farm and one (1), which was the control station, at a distance approximately 600m away from the cages. The rest of them were complementary stations to examine the impact of fish farming on the environment.

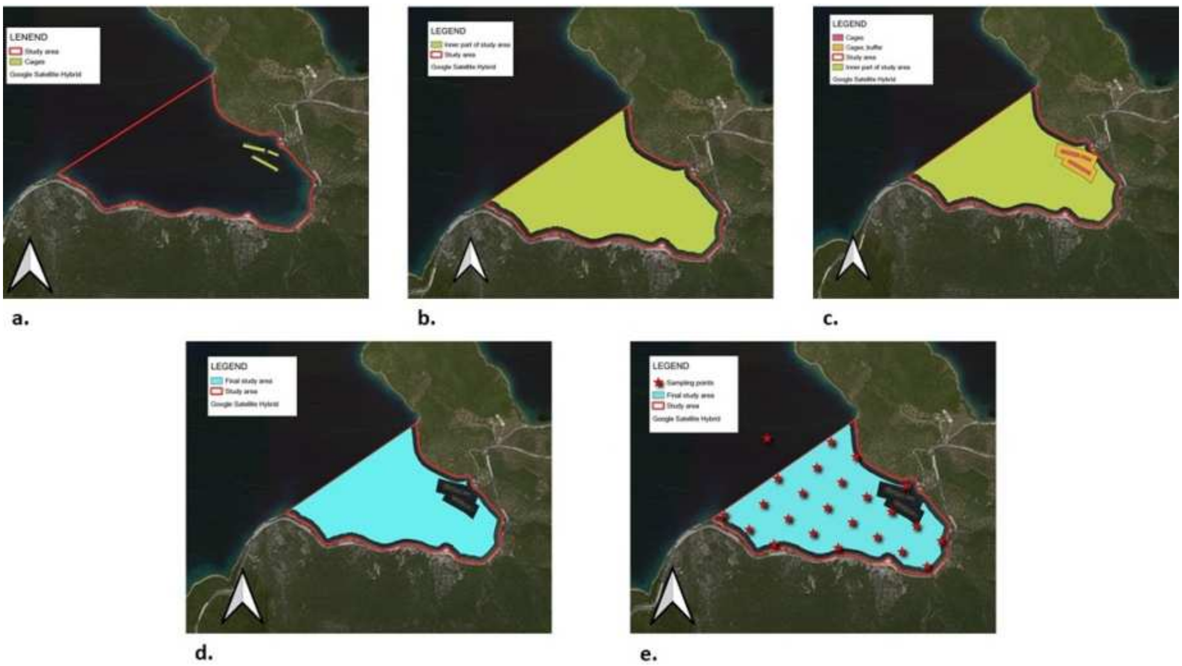

Figure 2. Sampling method using GIS

References

Iliopoulou P. (2017). Geographical Analysis, Hellenic Academic Libraries Link, Athens, p. 39-40.

Koutsopoulos K. (2009). Space Analysis Treatise: Theories and Methods Volume I, Papasotiriou, Athens, p. 164-165.

Neofitou N., Klaoudatos S. (2008). Effect of fish farming on the water column nutrient concentration in a semi-enclosed gulf of the Eastern Mediterranean. Aquaculture Research 39, 482-490.

Neofitou N., Papadimitriou K., Domenikiotis C., Tziantziou L., Panagiotaki P. (2019). GIS in environmental monitoring and assessment of fish farming impacts on nutrients of Pagasitikos Gulf, Eastern Mediterranean. Aquaculture 501, 62-75.

Neofitou N., Vafidis D., Klaoudatos S. (2010). The spatial and temporal effect of fish farming on benthic community structure in a semi enclosed gulf of the Eastern Mediterranean. Aquaculture Environment Interactions 1, 95-105.

THE EFFECT OF FISHMEAL REPLACEMENT BY *Zophobas morio* LARVAE MEAL ON PROXIMATE COMPOSITION OF GILTHEAD SEABREAM (*Sparus aurata*)

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Abstract
The present study was conducted in order to evaluate the effects of dietary fishmeal replacement by full- and low-fat *Zophobas morio* meal on whole body and muscle tissue proximate composition of *Sparus aurata* juveniles. Results indicate that dietary FM protein can be replaced up to 10% by full-fat or 30% by low-fat *Z. morio* meal without any major effect on body and muscle tissue composition of *S. aurata*.
Keywords: *Zophobas morio, insect, proximate, nutritional value, fishmeal*
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1.Introduction

Aquaculture is still on the search for suitable and sustainable alternative protein sources to replace fishmeal in aquafeeds. After their recent approval in the European aquafeed chain, insect proteins are considered as suitable alternatives to be used in aquafeeds since they are rich in essential amino acids (Henry *et al.* 2015). The use of superworm, *Zophobas morio*, as protein source in fish diets has not been adequately studied so far, despite its high nutritive value (Rumbos & Athanassiou 2021). In our growth trials with *Sparus aurata*, both full fat and defatted *Z. morio* larvae meal proved to be valuable in replacing dietary fishmeal protein without retarding growth (Asimaki *et al.* 2020; Asimaki *et al.* 2021). In order to better assess the efficiency of *Z. morio* protein in *S. aurata* nutrition we studied its effects on whole body and muscle tissue proximate composition of fish.

2. Materials and Methods

A total number of 540 *S. aurata* juveniles of 3.4g initial mean weight were allocated into 18 glass tanks (125L) within a closed recirculation seawater system and after an acclimatization period of 10 days divided into 6 dietary groups in triplicate tanks, each feeding on a different diet. Six isonitrogenous (52%) and isoenergetic (20 Mj/Kg) diets were formulated at which the fishmeal protein of the control diet (FM) was replaced by full-fat *Z. morio* larvae meal at 5% (ZFF5) and 10% (ZFF10) and by low-fat *Z. morio* meal at 10% (ZLF10), 20% (ZLF20) and 30% (ZLF30). After 100 days of feeding, four fish per tank were randomly selected for whole body proximate composition and four fish per tank for muscle tissue proximate composition according to AOAC methods. Specifically, moisture content was calculated by drying the samples in an oven at 105°C and ash content was determined with incineration at 600°C.

Crude protein content of the samples was determined by Kjeldahl analyses. To measure crude lipid content, lipids were extracted from the samples with petroleum ether in a Soxhlet extractor. The energy of the samples was measured with adiabatic bomb calorimetry. Comparison of means was performed by subjecting the data to one-way analysis of variance at a significance level of 0.05 using the IBM SPSS Statistics 24 statistical package. The significant differences between treatments were determined using Tukey's multiple comparison test.

3. Results and Discussion

Generally speaking, the replacement of dietary fishmeal by *Z. morio* did not alter extensively the body and tissue nutrient composition of *S. aurata*. The muscle tissue proximate composition of fish was unaffected (P>0.05) by the diet with the exception of the muscle energy content of fish fed the ZFF10 diet, which was significantly higher compared to that of the FM group, mainly due to its increased lipid content. This is an indication that the full-fat *Z. morio* meal may cause an increased lipid accumulation in the muscle tissue, although this did not occur in the whole body of these fish. Regarding the whole body composition, some significant differences were detected among the dietary groups. The body moisture of fish fed the diet ZLF10 was significantly decreased due to its increased body lipid content in these fish. However, body lipid contents did not differentiate among the groups indicating a similar lipid metabolism. ZLF10 fish had also a lower body protein content, but there was not any significant trend on body proteins with higher fishmeal replacement.

The fishmeal replacement by low-fat *Z. morio* at 30% led to increased body ash in fish that may indicate a negative effect of high inclusion levels of *Z. morio* on the mineral metabolism and deposition in fish. Literature on the use of *Z. morio* is extremely limited. Jabir *et al.* (2012) reported an insignificant effect of *Z. morio* as 25% fishmeal replacer on body composition of *Oreochromis niloticus*, but Alves *et al.* (2021) observed an increased body lipid and moisture content and a decreased body protein and ash content when the same species was fed on diets with 30% inclusion level of full-fat *Z. morio*. A decreased body protein and ash content with incremental dietary *Z. morio* was also observed in *Oncorhynchus mykiss* (Shekarabi *et al.* 2021). The present results indicate that dietary FM protein can be replaced up to 10% by full-fat or 30% by low-fat *Z. morio* meal without any major effect on body and muscle tissue composition of *S. aurata*.

Table 1. Whole body and muscle proximate composition (% of dry matter) and energy content (MJ kg^{-1}) of *S. aurata* fed with the experimental diets.

	FM	ZFF5	ZFF10	ZLF10	ZLF20	ZLF30
Whole body composition						
Moisture	67.6 ± 0.7^{ab}	69.1 ± 0.5^{a}	68.0 ± 0.7^{a}	65.7 ± 0.6^{b}	67.9 ± 0.7^{a}	67.4 ± 1.1^{ab}
Crude protein	53.4 ± 1.4^{ab}	56.2 ± 2.1^{a}	53.8 ± 1.5^{ab}	51.4 ± 1.3^{b}	54.0 ± 2.5^{ab}	53.0 ± 1.4^{ab}
Crude lipid	36.7±1.7	33.8±2.9	35.6±1.1	37.5±1.3	35.5±1.2	35.0±7.3
Ash	9.7 ± 0.2^{a}	9.9 ± 0.4^{ab}	9.9 ± 0.3^{ab}	9.9 ± 0.4^{ab}	9.7 ± 0.2^{ab}	10.6 ± 0.5^{b}
Gross energy	26.1±0.1	25.6±0.4	25.8±0.2	25.9±0.3	26.0±0.1	25.5±0.1
Muscle composition						
Moisture	75.4±0.3	75.0±0.9	74.8±0.9	74.9±0.5	74.0±0.5	74.9±0.3
Crude protein	83.3±1.4	82.3±1.9	81.4±0.6	82.2±0.9	81.3±2.0	83.3±1.2
Crude lipid	8.4±0.6	9.4±1.9	10.3±1.4	9.8±0.7	10.7±1.7	8.4±0.5
Ash	6.7±0.3	6.6±0.2	6.4±0.2	6.6±0.4	6.4±0.2	6.7±0.2
Gross energy	23.1 ± 0.3^{a}	23.3 ± 0.3^{ab}	23.8 ± 0.2^{b}	23.6 ± 0.1^{ab}	23.7 ± 0.2^{ab}	23.2 ± 0.1^{ab}

Values represent means ± st. deviation (n=3). Values within each row not sharing a common superscript letter are significantly different ($P<0.05$).

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References

Alves A.P., Paulino R.R., Pereira R.T., da Costa D.V., Rosa P.V. (2021). Aquaculture Research 52, 529-540.

Asimaki A., Psofakis P., Ekonomou G., Mente E., Rumbos C.I., Athanassiou C.G., Fountoulaki E., Henry M., Karapanagiotidis I.T. (2020). IFW 2020 - Insects to Feed the World Conference, 23-26 November 2020, Quebec, Canada. Journal of Insects as Food and Feed 6 Supplement 1, p. S24.

Asimaki A., Psofakis P., Neofytou M.C., Gkalogianni E.Z., Mente E., Rumbos C.I., Athanassiou C.G., Fountoulaki E., Henry M., Karapanagiotidis I.T. (2021). Proceedings of the European Aquaculture Society 2020, 12-15 April 201, Online, pp. 38-39.

Henry M., Gasco L., Piccolo G., Fountoulaki E. (2015). Animal Feed Science and Technology 203, 1-22.

Jabir A.R., Din M., Razak S.A., Vikineswary S. (2012). African Journal of Biotechnology 11, 6592–6598.

Shekarabi S.P.H., Mehrgan S.M., Banavreh A. (2021). Aquaculture Nutrition. 2021, 1–12.

Rumbos C.I., Athanassiou C.G. (2021). Journal of Insect Science 13, 1-11.

NATURAL ANTI-FOULING COATINGS ON KNOTTED NYLON FISH FARMING NETS

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Abstract
The study aims at the most effective corrosion protection of the fish farming cages' nets, using environmentally friendly anti-fouling agents, antimicrobials and coating substances of natural origin, in order to minimize the marine biological settlement avoiding the contamination of the aquatic environment with harmful for the biodiversity and product quality agents. The use of extracts with proven anti-fouling and antimicrobial action derived from edible plants, herbs and by-products of the agri-food industry was proposed, as well as the use of natural resins as a coating. The efficient coating based on homogeneity, roughness and fiber uniformity was evaluated and the effect of natural coatings, based on pine resin and EcoPoxy resin, on mechanical properties of knotted nylon fish farming nets was determined. The flexibility of the nets coated with oregano essential oil and pine resin was similar to the untreated nets and the copper coated samples. Maintaining the flexibility is very important, as a possible change would affect other parameters in the cages of fish farms.
Keywords: *marine biological settlement, pine resin, epoxy resin, oregano oil, flavonoids*
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Introduction

Fish farming sector is a pillar for the Mediterranean countries' economies. One of the biggest problems that fish farmers face is the marine erosion of cage nets, which leads to a significant reduction in production. Undesirable deposition and accumulation of marine organisms on the nets is a complex problem, since it destroys the cages and reduces the fish culture. The colonies of the corrosive organisms in the nets compete for feeding with the cultivated fish, release toxic substances and prevent the circulation of oxygen (Bloecher & Floerl 2020). The cost of replacing the nets is extremely high, while common methods of tackling biofouling, such as mechanical cleaning of the nets and corrosion-resistant coating with Cu_2O, have many drawbacks. The former method is labour intensive and the second one increases the levels of copper, which is toxic and is a source of contamination of fish farms, polluting not only the surrounding marine environment, but especially the farming, drastically affecting the quality of the fish produced and ultimately the health of consumers (Swain & Shinjo 2014). It is therefore necessary to find efficient and environmentally sustainable solutions to tackle the phenomenon of biofouling (Comas *et al.* 2021). The development of anti-corrosion coatings from bioactive substances of natural origin from herbs and by-products of the agri-food industry was suggested in this study, meeting the standards of the European Commission Biocidal Products Directives (EC Biocide Directive, 98/8 / EC). In this way, the health of the final consumer is directly benefited, as products contaminated with prohibited substances, such as copper and zinc, will not reach him. It also proposes an environmentally friendly method of corrosion protection that does not affect the ecosystem and reduces the long-term adverse effects of anti-corrosion methods currently used at European level. Finally, the proposed methodology does not require labor intensity, as in the mechanical cleaning of nets, and labor costs are significantly reduced.

Materials Methodology

A study and comparison of the mechanical properties of the original specimens with those coated through immersion in resin bath was conducted. The experimental methodology is presented in Table 1. Organic solvents (methanol and acetone) of analytical grade (Merck, CH) were used. Pure oregano oil from Organic Essential Oils-Zoi Filippou MEPE, GR and natural antioxidant flavonoid complex (Flavomix AX200, POLYPAN SA, GR) were used. The knotted nylon fish farming nets were obtained by DIOPAS SA, GR. Pine resin was naturally collected, while epoxy resin used was Epoxy Immersion Resin 1050 Resoltec, ALFAKEM SA, GR.

Table 1.Methodology

Process	Conditions
Immersion Bath preparation	20 min stirring at 10000 rpm at T=20°C. Resin (g):solvent ratio (ml) 1:20 further diluted in water until double volume, adding essential oil or FLAVOMIX AX200 in standard concentration 5% of the final mixture using proper emulsifiers. For the dilution of pine resin methanol was used while for the epoxy resin dilution acetone.
Immersion	Immersion of 30x30 cm net specimens in resin bath, T= 25°C, t= 1h
Drying	Drying of immersed specimens in vacuum dryer at 70°C for 3 h
Mechanical properties	ISO 1806:2002(E) using Zwick mechanical test device (Z2.5 / TN1S, Ulm, Germany). Each type of sample was measured 5 times. T=20°C, RH=65% and velocity 1000 mm/min

Results

In Table 2 the mechanical properties of coated and ucoated fish farming nets are presented. For the force at break and nomial strain at break wet net speciments were evaluated.

Table 2. Mechanical properties of coated and uncoated knotted nylon fish farming nets

Sample Nr	Type of coating	Dry half mesh width $w_{1/2}$ (mm)	Loops along the twine, n_l	Hardness, h*	Pretension P (N)	Force at break (N)	Nominal strain at break (%)
1	Pine resin	8	3	0.95	5.32	513	85
2	Pine resin+ Oregano oil	8	3	0.90	6.31	577	74
3	Pine resin+Flav.	9	3	0.88	8.21	623	79
4	Epoxy resin	6	3	1.05	5.42	501	81
5	Epoxy resin + Oreg. oil	8	3	0.96	8.30	658	69
6	Epoxy resin+Flav.	7	3	0.98	6.50	517	71
9	No coating	11	3	0.86	8.44	631	71
10	Copper	10	3	0.95	7.59	595	69

Conclusions

The flexibility of the nets coated with oregano essential oil and natural flavonoids complex and pine resin was similar to the untreated nets and the copper coated samples. Maintaining the flexibility is very important, as a possible change would affect other parameters in the cages of fish farms. Flexibility is sought so that the ripple energy is absorbed and not transmitted through the system to the cages. The weight of the coated nets, their hardness and the stability of the dimensions, which are important factors in nets' manufacturing have hardly changed. The results show that the antifouling treatment reduces the breaking strength of a netting material compared to the wet netting. The average strain at break was 75% for wet materials.

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References

Bloecher N., Floerl O. (2020). Efficacy testing of novel antifouling coatings for pen nets in aquaculture: How good are alternatives to traditional copper coatings? Aquaculture 519, 734-736.

Comas J., Parra D., Balasch J.C., Tort L. (2021). Effects of Fouling Management and Net Coating Strategies on Reared Gilthead Sea Bream Juveniles. Animals 11(3), 734.

Swain G., Shinjo N. (2014). Comparing biofouling control treatments for use on aquaculture nets. International Journal of Molecular Sciences 15(12): 22142-22154.

DIETARY SUPPLEMENTATION WITH ESSENTIAL OIL NANOEMULSION ON GROWTH PERFORMANCE AND BLOOD PROFILES OF GILTHEAD SEABREAM (*Sparus aurata*)

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Abstract
This study evaluated the effects of dietary supplementation with a nanoemulsion containing an essential oil mix on growth performance and blood profiles of *Sparus aurata*. The results showed that the inclusion of the nanoemulsion up to 5ml/kg in the diet did not have any major effect on feed palatability and acceptance by fish, while neither retarded nor enhanced fish growth. However, the essential oil mix had a clear immunostimulant effect.
Key words: *essential oils, nanoemulsion, nutrition, health, seabream*
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1. Introduction

Plant essential oils are known to have growth promoting, antibacterial, antifungal, antioxidant and anti-inflammatory properties (Sutili *et al.* 2018). Their benefits as feed supplements have been extensively studied in non-ruminants (Zeng *et al.* 2015), but research on fish is limited, although there is an increasing interest of the aquaculture industry to use them as environmental friendly and effective alternatives to synthetic antibiotics (Gholipourkanani *et al.* 2019). Thus, the aim of the present study was to investigate the effects of dietary supplementation with a nanoemulsion containing an essential oil mix on growth performance and blood profiles of *Sparus aurata*.

2. Materials & Methods

A commercial basal diet (Diet E0, 56% crude protein, 13% crude lipid) was used as a control. The basal diet was supplemented with a commercial nanoemulsion containing a mix of plant essential oils (oregano oil, lavender oil, clove oil, rosemary oil and absinthe oil) at 1ml/Kg (Diet E1), 3 ml/Kg (Diet E3) and 5 ml/Kg (Diet E5). The nanoemulsifier was incorporated into each diet by carefully spraying a corresponding concentration and then was allowed to be absorbed by the pellets for at least 3 hours under a fume cupboard. Triplicate groups of 30 *S. aurata* (mean weight 3.7±0.6 g) in a closed recirculation system were fed the diets at satiation two times per day for 61 days. At the end of the trial, blood samples, under anaesthesia, were taken by venipuncture from X fish/dietary group. Samples were collected in microtubes containing EDTA (ratio 1.26 mg/0.6 mL) as the anticoagulant agent. All samples were analyzed immediately by an automatic method (Fazio *et al.* 2019) to assess the haematological profile. ANOVA and Tukey's test were used to compare means among groups at a P level of 0.05 using the SPSS Statistics 24 package.

3. Results and Discussion

Survival, voluntary feed intake, growth and feed utilization were similar (P>0.05) among groups (Table 1) indicating that the nanoemulsion of plant essential oils at all tested levels did not have any major effect on feed palatability and acceptance by fish, while neither retarded nor enhanced fish growth. Increased dietary inclusion of the essential oil mix caused a significant increase in RBC, WBC, Hgb, Hct indicating an increased specific immune capacity and a modulation of several biological processes related to innate immune effector cells, such as leukocyte activation, neutrophil activation and neutrophil degranulation. Several studies have evaluated the use of various plant essential oils in the diets of farmed fish (reviewed by Sutili *et al.* 2018), including *S. aurata* (Perez-Sanchez *et al.* 2015). It is evident that plant essential oils can serve as growth and health promoting supplements (e.g. Zheng *et al.* 2009; Zeppenfeld *et al.* 2016), although there are studies showing an insignificant growth effect (Ebrahimi *et al.* 2020) as did the present study. Literature on the dietary supplementation of essential oils upon fish blood profile is extremely limited, but an increased number of WBC and other immune parameters have been reported previously in seabream (Firmino *et al.* 2021). Certainly, the effects are largely dependent on various factors including the origin and extraction process of the essential oil, the incorporation level and administration method, and the stability of the product amongst many other. In this context, the use of blends of essential oils may have a better effect than a single oil due to the synergistic interactions of the individual constituents, while the nanoemulsion process could enhance stability/quality of the supplement. However, this study did not reveal such benefits and further research is needed so to fully evaluate the use of plant essential oils in the diets of farmed fish.

Table 1. Growth performance and blood profile of *S. aurata* fed the experimental diets.

	E0	E1	E3	E5
Growth parameters				
Survival (%)	97.8 ± 1.92	98.9 ± 1.92	94.4 ± 6.94	91.67 ± 2.36
Feed intake (g/fish)	29.67 ± 1.68	28.22 ± 1.87	28.72 ± 2.72	26.21 ± 0.87
FBW (g/fish)	29.43 ± 1.02	27.60 ± 1.39	28.06 ± 2.31	25.97 ± 0.03
SGR (%/day)	3.40 ± 0.06	3.29 ± 0.08	3.32 ± 0.14	3.19 ± 0.01
FCR	1.05 ± 0.10	1.02 ± 0.02	1.02 ± 0.02	1.01 ± 0.03
Blood profile				
RBC (106 μ/L)	1.43 ± 0.13^{a}	1.69 ± 0.28^{b}	1.87 ± 0.54^{b}	2.15 ± 0.5^{c}
WBC (103 μ/L)	14.7 ± 2.8^{b}	9.6 ± 6.3^{a}	13.8 ± 6.1^{ab}	23.8 ± 5.0^{c}
Hgb (g/dL)	7.6 ± 0.6^{a}	6.4 ± 2.1^{a}	7.8 ± 0.4^{a}	9.3 ± 0.4^{b}
Hct (%)	18.1 ± 5.1^{a}	21.4 ± 2.0^{a}	24.7 ± 1.4^{ab}	27.2 ± 6.3^{b}
MCV (fl)	127 ± 10	127 ± 22	132 ± 7	127 ± 4
MCH (pg)	55.0 ± 2.5^{a}	38.9 ± 2.6^{b}	41.7 ± 3.7^{b}	43.3 ± 4.8^{b}
MCHC (g/dL)	42.0 ± 3.2^{a}	29.9 ± 2.4^{b}	31.6 ± 1.3^{ab}	34.2 ± 3.0^{ab}

Note: Values represent means ± standard deviation of triplicates. FBW, Final Body Weight; SGR, Specific Growth Rate; FCR, Feed Conversion Ratio; RBC, Red Blood Cell Count; WBC, White Blood Cell Count; Hb, Haemoglobin concentration; Hct, Haematocrit value; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration.

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References

Ebrahimi E., Haghjou M., Nematollahi A., Goudarzian F. (2020). Aquaculture 521, 734-909.

Firmino J.P., Vallejos-Vidal E., Balebona M.C., *et al.* (2021). Frontiers Immunololy 12, 625297. doi: 10.3389/fimmu.2021.625297

Gholipourkanani H., Buller N., Lymbery A. (2019). Aquaculture Research 50, 871–875.

Perez-Sanchez J., Benedito-Palos L., Estensoro I., Petropoulos Y., Calduch-Giner J.A., Browdy C.L., Sitja-Bobadilla A. (2015). Fish & Shellfish Immunology 44, 117-128.

Sutili F.J., Gatlin D.M., Heinzmann B.M., Baldisserotto B. (2018). Reviews in Aquaculture 10, 716–726.

Zeng Z., Zhang S., Wang H., Piao X. (2015). Journal of Animal Science and Biotechnology 6, 7.

Zeppenfeld C.C., Hernández D.R., Santinón J.J., Heinzmann B.M., da Cunha M.A., Schmidt D., Baldisserotto B. (2016). Aquaculture Nutrition 22(4), 933–940.

Zheng Z.L., Justin Tan Y.W., Liu H.Y., Zhou X.H., Xiang X., Wang K.Y. (2009). Aquaculture 292, 214-218.

EFFECTS OF FISHMEAL REPLACEMENT BY *Chlorella vulgaris* AND FISH OIL REPLACEMENT BY *Microchloropsis gaditana* AND *Schizochytrium* sp. BLEND ON WHOLE BODY PROXIMATE COMPOSITION OF EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Abstract
The present study was conducted in order to evaluate the effects of dietary fishmeal replacement by *Chlorella vulgaris* meal and of fish oil replacement by *M. gaditana* and *Schizochytrium* sp. blend on whole body proximate composition of seabass (*Dicentrarchus labrax*) juveniles. The results showed that whole body composition of fish did not significantly differ among dietary treatments, thus indicating a normal utilization of these algae meals.
Keywords: *Chlorella vulgaris*, *Microchloropsis gaditana*, *Schizochytrium sp.*, *Dicentrarchus labrax*, *proximate composition*
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1. Introduction

The need for dietary replacement of marine-origin resources by suitable alternatives still remains a major issue for aquaculture. Microalgae are regarded as promising alternatives that could potentially reduce dependence on conventional raw materials, thus ensuring sustainability standards in aquaculture (Shah *et al*. 2018). *Microchloropsis gaditana* and *Schizochytrium* sp. are rich sources of n-3 essential fatty acids, while *Chlorella vulgaris* is a rich in protein microalgae species. The present study evaluated the effects of fishmeal substitution by *C. vulgaris* and fish oil substitution by a blend of *M. gaditana* and *Schizochytrium* sp. on the whole body proximate composition of European seabass (*Dicentrarchus labrax*).

2. Materials and Methods

A total number of 630 juvenile seabass of 2.85±0.01 g initial mean weight were randomly distributed in triplicate groups (35 fish tank^{-1}, 3 tanks dietary treatment^{-1}) after an acclimatization period of 10 days to 18 closed seawater circulation system tanks of 125L capacity (T 21.0±1.0°C, pH 8.0±0.4, salinity 30±0.5 g/L, DO>6.5/L and total ammonia–nitrogen <0.1 mg/L). The groups were fed six different isoenergetic (21 MJ/Kg) and isonitrogenous (52% CP) diets, three of them identified as CM10, CM20 and CM30 were prepared by including *C. vulgaris* meal to replace approximately 10, 20 and 30% of fish meal protein from the Control diet. Two additional test diets were formulated substituting 50 and 100% of fish oil, based on the Control diet, by *M. gaditana* and *Schizochytrium* sp. dried biomass blend (SM50 and SM100). Over the entire experimental period of 11 weeks, fish were fed six days a week by hand to apparent satiation, twice per day.

At the end of the trial, fish were fasted for a period of 24 h before sampling. All fish were individually weighed after being euthanized with an overdose (1.0 mg L^{-1}) of 2-phenoxyethanol. Four fish were randomly selected from each tank (12 per treatment) and proximate composition was conducted to determine the nutrient composition of whole body of fish samples. Fish were minced into a meat grinder and homogenate subsamples of each fish were obtained. Thermal drying to constant weight in an oven at 105 °C for 24 h was applied to determine the moisture content. Crude protein content was determined by Kjeldahl analyses. Crude fat was determined by exhaustive Soxhlet extraction using petroleum ether. Ash content was determined by dry ashing in porcelain crucibles in a muffle furnace at 600 °C for 5 h and gross energy content was determined adiabatically using an oxygen bomb calorimeter.

3. Results and Discussion

Partial replacement of FM protein by *C. vulgaris* meal up to 30% and total FO replacement by *M. gaditana* and *Schizochytrium* sp. did not affect the whole body proximate composition of *D. labrax* juveniles (P>0.05) with the exception of the moisture content which was significantly higher in the CM-fed groups (P<0.05, Table 1). These results indicate that protein and lipids of the tested microalgae species are metabolized and utilized to a similar extent as fishmeal and fish oil denoting their nutritional suitability for *D. labrax*. Similar findings have been reported for fishmeal substitution by dietary *C. vulgaris* biomass up to 30% and total fish oil replacement by *M. gaditana* and *Schizochytrium* sp. in *Sparus aurata* diets by Metsoviti *et al*. (2018). Other studies with *D. labrax* have also indicated that even a total FO replacement by *Nannochloropsis* sp. or *Pavlova viridis* did not affect the moisture, lipid, protein and ash content of the fish (Haas *et al*. 2016). Although the same could not be said for omnivorous species such as *Oreochromis niloticus* and *Paralichthys olivaceus*, in which *C. vulgaris*, *M. gaditana* and *Schizochytrium* sp. have been shown to significantly alter the nutrient composition of the fish (Badwy *et al*. 2008; Qiao *et al*. 2014).

There was observed a reduced lipid content in the CM-fed groups, although not significant, compared to the Control that is attributed to the significantly lower moisture contents in these groups. Seong *et al*. (2019), reported a decreased lipid content in of *Pagrus major* with fishmeal and fish oil replacement by *Schizochytrium* sp. Overall, these findings suggest that *C. vulgaris*, *M. gaditana* and *Schizochytrium* sp. are promising alternatives for fishmeal and fish oil substitution in the diet of *D. labrax* which are complementary to our findings that the growth performance of *D. labrax* fed the tested microalgal diets was better to the fishmeal-fish oil control diet (Gkalogianni *et al*. 2020).

Table 1. Whole body proximate composition (g kg^{-1} on dry weight basis) and energy content (MJ kg^{-1}) of *D. labrax* fed the experimental diets

Parameters/dietary groups	Control	CM10	CM20	CM30	SM50	SM100
Moisture	65.29 ± 1.30^{a}	66.99 ± 2.32^{b}	67.36 ± 2.38^{b}	67.94 ± 1.09^{b}	64.60 ± 2.08^{a}	64.89 ± 1.56^{a}
Crude protein	49.24 ± 3.00	49.67 ± 2.69	49.93 ± 2.78	50.82 ± 2.67	46.52 ± 2.04	47.34 ± 3.15
Crude lipid	36.25 ± 2.97	33.48 ± 2.58	34.35 ± 3.19	32.22 ± 2.29	36.22 ± 2.74	40.26 ± 2.54
Ash	11.32 ± 1.09	11.31 ± 0.88	12.32 ± 1.38	12.38 ± 0.70	11.07 ± 0.89	10.33 ± 0.58
Gross energy (MJ kg^{-1})	25.78 ± 0.73	25.59 ± 1.31	25.31 ± 1.37	24.17 ± 0.61	25.54 ± 1.14	25.85 ± 0.85

Note: Values represent means ± standard deviation of quadruplicates and triplicates. Values within each row not sharing a common superscript letter are significantly different (P < 0.05).

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References

Badwy T.M., Ibrahim E. M., Zeinhom M. M. (2008). 8th International Symposium on Tilapia in Aquaculture 2008

Gkalogianni E.Z., Psofakis P., Asimaki A., Moustogianni A., Bouras S., Antoniadis D., Zarkadas I., Papapolymerou G., Katsoulas N., Karapanagiotidis I.T. (2020). Proceedings of the European Aquaculture Society, April 12-15, Virtual, p. 244-245

Haas S., Bauer J. L., Adakli A., Meyer S., Lippemeier S., Schwarz K., Schulz C. (2016). Journal of Applied Phycology 28(2), 1011-1021.

Metsoviti M.N., Gkalogianni E.Z., Katouni A.M., Rougkas G., Savvaki E., Psofakis P., Katsoulas N., Papapolymerou G., Karapanagiotidis I.T. (2018). Proceedings of the European Aquaculture Society and World Aquaculture Society (AQUA 2018), August 25-29, Montpellier, France, p. 508

Qiao H., Wang H., Song Z., Ma J., Li B., Liu X., Zhang L. (2014). Aquaculture Nutrition 20(6), 646-653.

Seong T., Matsutani H., Haga Y., Kitagima R., Satoh S. (2019). Aquaculture Research 50(9), 2460-2468

Shah M.R., Lutzu G.A., Alam A., Sarker P., Chowdhury M.K., Parsaeimehr A., Liang Y., Daroch M. (2018). Journal of Applied Phycology 30(1), 197-213

HEPATOSTEATOSIS DERIVED FROM PHYTOGENIC FEED ADDITIVES IN GILTHEAD SEABREAM *Sparus autata* L.

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Abstract
Seven isonitrogenous and isoenergetic diets were administered in gilthead seabream, two with essential cinnamon oil (*Cinnamomum zeylanicum*) (1% and 2% of the aquafeed), two with essential oregano oil (*Origanum vulgare*) (1% and 2% of the aquafeed), two with hempseed oil (*Cannabis sativa*) (1% and 2% of the aquafeed) and a control diet (no supplementation). Hepatocytes steatosis was found when *C. zeylanicum* 2% and *C. sativa* 2% were applied as feed additives, while the liver tissue of experimental fish showed no signs of pathology.
Key words: *Origanum vulgare*, *Cinnamomun zeylanicum, Cannabis sativa, liver, histology, Sparus autata*
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1. Introduction

Essential oils (EOs) represent a major group of phytogenic feed additives (PFA) and contain a wide variety of chemical compounds and interesting physicochemical characteristics with high added values respecting the environment, together with the limited side effects in animals. *O. vulgare* and *C. zeylanicum* have proved their growth-promoting, genoprotective, antistress, antioxidant, antidiabetic, antiinflammatory, antimicrobial, antiviral, antiparasitic, antineoplastic and immune modulatory effect (Abdel-Latif *et al*. 2020; Khafaga *et al*. 2020). Very few studies have examined hempseed oil effects in fish production as dietary additive, despite the fact that *Cannabis sativa* has been the focus of the attention of the scientific community all over the world (Pellati *et al*. 2018). Increased necrosis, liver vacuolization, regeneration and congestion are some of the abnormal histological changes on liver structure related to the use of medicinal plants as feed additives (Yigit *et al*. 2017). This study attempts to investigate the effects of phytogenic feed additives, as dietary additives, in marine fish such as gilthead seabream, an economically important fish species in Europe, in order to highlight the beneficial use of medicinal plants by the aquaculture industry. The aim of the present work was to assess changes in the liver histology of *Sparus aurata* fed two different dietary levels of *O. vulgare*, *C. zeylanicum* and *C. sativa*.

2. Materials and methods

630 gilthead seabreams (*Sparus aurata* L.) (5 ± 0.12g) were transferred to the experimental aquarium facilities of Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece. Throughout the experimental procedure, water quality and environmental conditions in the tanks were stable. All the procedures involving fish was performed according to the EU guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). Seven isonitrogenous and isoenergetic diets were formulated in three replicates, two with essential cinnamon oil (*C. zeylanicum*) (1% and 2% of the aquafeed), two with essential oregano oil (*O. vulgare*) (1% and 2% of the aquafeed), two with hempseed oil (*C. sativa*) (1% and 2% of the aquafeed) and a control diet (no supplementation). The feeding trial lasted 84 days. Liver tissue was then processed for routine histology, 5 μm sections were cut and dyed with hematoxylin & eosin for subsequent examination by means of light microscopy.

3. Results

Intrahepatic fat content was consistent with histopathological changes showing hepatocytes steatosis, when *C. zeylanicum* 2% and *C. sativa* 2% were applied as feed additives (Figure 1). Hepato-cellular vacuolation observed in liver tissue was caused by the accumulation of lipid droplets. Necrotic lesions and inflammatory cell infiltration were not observed and the liver tissue of fish in all other treatments showed normal histology.

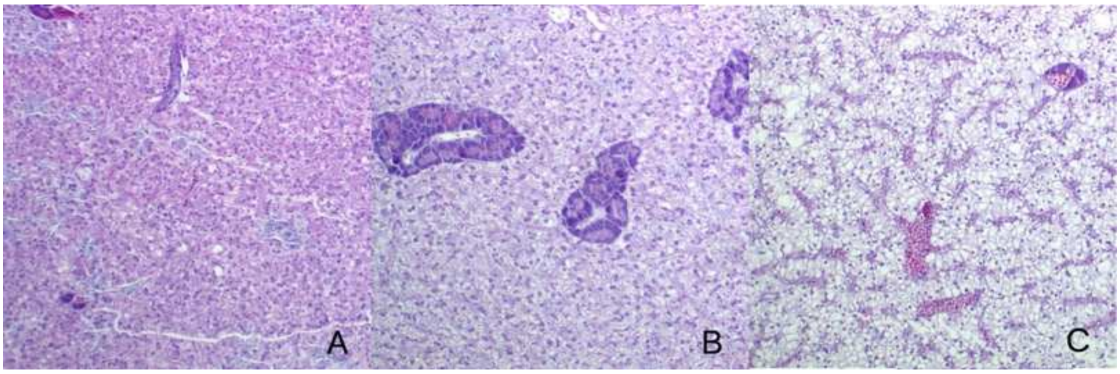

Figure 1. Representative liver histology (HE staining) of gilthead seabream. The histopathology in the case of gilthead seabream fed with *C. zeylanicum* 2% (B) and *C. sativa* 2% (C) revealed hepatosteatosis compared to control group, which was fed without supplement (A).

4. Discussion

In the present study, intrahepatic fat content was observed, when *C. zeylanicum* 2% and *C. sativa* 2% were applied as feed additives, but no necrotic tissue and inflammatory cell infiltration were found in all treatments. Negative effects including slightly necrosis or degeneration, on liver histology of rainbow trout (*Oncorhynchus mykiss*) were observed in the case of *O. onites* (Yigit *et al.* 2017). In contrast, the hepatopancreatic tissue had normal gross histomorphology, in the case of fish groups fed with *O. vulgare* essential oil (Abdel-Latif *et al*. 2020; Khafaga *et al.* 2020), while positive linear effect on the cytoplasmic percentage of hepatocytes and liver glycogen was mentioned in yellow tail tetra, *Astyanax altiparanae* (Ferreira *et al.* 2016). Dietary supplementation with cinnamon products appeared to improve liver histological conditions, decreasing lipid accumulation (Tartila *et al.* 2021). Histopathological changes may provide insight into the effects of unhealthy diets on various tissues in fish, while histological changes due to phytogenic feed additives are dose-dependent.

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References

Abdel-Latif H.M.R., Abdel-Tawwab M., Khafaga A.F., Dawood M.A.O. (2020). Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture 526, 735432.

Ferreira P.M.F., Caldas D.W., Salaro A.L., Sartori S.S.R., Oliveira J.M., Cardoso A.J.S., Zuanon J.A. S. (2016). Intestinal and liver morphometry of the Yellow Tail Tetra (*Astyanax altiparanae*) fed with oregano oil. Anais Da Academia Brasileira de Ciências 88, 911–922.

Khafaga A.F., Naiel M.A.E., Dawood M.A.O., Abdel-Latif H.M.R. (2020). Dietary Origanum vulgare essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (*Cyprinus* carpio). Aquatic Toxicology 228, 105624.

Pellati F., Brighenti V., Sperlea J., Marchetti L., Bertelli D., Benvenuti S. (2018). New Methods for the Comprehensive Analysis of Bioactive Compounds in *Cannabis sativa* L. (hemp). Molecules 23, 2639.

Tartila S.S.Q., Jusadi D., Setiawati M., Fauzi I.A. (2021). Evaluation of dietary supplementation with cinnamon products on growth, blood composition, liver structure, and meat quality of striped catfish (*Pangasianodon hypophthalmus*). Aquaculture International (in press)

Yigit N.O., Diler O., Koca S.B., Gormez O. (2017). Effect on Histology and Nutrient Digestibility of Supplemented Origanum onites Essential Oil to Rainbow Trout Diets (*Oncorhynchus mykiss*). Indian Journal of Pharmaceutical Education and Research 51, 262-267.

THE EFFECTS OF FISHMEAL REPLACEMENT BY DIETARY *Zophobas morio* LARVAE MEAL ON LIVER AND INTESTINAL HISTOLOGY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Abstract
Dietary fishmeal replacement at 10% by *Zophobas morio* larvae meal did not affect liver and intestinal histomorphology of *Sparus aurata*.
Key words: *land animal proteins, insects, fishmeal, aquaculture*
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1. Introduction

Insect meals have been proved as suitable proteins for fishmeal replacement in the diets of most farmed fish species (Henry *et al.* 2015). Most studies with insect proteins have evaluated fish growth and feed utilization, but little is known about their effects on the functionality of the digestive system. A functional digestive system is a prerequisite for the optimal growth of fish with liver and intestine being the main organs of nutrient digestion and metabolism. Thus, studying possible effects and alterations in the histomorphology of these tissues is fundamental for the evaluation of the use of insect proteins as fishmeal replacers in the diet of farmed fish species, including *S. aurata*.

2. Materials and Methods

S. aurata juveniles of 3.4 g initial mean were fed three isonitrogenous (52%) and isoenergetic (20 Mj/Kg) diets at which the fishmeal protein of the control (FM) was replaced by either full-fat (ZFF10) or low-fat (ZLF10) *Z. morio* larvae meal at 10%. After 100 days of feeding, 12 fish/dietary group were euthanized and samples of liver and midgut amples were first fixed in 10% buffered formalin, dehydrated in a graded series of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 5–7 μm were mounted and stained with haematoxylin and eosin. The sections were observed by light microscopy under a total magnification of 100X and 400X. A digital camera affixed to the microscope was used to acquire histological micrographs. Liver histomorphology was evaluated based on possible nuclear displacement, lipid accumulation and vacuolization, signs of steatosis, thrombosis and hemorrhages. Intestinal histomorphology was evaluated based on the length and width of microvilli, number of goblet cells and signs of enteritis.

3. Results and Discussion

Histological observations of liver sections showed that in all dietary groups the majority of the appeared in the center of the hepatocytes, having prominent heterochromatin and nucleolus (Fig. 1). However, many lipid droplets of varying in size were evident in the hepatic parenchyma in all dietary groups that led to an extended steatosis in many fish. In addition, hemorrhagic symptoms and thrombosis were detected in many fish from all dietary groups. In comparison, the degree of lipid vacuolization and of hepatic alterations was similar in all fish no matter the type of diet fed. This high hepatic lipid accumulation is due to the excessive dietary intake of energy and lipids as all fish, either feeding on fishmeal or *Z. morio* meal, exerted a high feed intake (Asimaki *et al.* 2020; 2021) indicating the high palatability of the insect meal. It is known that excessive dietary intake of energy and lipids is leading to high hepatic lipid accumulation due to a reduced ability of hepatocytes to β-oxidize them, thus leading to large lipid droplets and subsequent steatosis (Spisni *et al.* 1998). This fact probably masked the effects of *Z. morio* and further studies are required in order to enlighten the impacts of *Z. morio* in the liver histomorphology of *S. aurata*.

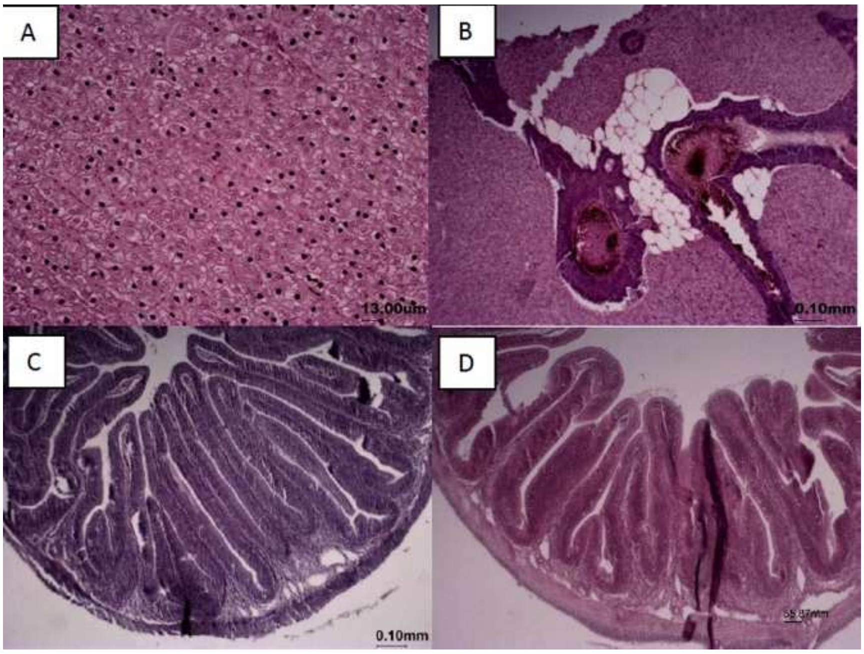

Figure 1: Histological examination of *S. aurata* fed the experimental diets (FM, ZFF10 & ZLF10). A, lipid vacuolization observed in all groups; B, steatosis and thrombosis observed in all groups; C, midgut of FM groups; D, midgut of ZFF10 – prolonged microvilli.

As far as the effects of *Z. morio* on the midgut of *S. aurata* are concerned, all dietary groups of fish presented a normal intestinal morphology with no signs of inflammation (Fig. 1). Enterocytes were distinct, while goblet cells and apical epithelial vacuoles were normally present. Fish fed the ZFF10 and ZLF10 diets had significantly (P>0.05) increased length of intestinal microvilli compared to fish fed on FM, while the ZFF10 had fewer goblet cells. Microvilli elongation is indicating a lower nutrient digestion of *Z. morio* and a need for a greater absorbent surface. Up to date, very little is known on the effects of insect meals on the liver and intestine histomorphology of fish. Mikołajczak *et al.* (2020) showed that a high dietary FM replacement by *Z. morio* did not cause any adverse impacts gut histomorphology of sea trout. Lock *et al.* (2016) did not observe any effects on liver and intestinal histology of *Salmo salar* feeding on diets with high FM replacement by *Hermetia illucens*, but Zarantoniello *et al.* (2020) reported hepatic steatosis in *Danio rerio*.

Acknowledgements

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References

Lock E.R., Arsiwalla T., Waagbø R. (2016). Aquaculture Nutrition 22, 1202-1213.
Mikołajczak Z., Rawski M., Mazurkiewicz J., Kierończyk B., Józefiak D. (2020). Animals 10, 1031.
Zarantoniello M., Randazzo B., Gioacchini G., Truzzi C., Giorgini E., Riolo P., ...,Lucon-Xiccato T. (2020). Scientific reports 10, 1-16.

NERVOUS NECROSIS VIRUS PERSISTENT INFECTION OF SEA BASS (*Dicentrarchus labrax*) AFFECTS INTERFERON PATHWAY-RELATED GENE TRANSCRIPTION

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Abstract
The main innate defense mechanisms of antiviral response include the production of the interferon type I and II peptides and Mx protein. Aim of the present study was to evaluate the humoral response elicited as well as the modulation of interferon pathway-related genes in European sea bass challenged with nervous necrosis virus (NNV) in specific time points, in a long term study. Fish were experimentally infected and monitored for 28 days. Mortalities, brain viral loads and specific anti-NNV antibodies were determined. Head-kidney tissues were aseptically excised and subjected to qPCR to assess interferon pathway-related genes expression in NNV-infected fish. Non-infected fish and fish immunized with poly I:C were served as negative and positive control, respectively. Infected fish presented clinical signs 3 dpi and mortality reached 20% on 5-7 dpi. The highest brain viral load was observed 7 dpi. In NNV-infected fish, all three genes (MxA, ISG12 and IRF7) had no statistical differences in expression levels at early time points (<12hpi), in contrast with poly I:C induced responses for the MxA and ISG12 genes. Noteworthy, the infected fish appeared to be immunologically active at 7- and 14-dpi, providing information for fish immunological status on persistent infection fighting. IgHM gene was also highly expressed 14 and 28 dpi, followed by a high anti-NNV IgM level in the serum of infected fish. Knowing the long term effects of viral infection on fish immune responses and B-cell activation will help to get more insight into the host mechanisms responsible for NNV infection progress in sea bass.
Keywords: *Dicentrarchus labrax*, *b-nodavirus, viral nervous necrosis, interferon pathway, immunoglobulin IgM*
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1. Introduction

Host-pathogen interactions concerning viral infections of teleost fish are under intense investigation as a major problem concerning aquaculture. The onset of an infectious disease in a specific host depends both on pathogen virulence and the host immune response (Moreno *et al.* 2020). The effectiveness of immune defense mechanisms is tightly related to the host health and it can be assessed *in vivo* by analyzing antibody responses and immune-related genes expression. Nervous necrosis virus (NNV) is the causing agent of viral nervous necrosis (VNN), or vacuolating encephalopathy and retinopathy (VER) or encephalomyelitis, a disease resulting in rate mortalities up to 100% in more than 120 different species from marine and freshwater environments. NNV belongs to the genus Betanodavirus of the Nodaviridae family. The clinical symptoms of NNV infection include abnormal swimming behavior, loss of appetite, swim bladder hyperinflation and coloration abnormalities, resulting eventually in the death of infected hosts (Bandín *et al.* 2020). The European sea bass (*Dicentrarchus labrax*), a major NNV host, is a teleost fish found in the Mediterranean and is economically important since it is ranked fourth in European aquaculture production.

2. Materials and Methods

Healthy non-vaccinated fish were experimentally infected with NNV originally isolated from naturally infected *D. labrax* (genotype: RGNNV (Toubanaki *et al.* 2015). Sea bass were challenged by intramuscular injection with 10^{11} $TCID_{50}$ mL^{-1} of 100 μL NNV-containing supernatant. Negative control group was mock-challenged with 100 μL PBS and positive control group utilized polyriboinosinic:polyribocytidylic acid (p(I:C)) as viral infection mimics. Sampling was performed at 0, 3, 6, 12, 24 and 48 hours post infection (hpi), followed by 3, 4, 7, 14 and 28 days post infection (dpi). At each time point fish were anesthetized and weighed. Blood, head kidney (HK) and brain (B) tissues were removed aseptically. Mortality was recorded daily and RT-qPCR was used for viral load quantification. Total RNA was extracted from HK and B, and 5 μg RNA were used for cDNA synthesis. SYBR Green-based real-time PCR reactions were performed in triplicate. Finally, the specific anti-NNV IgM levels were determined on each group sera utilizing a sandwich-ELISA (Bakopoulos *et al.* 1997).

3. Results – Discussion

Typical signs of the disease were observed in NNV-infected fish from day 3, including erratic swimming, disorientation and skin discoloration. Daily mortalities reached a peak on days 5-6 while they were minimized after day 12. No mortalities were registered in negative and positive control groups. Viral RNA was detected in NNV-fish 3 dpi, while the highest levels of viral load were observed on 7 dpi. The viral load at the final time point (28 dpi) increased while the mortality has been ceased, suggesting that the surviving fish are carrying virus particles. Further studies for virus propagation from isolated brains on cell cultures to confirm pathogenicity will reveal whether the surviving fish are asymptomatic carriers with high viral loads almost one month following the initial infection or they have delayed virus clearance from the brain. The expression level of B lymphocyte (IgHM) related gene were assessed to analyze B cell immunity. IgHM expression increased on 6-12 hpi, slighlty decreased at the following time points and began to increase again on 4 dpi. The expression levels increased significantly 14 dpi and finally at 28 dpi the IgHM levels appeared lower but highly above the basal level. In accordance, the anti-NNV IgM specific activity in fish sera was elevated 14 dpi for the NNV-infected group and was significantly higher compared to the control groups.

NNV infection induces various gene expressions in both innate and adaptive immune responses. The interferon (IFN) pathway, a major antiviral response system, plays an important role in the suppression of NNV replication in the host cell and it is involved in the persistence of NNV infection. IFN activates the Mx gene to resist the NNV infection by interfering the viral RdRp activity, while interferon-induced genes (ISGs) mediate antiviral resistance. In particular, ISG12 ubiquitin-like modifier (ISG12) is involved in activating the intrinsic apoptosis pathway and its antiviral activity has been demonstrated on high vertebrates and fish. The interferon regulatory factor 7 (IRF7) has been identified as a primary transcription factor priming the type I IFN response. MxA, ISG12 and IRF7 genes expression was assessed in the present study. In NNV-infected fish, all three genes had no statistical differences in expression levels at early time points (<12hpi), in contrast with p(I:C) induced responses for the MxA and ISG12 genes. Low IFN-related genes expression was observed up to 4dpi where most fish appear infected and mortalities are recorded. The MxA expression levels significantly increased 7 dpi and the IRF7 expression levels were, also, slightly increased on 7 and 14 dpi. The ISG12 expression levels increased on 7 dpi whereas a dramatic increase in ISG12 expression 14 dpi. The observed immune response activation in late time points for the studied IFN-related genes, offers a glimpse in host attempts to overcome persistent NNV infection and is currently under further investigation.

Acknowledgements

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References

Bakopoulos V., Volpatti D., E., R., Galleotti M., Adams A. (1997). Development of an ELISA to detect *Pasteurella piscicida* in culture and in 'spiked' fish tissue. Aquaculture 153, 359-366.

Bandin I., Souto S. (2020). Betanodavirus and VER Disease: A 30-year Research Review. Pathogens, 9, 106.

Moreno P., Gemez-Mata J., Garcia-Rosado E., Bejar J., Labella A.M., Souto S., Carmen-Alonso M. (2020). Differential immunogene expression profile of European sea bass (*Dicentrarchus labrax, L.*) in response to highly and low virulent NNV. Fish & Shellfish Immunology 106, 56-70.

Toubanaki D.K., Margaroni M., Karagouni E. (2015). Development of a Novel Allele-Specific PCR Method for Rapid Assessment of Nervous Necrosis Virus Genotypes. Current Microbiology 71, 529-39.

EFFECT OF STOCKING DENSITY ON THE FEEDING BEHAVIOR OF SEA BREAM (*Sparus aurata* L.) ADMINISTRATED WITH PHYTOGENIC FEED ADDITIVES

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Abstract
Feeding behavior was examined in fish held in tanks under three different rearing densities combined with the administration of cinnamon as feed additive at a rate of 2% for 15 days. Tracking and speed analysis of fish performed with ImageJ. Results showed that in all groups ingestion rates were similar, while significant differences between densities were not observed. Fish performed the same in terms of velocity in the three tested densities and significant differences were not observed. Fish fed with cinnamon performed faster growth at low densities. In conclusion, cinnamon as a feed additive combined with low densities can enhance welfare in intensively farmed sea bream juveniles.
Key words: *Stocking density, Sparus aurata, sea bream, phytogenic feed additives, feeding behavior.*
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1.Introduction

Stocking density is a major factor in fish welfare control and becomes even more important as aquaculture target is high productivity, where high densities in a limited space are usually applied. Studies carried out in laboratory conditions have shown that the effect of stocking density on fish welfare differs between species. At the same time, feeding with phytogenic additives (PFAs - Phytogenic Feed Additives) has been shown to enhance appetite stimulation, immune response to diseases and fish growth. The aim of the present study was to examine the feeding behavior of sea bream through observation under the effect of three stocking densities in combination with the administration of cinnamon as a feed additive.

2.Materials and methods

120 juvenile seabreams (initial mean weight 15.7±3.69 g) were stocked in six tanks (130 L), with closed water circulation system, mechanic-biological filter and continuous oxygen supply. Duplicate groups (CONTROL and CIN) with 10, 20 and 30 fish per tank were fed with commercial pellets and with pellets plus cinnamon as an additive at a rate of 2% respectively. All fish adapted well to the feeding regimes, and no mortalities were recorded throughout the experimental period. The duration of the experiment was 2 weeks. Action camera and ImageJ were used to analyze feeding behavior. For fish tracking in the tanks as well as for the calculation of the speed and the distance covered, manual tracking was chosen.

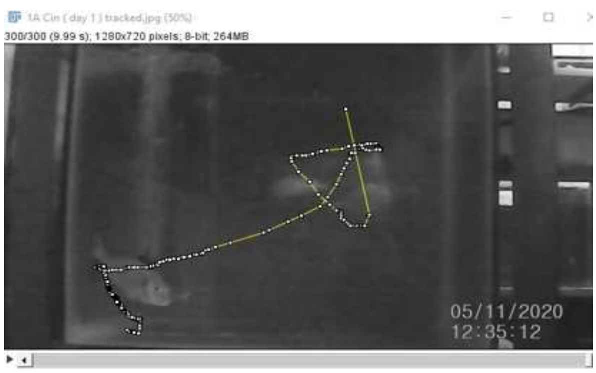

Image 1. Tracking model of sea bream juveniles fed with cinnamon as feed additive.

3.Results

Results of the feeding behavior (Ingestion, Dis-interest, Intake-Rejection-Recollection) are presented in Figures 1, 2, 3 and 4, respectively. Ingestion rates of feed for treatments and controls reached 100%, while there was no significant difference in ingestion between treatments and control for each density (P>0.05). Similar patterns were observed between treatment and control groups regarding the velocities of fish in the three densities as shown in Figures 5 and 6.

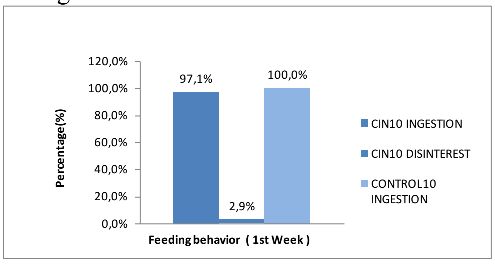

Figure 1. Average ingestion rates in groups CIN, CONTROL (stocking density 10, 1st week)

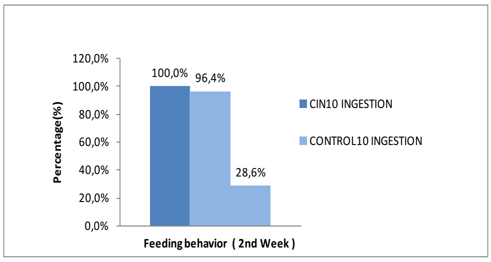

Figure 2. Average ingestion rates in groups CIN, CONTROL (stocking density 10, 2nd week)

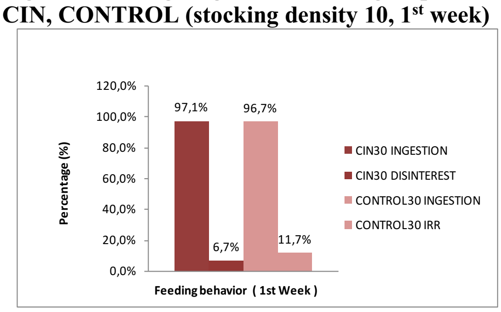

Figure 3. Average ingestion rates in groups CIN, CONTROL (stocking density 30, 1st week)

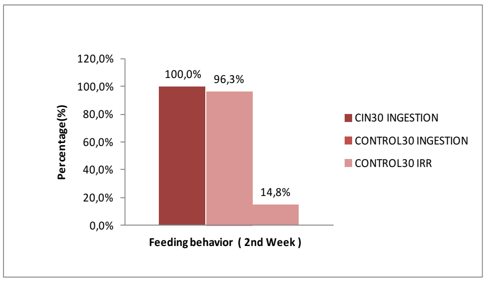

Figure 4. Average ingestion rates between groups CIN, CONTROL (stocking density 30, 2nd week)

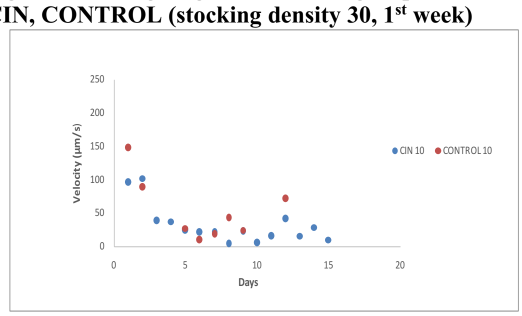

Figure 5. Velocity in groups CIN, CONTROL with stocking density 10 (15 days)

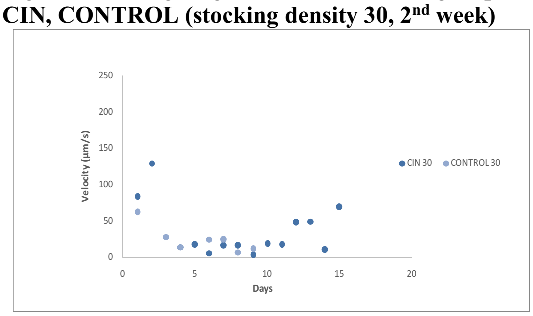

Figure 6. Velocity in groups CIN, CONTROL with stocking density 30 (15 days)

4.Discussion

In the present study, there were no significant differences in ingestion rates between different stocking densities tested. However, low stocking densities seem to have a positive effect on the general behavior and welfare of sea bream juveniles. Several studies have shown that low stocking densities reduce stress and enhance growth (Ashley 2006; Tort *et al.* 2010; Lopez *et al.* 2019; Fattah Abdel *et al.* 2020). Hierarchies were also observed, which is proven by many studies as a social mechanism of *S. aurata* for feed competition. In conclusion, low stocking densities in combination with PFAs in feed, seem an effective management strategy for intensively farmed juvenile sea bream (Bulfon *et al.* 2013).

References

Ashley J.P. (2006). Fish Welfare: Current issues in aquaculture. Applied Animal Behaviour Science., 104, 199 -235

Bulfon C., Volpatti D., Galeotti M. (2013). Current research on the use of plant derived products in farmed fish. Aquaculture Research, 46, 513-551

Fattah Abdel F.A., Ahmed A. F., Salem Y. Al Sadik, Mohammed H.H., Mohamed I.Y., Said N.E. (2020). Egyptian Journal of Aquatic Biology and Fisheries, 24, 539 -560

Lopez A.P., Alvarez N.J., Pons J.A., Reig L., Carella F., Carrasson M., Roque A. (2019). Linking stocking densities and feeding strategies with social and individual stress responses on gilthead seabream (*Sparus aurata*). Physiology & Behavior 213:112723. Elsevier BV. doi:10.1016/j.physbeh.2019.112723

Tort, L., Pavlidis, M.A., Woo, N.Y.S. (2010). Stress and welfare in sparidfish. In: Pavilidis, M.A., Mylonas, C.C. (Eds.), Sparidae: Biology and Aquaculture of Gilthead Seabream and Other Species. Wiley-Blackwell, New Delhi, India, pp. 75–94

CAROTENOID IDENTIFICATION AND QUANTIFICATION IN THE SKIN OF *Pagrus pagrus* USING DIFFERENT EXTRACTION METHODS

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Abstract
Wild Mediterranean *Pagrus pagrus* and aquaculture reared *Pagrus major* fish were acquired from Central Aegean and a local fishmonger in Greece, respectively. The carotenoid profile of their skin was analyzed employing UPLC-QTOF MS/MS, using different homogenization and extraction methods, followed by saponification procedure or without. To improve the saponification procedure different potassium hydroxide (KOH) concentrations were tested. The results indicated that the combined use of mortar and pestle with the unsaponified extraction method 4 showed the highest yield of extracts (5.02%) and measured concentrations of astaxanthin (12.72 mg/kg) and canthaxanthin (1.05 mg/kg) in *P. pagrus* skin.
Keywords: *Pagrus pagrus, skin, carotenoids, extraction, UPLC-Q-TOF*
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Introduction

Aquaculture plays an important role in the food industry. Gilthead seabream (55%) and European sea bass (42%) are the main species produced in Greek aquaculture, while among the other cultivated species, *Pagrus major* is of great significance according to Federation of Greek Maricultures (FGM 2019). Fish skin coloration plays an important role as a quality aspect for the consumers. da Costa & Miranda Filho (2020) concluded that its characteristic color is due to carotenoids, that tend to reside in skin cells called chromatophores. Noori & Razi (2017) pointed out that wild fish populations of *P. Pagrus* consume carotenoid-rich prey, unlike their farmed counterparts, that require the inclusion of pigment additives in their aquafeeds.

The objective of the trial was to compare different homogenization and extraction methods followed by a saponification procedure or without, for assessing the carotenoid pigments mainly astaxanthin, canthaxanthin and β-carotene, in the skin of cultured *P. major* and wild *P. pagrus*.

2. Material and Methods

Wild *P. pagrus* samples were fished and transported from Central Aegean to HCMR laboratory in Athens, while aquaculture reared *P. major* fish (n=3) were acquired from a local fishmonger. At first, their scales were removed, and the skin was separated and stored at -20^{o}C. Two homogenization methods were tested and compared for recovering fish skin carotenoids. Pools of skin were homogenized using a homogenizer (Kinematica AG POLYTRON PT MR 1600E) or with a mortar and pestle.

Four different extraction protocols were used for the identification and quantification of fish skin carotenoids, mainly astaxanthin, canthaxanthin and β-carotene. In each extraction method, two pool skin samples from each species were prepared. The carotenoids extraction from fish skin was accomplished based on modified methods published by Leclercq *et al*. (2010), Jorjani *et al*. (2018) and Dananjaya *et al*. (2019) as shown in Figure 1. Half of the skin extracts from each method were saponified according to Larsen & Christensen (2005), using different concentration levels of aqueous KOH (1%, 5%, 10%), while the rest remained unsaponified.

Furthermore, a bacterial reference material (*Paracoccus carotinifaciens)* aided in the identification of additional carotenoids. All analyses were performed in duplicates.

Chromatographic separation of saponified and unsaponified samples was performed on an Acquity UPLC H-Class system (Waters Corp., Millford, MA, USA), coupled with a quadrupole-orthogonal acceleration Time of Flight mass spectrometer (Q-oaTOF Premier, Waters Micromass, Manchester, UK). MassLynx 4.1. software by Waters was used for data acquisition and analysis.

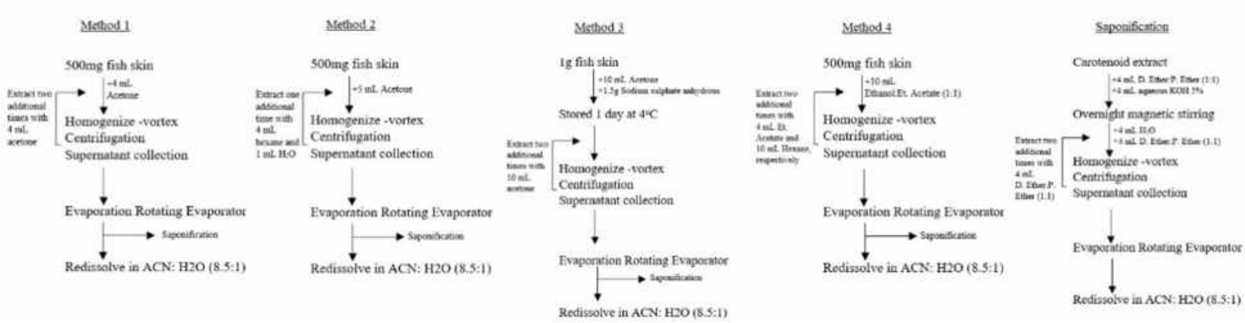

Figure 1. Carotenoid extraction methods and saponification procedure

3. Results and Discussion

The saponification of samples from *P. major* skin with 5% KOH revealed a higher astaxanthin concentration (10.05 mg/kg) compared to 1% and 10% KOH (6.04 and 6.42%, respectively), while canthaxanthin was not detected in any sample of the tested concentrations. However, the results showed that the combined use of mortar and pestle followed by the unsaponified method 4 presented the highest concentrations of astaxanthin and canthaxanthin (12.72 mg/kg and 1.05 mg/kg, respectively) in *P. pagrus* skin. Beta-carotene was not detected in any saponified or unsaponified sample from both species. According to our preliminary results, it is possible to assume that the saponification procedure had an obvious impact on all analyzed samples, decreasing their final extracted astaxanthin concentration. This is contributed to the fact that saponification may lead to different rates of carotenoid degradation, isomerization and reduced content, depending on the conditions of the procedure (temperature, volume and concentration of KOH, etc.), as well as the type of the analyzed sample. Moreover, the screening of carotenoid pigments extracted from *P. carotinifaciens* by MS/MS, aided in the identification of two additional pigments, adonirubin and adonixanthin in *P. pagrus* skin. The remaining analyses of *P. major* skin are pending.

Acknowledgements

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References

da Costa, D.P., Miranda-Filho, K.C. (2020). The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. Review in Aquaculture. 12-3, 1567–1578.

Dananjaya, S. H., Manjula, P., Dissanayake, A. S., Edussuriya, M., Radampola, K., Park, B. K., De Zoysa, M. (2019). Growth performance and color enhancement of goldfish, *Carassius auratus*, fed diets containing natural dyes extracted from annatto (*Bixa orellana*) seeds. Journal of Applied Aquaculture, 1–17.

FGM (2019). Federation of Greek Maricultures, Aquaculture in Greece 2019 annual report. https://www.fgm.com.gr/uploads/file/FGM_19_ENG_WEB_spreads.pdf (accessed: 15-07-2021).

Jorjani, M., Sharif Rohani, M., Mirhashemi Rostami, A., Ako, H., Tan Shau Hwai, A. (2018). Pigmentation and growth performance in the blue gourami, *Trichogaster trichopterus*, fed marigold, *Calendula officinalis*, powder, a natural carotenoid source. Journal of the World Aquaculture Society, 50(4), pp.789-799.

Larsen E., Christensen L. P. (2005). Simple Saponification Method for the Quantitative Determination of Carotenoids in Green Vegetables. Journal of Agricultural and Food Chemistry 53, 17, 6598–6602.

Leclercq, E., Dick, J., Taylor, J., Bell, J., Hunter, D., Migaud, H. (2010). Seasonal Variations in Skin Pigmentation and Flesh Quality of Atlantic Salmon (*Salmo salar* L.): Implications for Quality Management. Journal of Agricultural and Food Chemistry, 58(11), pp.7036-7045.

Noori, A., Razi, A. (2017). Effects of dietary astaxanthin on the growth and skin and muscle pigmentation of sexually immature rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Teleostei: salmonidae). Iranian Journal of Ichthyology 4 (4), 361–374.

PRELIMINARY RESULTS OF FEEDING BEHAVIOUR OF *Holothuria polii*: REMAINING TIME IN THE SANDY SUBSTRATE AND AVAILABILITY OF THE ORGANIC MATTER

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Abstract
The Holothuria or sea cucumber's commercial significance is constantly rising, which results to the fishing of more individuals from wild, per year. The purpose of the present work was to estimate the time *H. polii* remains in the sandy substrate, during the bright hours of the day, compared to the availability of organic materials in the sand. To achieve this goal, 9 groups of sea cucumbers were used (3 conditions x triplicate) under natural light conditions. At first triplicate (control) there was no change of the sand, at second (group A) the sand was replaced only once after fourteen (14) days and at third (group B) the sand was replaced 3 times a week. Their behavior was recorded by a camera, in time lapses for a period of 28 days. The conclusion was that *H. polii* consume their food during the day-time. The results showed that the average time that *H.polii* remained in the sand was related to and actually increased following the frequency of the sand changes. On the contrary, the concentration of the organic materials in the substrate (sand) was decreased at stable rate, independently of the sand replacement. In conclusion, the time that the *H. polii* remains in the sand is not related to the availability of organic matter in the sediment during the day, so further investigation is necessary to be conducted.
Keywords: *Holothuria polii,* feeding behavior, organic matter, sediment
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Introduction

Sea cucumbers can live in sediments all over the word, on rocky or sandy areas, in hot or cold waters. They are organisms that consume sediment and feed on the organic matter in the sand (Hickman et al. 2001). They play an important role for the benthic environment because of the bioturbation and stirring of the sediments, thus preventing anoxic conditions. Five hundred species have been identified till now, 7 of them in the Mediterranean Sea. The most common species in Mediterranean are: *Holothuria (Holothuria) mammata, Holothuria (Holothuria) tubulosa, Holothuria (Panningothuria) forskali, Holothuria (Platyperona) sanctori and Holothuria (Roweothuria) polii* (Valente *et al*. 2014).

Expanded commercial interest in recent years has led to increased scientific activity in physiology and behavior of these species. In the present study, the cryptic behavior of *Holothuria polii* (Delle Chiaje 1824) was investigated in sandy substrates containing different concentrations of organic material. More specifically, an attempt was made to correlate the time the animals spend in the sand during the daylight period of the day, while they are feeding, with the availability of organic material in the sediment.

Materials and Methods

The experiment lasted for 28 days, followed 3 conditions (Witness = control, group A, group B), 3 replicates per condition and 10 adult animals per tank (500 l) under natural lighting conditions. In first group of tanks (control) no change of sand was made throughout the experiment, in second (Group A) the sand was replaced on the 14th day and in third group (Group B) the sand was replaced 3 times per week. The replacement of the sand was intended to enrich the substrate with new organic material in order to feed the holothurians. The behavior of Holothurians was recorded via a digital camera (Samsung SMX-F30 camcorder), using time laps (1 frame per 5 seconds) for 28 days. The organic material (organic carbon in g) of the sediment in each tank was calculated through samples of surface sediment taken at regular intervals throughout the experiments.

Results - Conclusions

According to related bibliography *H.polii* feeds during the day, so their daily activity also reflects their feeding activity. It was considered that the time spent in the sandy substrate was proportional to their feeding time and depended on the amount of organic material in the sand (Siegenthaler *et al*. 2015).

The results showed that, among the three conditions, there were statistical differences in the length of time the holothurians remained in the sand. It was observed that the more frequent the change of sand in the tank, the longer the time spent on the substrate and not on the tank walls (Table 1).

Table 1: Average time *H.polii* spends on the sediment during the day.

	Control	Group A	Group B
Average time (in sec) *H.polii* spends on the sediment	64,45 ± 31,3	84,14 ± 28,70	115,8 ± 23,77

Analysis of sediment samples showed that organic material decreases at a constant rate regardless of the frequency of substrate replacement (Fig 1). Data analysis showed that there are no statistically significant differences in the amount of organic material in the sand between the three different conditions. Thus, the assumption that the time spent in the sand is related to the concentration of the organic material of the substrate is not proved.

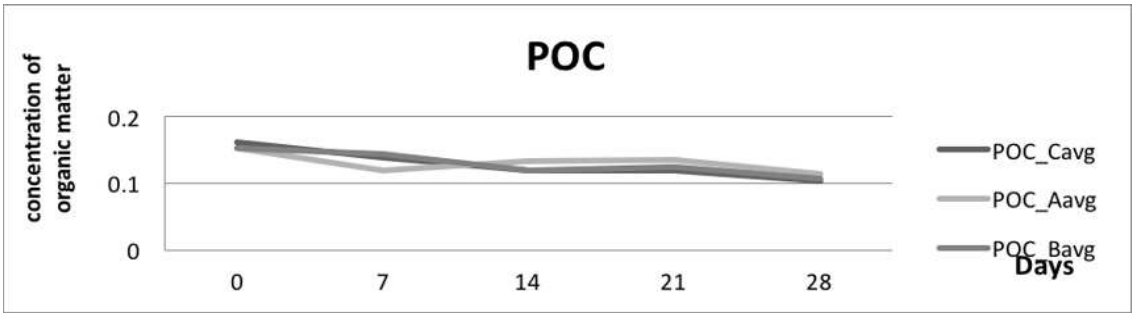

Figure 1: Assemblage of organic matter for 3 groups during the time of the experiment.

In conclusion, Holothuria are organisms that their behavior is directly influenced by the components of the substrate. When they receive the appropriate amount of food, they stop feeding but do not move outside the sandy substrate of the tank, they remain within it. Therefore, holothuria may be affected by certain components of the substrate, but the amount of organic material is not one of them. Further investigation is necessary to be conducted.

References

Hickman C.P., Roberts S. & Larson A. (2001). Integrated Principles of Zoology. Eleventh Edition, McGrow-Hill, New York, ISBN 0–07–118077–X.

Siegenthaler A., Cánovas F. & González-Wangüemert M. (2015). Spatial Distribution Patterns and Movements of *Holothuria arguinensis* in the Ria Formosa (Portugal). Journal of Sea Research 102: 33–40.

Valente S., Serrão E.A. & González-Wangüemert M. (2014). West versus East Mediterranean Sea: Origin and Genetic Differentiation of the Sea cucumber *Holothuria Polii.* Marine Ecology, 485: 95.

THE EFFECT OF FEEDING FREQUENCY ON SEABASS DAILY FOOD CONSUMPTION CO-CULTURED WITH LETTUCE IN AN AQUAPONIC SYSTEM

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Abstract
Aquaponics combines fish farming in recirculation aquaculture systems (RAS) and plant cultivation in hydroponic tanks. A total number of 171 seabass individuals (*Dicentrarchus Labrax*) with an average initial weight of 6.8 ± 0.1 g and an average initial length of 8.6 ± 0.05 cm were divided into 3 autonomous recirculation aquaponic systems. Each system consists of three 100L tanks and had 19 individuals in each tank. Three different feeding frequency treatments (2, 4 and 8 meals/day) were used for 45 days. In addition, 24 lettuce plants (*Lactuca sativa*) were used in the system which had an average initial height of 11.8 cm and were placed in a 26 L hydroponic culture tank according to media bed methods. The results showed that seabass showed higher food consumption (% body weight) when fed daily with 4 or 8 meals compared to seabass fed 2 meals per day (ANOVA, $p < 0.05$).
Keywords: *Freshwater aquaponic system, food consumption of seabass (D.labrax), Feeding frequency.*
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1. Introduction

Aquaponic is a combined system of fish and plant cultivation. It is also characterized as a sustainable method as it provides increased quality vegetables (leafy vegetables, fruiting vegetables, herbs) and animal organisms (fish, crustaceans) throughout the year. The main problem in aquaculture is water quality degradation caused by the accumulation of culture waste and attributed to fish excretes nitrogenous waste such as ammonia is directly discharged into the aquatic environment (Rackocy *et al.* 2006). Francis-Floyd *et al.* (1996) reported that ammonia becomes the second limiting factor after oxygen affecting fish growth and become from fish diets, food quantity, protein-containing and feeding frequency, while fish can absorb 20-30% of feed nutrients and excrete nitrogen at 80%, as ammonium form and organic protein. Seabass (*D.labrax*) belongs to commercial euryhaline species, widely used in Mediterranean aquaculture. Adapted in a wide range of salinity and can be used successfully in freshwater aquaponics systems (Nozzi *et al.* 2016; Stathopoulou *et al.* 2021) or brackish aquaponic systems (Tassiou 2019) and grew well without problems. The aim of the present study was to investigate the effect of three daily feeding frequencies (2 meals/d, 4 meals/d and 8 meals/d), on seabass food consumption co-cultured for 45 days in a freshwater aquaponic system with lettuce plants

2. Material and Methods

The experiment was carried out at the Laboratory of aquaculture-lab-scale aquaponics area, Department of Ichthyology and Aquatic Environment, (University of Thessaly, Greece). 171 sea bass individuals with a mean initial weight and initial length of 6.8 ± 0.1 gr and 8.6 ± 0.05 cm were divided into 3 autonomous aquaponic recirculation systems of a total volume of 500 L. 19 individuals per fish tank were fed daily a commercial food in three different feeding frequencies treatments (2 meals/d, 4 meals/d and 8 meals/d). The experimental set-up and conditions were described previously by Stathopoulou *et al.* (2021). To investigate seabass food consumption uneaten food and faeces were collected every day by siphoning for 45 days and separated by a 0.2-0.5 mm mesh planktonic net. The samples were separated according to shape and colour into two groups one was the uneaten food and the other the faeces group. They were placed into 250mL round bottom porcelain evaporation capsules and then in an oven at 105^{o} for 24 hrs to determine the dry matter. The samples were cooled in a desiccator and reweighed. Water stability was calculated as the percentage difference in sample weight after re-weighing and expressed as % loss of dry: Leaching factor (%) = [100-(dry matter of food offered - dry matter of food offered after 24 hrs)]/dry matter of food offered. Food consumption was determined according to the equation: Food consumption (gr) =[Food offered-(Food offered *leaching factor/100)]-uneaten food. Seabass growth performance was calculated according to the indexes described by Stathopoulou *et al.* (2021). At the end of the experiment food and faeces were analysed for chemical as described by AOAC (1995). Data were checked for normality (Kolmogorov-Smirnov), homogeneity (Levene test) and comparisons of means (One way ANOVA). Data were considered statistically significant at $p<0.05$ (Zar 1999). Statistical analyses were carried out using the software package IBM SPSS Statistics V25.

3. Results

Seabass food consumption is presented in Table 1. At the start of the experiment, there were no statistically significant differences in the initial body weights and lengths of seabass (ANOVA, $p>0.05$) for all treatments. Food consumption was statistical significant higher when seabass fed daily 4 meals and 8 meals compared with 2 meals, (ANOVA, $p<0.05$) (Table 1). Daily food consumption was statistically significantly lower when seabass fed with 2 meals per day compared to 4 meals and 8 meals per day (ANOVA, $p<0.05$) (Table 1).

Table 1. Sea bass, Daily food intake and food consumption of sea bass cultured in aquaponics for 45 days and fed daily with 2 meals, 4 meals and 8 meals.

	2 meals/d	**4 meals/d**	**8 meals/d**
Initial body weight (Win ,gr)	6.8 ± 0.18^{a}	6.8 ± 0.17^{a}	6.8 ± 0.15^{a}
Mean daily food offered (wet weight) (g)	$6{,}36\pm0{,}18^{a}$	$7{,}99\pm0{,}009^{b}$	$8{,}11\pm0{,}06^{b}$
Mean daily food offered (dry weight) (g)	$6{,}23\pm0{,}18^{a}$	$7{,}85\pm0{,}07^{b}$	$7{,}97\pm0{,}06^{b}$
Food consumtpion (gr)	$4{,}91\pm0{,}15^{a}$	$6{,}26\pm0{,}11^{b}$	$6{,}31\pm0{,}04^{b}$
Daily mean food consumtpion (% body weight)	$1{,}66\pm0{,}06^{a}$	$2{,}12\pm0{,}05^{b}$	$2{,}11\pm0{,}05^{b}$
Daily Feeding rate %	$3{,}87\pm0{,}06^{a}$	$3{,}95\pm0{,}05^{a}$	$3{,}92\pm0{,}06^{a}$

Data were expressed as mean ± S.E.M (n=57, $n_{daily\ feeding\ ratio}$=108). Means in a row followed by the same superscript are not statistically significantly different ($p>0.05$).

4. Discussion

The present study, to the knowledge of the authors, is the first time that investigates seabass food consumption in freshwater aquaponics systems under three different daily feeding frequencies (2 meals /d, 4 meals /d and 8 meals /d) within 45 days. The results of the present study indicated that seabass fed 2 meals per day consumed significantly lower food (ANOVA, $p<0.05$) than those fed 4 or 8 meals per day. The food consumption results from the present study are in agreement with those results reported by Tasiou (2019) for seabass juvenile cultured in a brackish aquaponic system with 3 different salinities (20 ppt, 14 ppt, 8 ppt) and fed 3 meals per day (food consumption under the three salinities 5.9gr). When seabass fed 2 meals/day showed a lower food consumption compared to food consumption reported from Tasiou (2019). Mean daily food consumption (% body weight) ranged from 1.66±0.06 % b.w for seabass fed 2 meals/day, and 2.1±0.05 % b.w for seabass fed 4 and 8 meals per day and was higher from daily food consumption (1.6 gr) reported by Marais & Kissil (1979) in 200L aquarium for 80 days.

References

Francis-Floyd R., Watson C., Petty D., Pourder D. B. (1996) Ammonia in aquatic systems. University of Florida, Department of Fisheries and Aquatic Science, Florida Coop,Ext. Serv. FA-16, 4 pp.

Marais, J.F.K., and Kissil,G.W.(1979). The influence of energy level on the feed intake, growth, feed conversion and body composition of *Sparus aurata*, *Aquaculture*, 17:203-219.

Nozzi, V., Parisi, g., Crescenzo, D., Giordano, M., Carnevali, O. (2016). Evaluation of *Dicentarchus labrax* Meats and the Vegetable Quality of *Beta vulgaris* var cicla Farmed in Freshwater and Saltwater Aquaponic Systems, *Water,* 2016, 8, 423.

Rakocy, J.E, Masser, M.P., Losordo, T.M. (2006). Recirculating aquaculture tank production systems: aquaponics- integrating fish and plant culture, Southern Regional Aquaculture Center, vol. 454, pp. 1-16.

Stathopoulou, P., Berillis, P., Vlahos, N., Nikouli, E., Kormas, K., Levizou, E., Katsoulas, N. and Mente, E., (2021). Freshwater-adapted Seabass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. *PeerJ*, 9, p.e11522.

Tasiou K. (2019). The production process of sea bass in an aquaponic system. MSc thesis. Department of Ichthyology and Aquatic Environment, University of Thessaly, pp. 85.

Zar J. H. Biostatistical Analysis; Prentice-Hall: London,UK, (1999), P. 718.

EMPLOYMENT AND SOCIO-DEMOGRAPHICS OF GREEK AQUACULTURE SECTOR

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Abstract
Greece represents 10% of the total EU aquaculture production volume and is also the main seabream and seabass producer, covering 47% of the total EU value. Consequently, the sector of aquaculture has a significant contribution in employment terms for Greece.. A very important element for the aquaculture sector employment is that many jobs are created in remote areas of the Greek territory, mainly in islands, which contributes significantly to the economic development of local communities. Aquaculture creates jobs in 10 of the 13 Regions of Greece, with the Regions of Western Greece, Central Greece, Peloponnese, and Attica representing the greatest percentage. Given the important role of aquaculture in the Greek economy, this paper will attempt to describe the sector's employment structure and compare the respective socio-demographic variables of aquaculture between Greece and EU.
Keywords: *Aquaculture, employment, age, gender, education, nationality.*

1. Introduction

According to the European Commission data, among EU countries that demonstrate aquaculture activity, Greece ranks 5th in total number of employees in that sector, behind Spain, France, Poland, and Italy (STECF 2018). Almost 18% of aquaculture companies in Greece are large capital companies, responsible for 80% of the total aquaculture products sales and, according to Hellenic Ministry of Rural Development and Food, although most of the aquaculture companies are SMEs, the few large ones employ more than half of the sector's employees (Ministry of Rural Development and Food 2018). These enterprises represent mainly the marine fish farming sector (seabass and seabream mostly), while the freshwater and shellfish aquaculture sector consist mainly of small family businesses. While the average number of employees in the sector for the last 15 years rises to almost 4.500, it is also estimated that the total number of employees in the Greek aquaculture sector, taking into account not only the directly related jobs but also the indirect jobs created by the support services of the industry (fish feed, technical equipment, packaging, transport, etc.), amounts to a total of 12.000 employees, directly and indirectly employed (including scientific, technical and skilled labor staff) (Federation of Greek Maricultures, 2019).

2. Material and Methods

Based on data from Hellenic Statistical Authority (HSA) and European Commission aquaculture sector research reports for Greece for the 2002-2020 period (STECF 2018; 2020), this paper studies the respective employment of Greek aquaculture sector in total and average numbers by water category following the ELSTAT categorization. Furthermore, it studies the socio-demographics of the aquaculture sector, comparing them to the respective socioeconomic variables of EU countries with aquaculture activity according to the European Commission economic reports. Microsoft Excel was used for data processing and creation of the figures.

3. Results

3.1 Employment in aquaculture, by water category

Greece demonstrates aquaculture activity in fresh, brackish, and marine water. The timetable of aquaculture employment by water category in Greece, according to ELSTAT data for the period 2002 -2018, is depicted in Figure 1. Figure 2 shows the change in employment rates by water category, following the expansion of marine water aquaculture during the 2002-2018 period

3.2. Socio-demographics of Greek aquaculture sector in comparison to EU.

According to the Economic Report of the EU Aquaculture sector by the Scientific, Technical and Economic Committee for Fisheries for 2018, in Greece male workers dominate the aquaculture sector as they account for 89% of the total employment (STECF 2020). Regarding the aquaculture sector's employment distribution by age, the 40-63 years segment represents the largest percentage (41%). Based on the educational level, medium education (Upper Secondary School and Post-secondary non-tertiary education), corresponds to 38%, followed by low education (31%). Finally, on the aquaculture sector's Nationality distribution, Greece demonstrates the largest percentage in the National category (54%), followed by the Non-EU/EEA (20%) (STECF 2020). The following Figures (Figure 3-6), show the comparison of social variables in Aquaculture sector employment between Greece and the EU total.

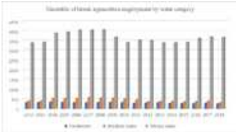

Figure 1. Sea bass & sea bream production in Greece during 2002-2018.

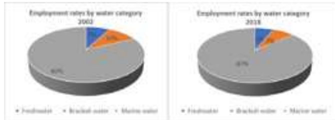

Figure 2: Employment rates by water category 2002 and 2018

4. Discussion

Regarding the employment by water category analysis, the predominance of marine aquaculture techniques in Greece is confirmed since, for the 2002-2018 period, the initial percentage of 82% of total employment corresponding to marine water aquaculture in 2002, has risen to 87% in 2018 while fresh and brackish water techniques employ 6% and 7% of the workforce respectively.

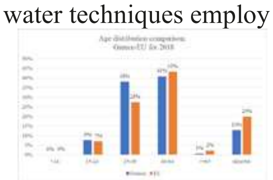

Figure 3: Age distribution comparison: Greece-EU for 2018

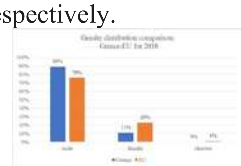

Figure 4: Gender distribution comparison: Greece-EU for 2018

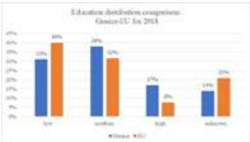

Figure 5: Education distribution comparison: Greece-EU for 2018

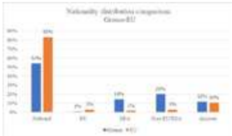

Figure 6: Nationality distribution comparison: Greece-EU for 2018

On the socio-demographic aspect of the Greek aquaculture sector, men constitute the largest part of the workforce (89%), while working women are usually employed in hatcheries and packaging companies, as well as in secondary activities such as mussel deshelling and fisheries processing and represent the rest 11% of aquaculture employment in the sector while in EU the difference is reduced by 12%. The age distribution charts show that while the percentage of employees of more than 40 years of age are almost similar in Greece and EU, Greece employs almost 11% more in the 15-39 categories. On the education distribution, Greece employs more people of medium and especially of higher education than total EU (38% to 32% and 17% to 8% respectively).Furthermore, on nationality distribution, Greece demonstrates significantly more employees from European Economic Area (EEA) and Non-EU/EEA countries than EU totals (34% to 4% respectively). Last, according to EC aquaculture economic reports, Greece ranks 4th on Full-Time Equivalent (FTE) number of employees and 5th on total employment number among EU countries with aquaculture activity in 2018, and 8th on average wage per FTE.

References

European Commission, 2016. Tapping into blue growth: the way forward for European aquaculture. Brussels, European Union.

Federation of Greek Maricultures, 2019. Aquaculture in Greece, Recovered from https://www.fgm.com.gr/uploads/file/FGM_19_GR_WEB_Spreads(4).pdf.

Hellenic Ministry of Rural Development and Food, 2018. Annual data collection report for the fisheries and aquaculture sector. Year of reference: 2018, Kavala: Ministry of Rural Development and Food.

Hellenic Statistical Authority, 2019. Survey on Aquaculture, recovered from https://www.statistics.gr/en/statistics/

STECF - Scientific, Technical and Economic Committee for Fisheries, 2018. Economic Report of the EU Aquaculture sector (STECF-18-19), Luxembourg: Publications Office of the European Union.

STECF - Scientific, Technical and Economic Committee for Fisheries, 2020. Economic Report of the EU Aquaculture sector (STECF-20-12), Luxembourg: Publications Office of the European Union.

OPTIMISATION STUDIES FOR THE COMMERCIAL PRODUCTION OF *Arthrospira platensis*

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Abstract
Arthrospira platensis (Spirulina) is the most cultivated microalga worldwide. Most farms produce Spirulina in open raceway ponds exerting little control on the process. Strain selection, therefore, along with media improvements are crucial for the farming economics. In this study we tested two strains, namely SAG 257.80 and SAG 85.79 under high or low light representing high and low production season using full or half strength of bicarbonate in the cultivation medium. Results show that although at high season the former strain might be more productive at exponential growth (supporting continuous growth at such dilution rates) SAG 85.79 scores much better at batch modes (higher yields and productivities in the long run). Bicarbonates in the medium could be halved with no effect on growth or yields offering significant cuts for the farming expenses.
Keywords: *Arthrospira, Spirulina, growth, light, carbonates*
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1. Introduction

Arthrospira platensis (=*Spirulina platensis* Geitler 1925) Gomont 1892 (Cyanobacteria, Oscillatoriales), is a filamentous cyanobacterium able to grow in tropical and subtropical alkaline lake waters e.g., Lake Chad in African or Lake Texcoco in Mexico. *A. platensis* is known for its beneficial dietary effects, including antioxidant and antimutagenic properties (Capelli & Cysewski 2010). *A. platensis* is permitted to be sold as food supplement since 1981 by the United States Food and Drug Administration (FDA) because of its ability to produce large quantities of valuable products such as phycocyanin (Estrada *et al.* 2001) and ω-6 and ω-3 polyunsaturated fatty acids (Cohen *et al.* 1987; Deshnium *et al*. 2000).

A. platensis is grown in both open and closed systems. The large-scale production output of *A. platensis* biomass depends on many factors, the most important of which are temperature and light (Vonshak 1997). In outdoor systems both these factors are hardly regulated while they are also exposed to contaminants. In Greece, where all Spirulina farms are operating open raceways, some control is offered by housing them in greenhouses (rain, dust, shading) and the use of heating (geothermal water or biomass combustion). More control could be exerted on other factors such as culture media and strain selection.

In this study we used two different strains widely popular for mass cultivation against full or half strength media regarding the most expensive component which is bicarbonate soda ($NAHCO_3$).

The strains used were *A. platensis,* SAG 85.79 and SAG 257.80 at high light representing high season (mid-April to mid-October) and low light for the low season keeping the temperature constant to 30°C representing the average value encountered in most farms during the production.

Statistical evaluation of results was carried out using XLStat (Addinsoft): ANOVA was used for factor effects and Tukey's HSD test to compare means.

2. Materials and methods

Two strains were used for testing outputs at the different culture conditions chosen, i.e., SAG 85.79 isolated from a natron lake in Chad and SAG 257.80 isolated from Huacachina desert lake (oasis) in Peru. The former is considered a high growth strain while the latter a very tolerant one to climatic adversities. All strains were acquired from the University of Goteborg's extensive culture collection.

The experiment was carried out in aerated 2 L tubular flasks (6 for each strain) arranged against fluorescent flasks of 30 W/m (Phillips 865T8) to provide either 200 $\mu mol\ photons\ m^{-2}s^{-1}$ (HL) or 60 $\mu mol\ photons\ m^{-2}s^{-1}$ (LL) to simulate high and low season's light intensity. Temperature was kept constant at 30°C placing the flasks in a 50 L aquarium halfway filled with water and a standard heater. Aeration was provided constantly by a Hailea air pump to agitate water and remove excess oxygen produced. The culture medium used was the standard Zarouk's medium at full (16,7 g/L) and half (8,35 g/L) strength of sodium bicarbonate ($NaHCO_3$). pH was monitored daily by a Hach-Lange portable pH meter.

Growth was assessed by handcrafted Secchi disc in a volumetric cylinder calibrated for dry weight through a standard curve. For dry weight aliquots were taken via vacuum filtration on tarred GF/C filters rinsed thoroughly with ammonium formate (NH_4HCO_2 4%) and desiccated to dryness in a Mettler incubator at 60°C.

For assessing the output, the following calculations were carried out: for growth, the specific growth rate μ=ln(N_2/N_1)/day_2-day_1 was used to estimate the exponential μmax (per day); and the highest biomass concentration observed (NX_{max}, g/L). For productivity, the Yield (NX_{max}-NX_0 g/L) and the volumetric productivity (Yield/day, g/L/day) for each strain.

3. Results and Discussion

Results are shown in Table 1. Regarding growth, both strains exhibited good growth not statistically different at high light, while at low light SAG 85.79 was almost twice faster. Therefore, at low season this strain may have an advantage over the other at continuous modes. Soda content did not have any effect at high light while at low light full strength was giving much better output, especially for the strain from Peru.

SAG 85.79 yielded almost doubled figures in biomass produced either under high or low light in comparison with SAG 257.80. Hence, for batch cultivation mode this strain seems more prolific in both high and low season. Soda content of the culture medium did not have any effect on either yield or productivity. Moreover, pH data on cultivation did not show any difference between cultivation on half or full strength therefore, farmers could half its content in initial fertilisation of the water when the cultivation starts. This might offer a significant cut in the overall expenses for each production cycle.

Table 1. Cultivation of different strains of Arthrospira platensis under different light intensities and media strength.

strain	$NaHCO_3$	Light Intensity	μ_{max} per day	*STD*	NX_{max} g/L	*STD*	Yield g/L	*STD*	Productivity g/L/day	*STD*
SAG 85.79	100%	HL	0.329	*0.014*	1.239	*0.030*	0.843	*0.030*	0.113	*0.007*
SAG 85.79	50%	HL	0.322	*0.007*	1.262	*0.008*	0.866	*0.008*	0.116	*0.010*
SAG 85.79	100%	LL	0.231	*0.060*	0.981	*0.013*	0.585	*0.013*	0.065	*0.001*
SAG 85.79	50%	LL	0.180	*0.022*	0.986	*0.005*	0.590	*0.005*	0.072	*0.018*
SAG 257.80	100%	HL	0.365	*0.001*	0.680	*0.022*	0.420	*0.022*	0.065	*0.004*
SAG 257.80	50%	HL	0.347	*0.000*	0.692	*0.008*	0.432	*0.008*	0.062	*0.001*
SAG 257.80	100%	LL	0.169	*0.025*	0.554	*0.016*	0.294	*0.016*	0.039	*0.002*
SAG 257.80	50%	LL	0.096	*0.000*	0.543	*0.002*	0.283	*0.002*	0.040	*0.000*

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References

Capelli, B. and Cysewski, G.R., 2010. Potential health benefits of *Spirulina* microalgae. *Nutrafoods*, *9*(2), pp.19-26.
Cohen, Z., Vonshak, A. and Richmond, A., 1987. Fatty acid composition of *Spirulina* strains grown under various environmental conditions. *Phytochemistry*, *26*(8), pp.2255-2258.
Deshnium, P., Paithoonrangsarid, K., Suphatrakul, A., Meesapyodsuk, D., Tanticharoen, M. and Cheevadhanarak, S., 2000. Temperature-independent and-dependent expression of desaturase genes in filamentous cyanobacterium *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC 9438). *FEMS Microbiology Letters*, *184*(2), pp.207-213.
Vonshak, A. ed., 1997. Spirulina platensis (Arthrospira*): physiology, cell-biology and biotechnology*. CRC press.

PRELIMINARY RESULTS OF BRACKISH AQUAPONICS WITH SEA BASS (*Dicentrarchus labrax*)AND OPPOSITE-LEAVED SALTWORT PLANT OR AGRETTI (*Salsola soda*)

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Abstract
The ever-increasing salinity of water on the planet creates the need for research into the cultivation of species with high commercial and nutritional value. In this context, sea bass (*Dicentrarchus labrax*) was co-cultured with the halophyte agrettior opposite-leaved saltwort plant (*Salsola soda*), which seems to have significant health benefits and emerging commercial valuein a brackish aquaponic system. The aim of the present study was to investigate the effects of three different salinities (3 ppt, 7ppt, 14ppt) on seabass and halophyte agretti growth performance and survival rate. In total, 126 seabass individuals were used with an average weight of 5.79 ± 0.89 g and an average length of 8.44 ± 0.53 cm. In addition, 18 plants with an average height of 17.07 ± 0.17 cm were used at the aquaponic system. The results showed that the growth performance of the seabass was higher at 14ppt (SGR=3.23±0.08 %/d) than 7ppt (SGR=3.08±0.06 %/d) and 3 ppt (SGR 3.08 %/d).Moreover, growth of halophyte agretti (height increase) appeared to be statistically significant higher in the 7ppt salinity than in the 14 ppt and 3ppt($p < 0.05$).
Keywords: *Brackish aquaponics, sea bass, agretti, Salsola soda, Dicentrarchus labrax*
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1. Introduction

In recent years, human activities have led to a gradual increase in salinity in the flowing waters of the planet (Colla *et al.* 2006), as a result traditional plant crops are facing many growth problems. Plants such as agretti (*Salsola soda*) which tolerate the presence of salt in the water and have significant health benefits (Iannuzzi *et al.* 2020) can be an ideal candidate for brackish aquaponic systems. The purpose of this study is to evaluate the growth performance oftwo high commercial value species sea bass (*Dicentrarchus labrax*) and agretti (S*alsola soda)*in a brackish aquaponic system.

2. Materials and Methods

The experimental process lasted 45 days. The experiment was conducted in the facilities of the Department of Ichthyology and Aquatic Environment at the University of Thessaly.Three salinity treatments 3 ppt, 7 ppt and 14 ppt were used, respectively.Three autonomous small-scale aquaponic systems (500 L) consisting of 9 fish tanks (120 L), 3 hydroponic tanks (100 L) and 3 sump biofilters (120 L) as briefly described by Stathopoulou *et al*. (2021) were used for the trial.In total, 126 seabass individuals with an average weight of 5.79 ± 0.89 g and an average length of 8.44 ± 0.53 cm were used and were divided into 14 individuals per fish tank (42 fish/treatments).Moreover, 18 individuals of halophyte agretti were divided into 6 stolons in each system with an average initial height of 17.07 ± 0.17 cm. Fish were fed *at libitum* two times per day (9 a.m. and 4 p.m.). with a commercial diet (protein 56%, fat 13%, ash 13%). pH, temperature and oxygen saturation were measured daily, while TAN (total ammonia nitrogen), nitrite (NO_2^-) and nitrate (NO_3^-) were measured twice a week with a Tropic Marin® test kit in the influent and effluent of the grow bed. In addition, every 15 days, fish were anaesthetized with an 0.20 mg/L MSS 222, and fish body weights and lengths were measured. Every 15 days measurements of height and number of side branches of the plants were taken. At the end of the experiment,Specific Growth Rate [SGR (%/day)=[(ln Wfin − ln Wi)/duration of experiment] × 100] where Win and Wfin are the initial and final weight of the fish respectively, fish weight gain[(WG (g)=Wfin-Win)], survival rate of the fish[S=((final number of fish − initial number of fish)/initial number of fish) × 100] and height difference of the plants [dH=Final height of plants-initial height of plants]were calculated (Mente *et al*. 2016; Vlahos *et al.* 2019). Data were checked for normality (Kolmogorov-Smirnov/Sapiro-Wilk), homogeneity (Levene test) and comparisons of means (One way ANOVA). Data were considered statistically significant at $p<0.05$ (Zar 1999). Statistical analyses were carried out using the software package IBM SPSS Statistics V26.

3. Results

Fish growth performance is presented in Table 1. At the start of the experiment, there are no significant differences in the means of the seabass initial body weights and lengths and plants height ($p>0.05$) for both treatments. At the end of the experiment,SGR ranged between 3.08±0.06 %/d and 3.23±0.08%/d and there were no significant differences between the treatments ($p>0.05$).WG was statistically significant lower at 3ppt than 14 ppt and 7 ppt salinity treatment ($p<0.05$).At the end of the experiment agretti growth performance (Final Height and dH) was no statistically significant different between treatments($p>0.05$) although it was higher at the 7ppt group (Table 1).

Table1. The growth performance of sea bass and agretti co-cultured in a brackish aquaponic systems for 45 days.

	14 ppt	7 ppt	3 ppt
	Seabass growth performance		
W_{in} (g)	6.78± 0.15^{a}	6.71±0.15^{a}	6.62± 0.15^{a}
W_{fin} (g)	29.15±0.77^{a}	27.38±0.72^{a}	26.47±0.58^{b}
L_{in} (cm)	8.49±0.08^{a}	8.47±0.08^{a}	8.35±0.09^{a}
S (%)	100^{a}	100^{a}	100^{a}
WG (g)	22.38±0.80^{a}	20.67±0.71^{a}	19.84±0.54^{b}
SGR (%/d)	3.23±0.08^{a}	3.11±0.50^{a}	3.08±0.06^{a}
Salsola soda growth performance			
H_{in}(cm)	17.03±0.03^{a}	17.15±0.76^{a}	17.02±0.83^{a}
H_{fin}(cm)	38.67±3.22^{a}	41.17±3.08^{a}	32±0.97^{a}
dH (cm)	21.63±3.24^{a}	24.02±3.09^{a}	14.98±0.97^{a}

Data were expressed as mean ± S.E.M (n_{fish}=78, n_{plants}=54). Means in a row followed by the same superscript are not statistically significantly different ($p>0.05$).

4. Discussion

The results of present study showedthat salinity did not affect the survival rate of seabassandagretticocultured in a brackish aquaponic system (ANOVA, $p>0.05$). According to the results, SGR ranged from 3.08 %/d for 3 ppt, 3.11%/d for 7 ppt and 3.23% /d for 14 ppt, and are in aggrenmentwith those reported by Tasiou (2019), Stathopoulou *et al*. (2021) reported lower SGR (1.44-1.90 %/day) for sea bass growing in a freshwater aquaponics system. This is the first time that *S. soda* is cultivated in an brackish aquaponic system as the main plant. Colla *et al*. (2006) used agretti as a desalinating companion plant combined with greenhouse pepper (*Capsicum annuum*) and their results show that agretti could be an attractive strategy for the future in order to limiting yield reduction. The conclusion is that the combination of sea bass and agretti in brackish aquaponics system could be create a new path for alternative and eco-friendly crops in near future.

5. References

Colla G., Rouphael Y., FallovoC., Cardarelli M., GraifenbergA. (2006) Use of Salsola soda as a companion plant to improve greenhouse pepper (*Capsicum annuum*) performance under saline conditions, New Zealand Journal of Crop and Horticultural Science, Vol. 34: 283-290.

Iannuzzi A. M., Moschini R., De Leo M., Pineschi C., Balestri F., Cappiello M., Braca A., Del-Corso A. (2020) Chemical profile and nutraceutical features of *Salsola soda* (agretti): Anti-inflammatory and antidiabetic potential of its flavonoids, Food Bioscience, 37, 100713.

Mente, E., Solovyev, M.M.,Vlahos,N., Rotllant, G., Gisbert,E (2016). Digestive enzymes activity during initial ontogeny and after feeding diets with different protein sources in zebra cichlid *Archocentrusnigrofasciatus*. Journal of World Aquaculture Society, 48, 5, 831-848.

Stathopoulou P, Berillis P, Vlahos N, Nikouli E, Kormas KA, Levizou E, Katsoulas N, Mente E. (2021) Freshwater-adapted sea bass *Dicentrarchus labrax* feeding frequency impact in a lettuce *Lactuca sativa* aquaponics system. PeerJ,*9*:e11522.

Tasiou K. (2019). *The production process of sea bass in an aquaponic system*. MSc thesis. Department of Ichthyology and Aquatic Environment, University of Thessaly, pp. 85.

Vlahos N., Levizou E., Stathopoulou P., Berillis P., Antonopoulou E., Bekiari V., Krigas N., Kormas K ., and Mente E. (2019). An Experimental Brackish Aquaponic System Using Juvenile Gilthead Sea Bream (*Sparus aurata*) and Rock Samphire (*Crithmum maritimum*). *Sustainability* 11(18). DOI: 10.3390/su11184820.

Zar J.H. (1999).Biostatistical Analysis. Prentice-Hall. International Editions, London, UK.

PERSPECTIVES OF FRESH WATER AQUACULTURE IN GREECE

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Abstract
The contribution of fishery to a plethora of fields renders it exceptionally significant globally. In Greece it is estimated that 63% of the country's production in fishery products stems from aquaculture and the remaining 37% from fishery. In the present study takes place a data analysis concerning the quantity and production values of the freshwater aquaculture and especially for 'trout culture' (*Oncorhynchus mykiss*) in Greece. The purpose of the present study is to present a historical overview of the aforementioned data of trout culture covering a time period of 30 years, the presentation of the geographical distribution of the specific aquacultures and the emergence of the aquaculture sector significance as a major growth pillar for the Greek economy and its positive contribution to the country's trade balance, as it exceeds the corresponding imports.
Key words: aquaculture *inland waters, trout culture, Oncorhynchus mykiss, Greece*
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1. Introduction

The global population increase in conjunction with climate change comprise a challenge to food security provision and put pressure on natural resources such as the soil and waters (Lacy *et al*. 2017). Fishery activities play a key role in this global challenge. However, the promotion and growth of aquaculture is affected by biological, technical, financial, institutional, and environmental factors including consumer protection (European Commission 2017; Guillen *et al*. 2019). The global fish production is estimated to have risen by 2,1% in a total of 178.8 million tons, increasing the global fish consumption to an average of 20.7 kg fish per person in 2018 (FAO 2019).

In 2017 the amount of fishery products in Greece rose to 638 tons in brackish waters, worth 2,463,000 (euros), in marine waters the amount was 122,446 tons, worth 531,369,000 (euros) and in fresh waters 2,337 tons, worth 11,656,000 (euros). An estimated 63% of the country's fishery products stem from aquaculture and 38% from fishery. Inland water aquaculture production reach only 2% that come mainly from small family businesses units producing 'rainbow trout', while only 1% of the production stems from sea lagoons (European Commission 2015; ELSTAT 2017).

2. Materials and Methods.

The search, collection, and categorization of data regarding the geographical distribution and the operation of inland waters aquacultures in Greece took place from the Hellenic Republic Ministry of Rural Development and Food. Two data bases were used, Web of Science https://www.sciencedirect.com/, Google Scholar https://scholar. google.com/ for the period 2016 to 2020 and organized fora were emailed such as: Hellenic Statistical Authority, (FAO) http:// www.fao.org/fishery/statistics/en. Methods of graphical data presentation (chronological charts), Arithmetic descriptive measures, the Trend analysis (linear regression method) and the prediction method with the ARIMA (p,d,q) model were used by means of Microsoft Office Excel Version 7 and Minitab (version 19, Minitab Inc., State College, PA, USA).

3. Results and Discussion

The number of trout culture units in each region in detail are: in Arkadia 1, in Arta 4, in Achaia 5, in Viotia 2, in Grevena 2, in Drama 3, in Evrytania 2, in Ilia 1, in Imathia 2, in Irakleio 2, in Thesprotia 3, in Ioannina 38, in Kavala 1, in Kastoria 4, in Lakonia 1, in Magnisia 1, in Messinia 1, in Xanthi 1, in Pella 3, in Preveza 5, in Serres 3 and in Fthiotida 1.

The contribution of rainbow trout (*Oncorhynchus mykiss*) to the production of aquaculture (1985-1987) remained almost stable at 2.82% of the total production (71,005 tons) in 1985 in aquacultures at 2.80% in 2017, the fish amount reached 1,989 tons in 2017 from 2,000 tons in 1985, with the average amount reaching 2,152 tons.

As depicted in Figure 1, the production performance of rainbow trout for the period 1985-2017 noted an unstable reaction, presenting continuous fluctuations in the annual fish production.
Meanwhile, the prices for rainbow trout (*Oncorhynchus mykiss*) from aquaculture increased from 4.10 U.S. dollars per kilo in 2016 to 4.44 U.S. dollars per kilo in 2017, while the average price between 1985 and 2017 reached 3.31 $/Kg. The growth rate for the measurement period (1985-2017) of the amount (71,005 tons) was estimated (CAGR)= -0.02% and value (CAGR)= 0.4% of total value (263,300,00$). Figure 2 presents the maximum production value 4.3$/Kg, recorded in 2013, value 8,702,000 ($) from a production of 2,016 tons.

In Figure 3 is presented the prediction of the production volume for the period 34 (2018) amount 1,723 tons, period 35 (2019) amount 1,750 t and period 36 (2020) 1,699 tons.

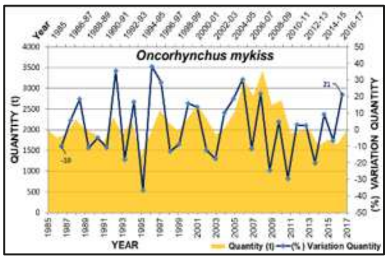

Figure 1. Production in tons of *Oncorhynchus mykiss* in Greece and percentage variation of the amount of aquaculture per year.

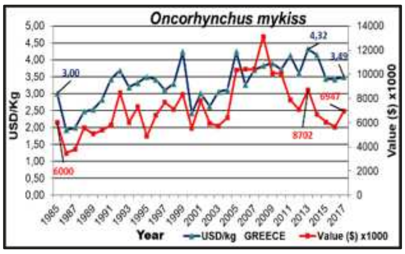

Figure 2. Price fluctuation in comparison with production evolution of *Oncorhynchus mykiss* in Greece.

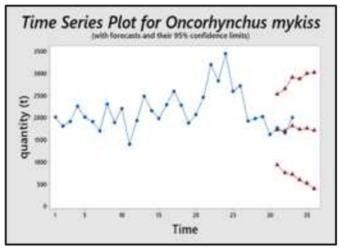

Figure 3. Prediction diagram of the production volume of aquaculture (*Oncorhynchus mykiss*) by using ARIMA (3, 1, 1) from 1985-2020.

Conclusions

The present study provides a general overview of the production and value of inland water aquaculture and especially of rainbow trout *Oncorhynchus mykiss* over the last thirty-three years in Greece. The *Oncorhynchus mykiss* aquaculture presents a rapid production increase, based on business activity with the corresponding branches attracting capitals from national and international sources. It is noted that during the period 2009-2017, the economic crisis which the country went through is also reflected in the production route and growth of the aquaculture sector. The results provide valuable knowledge on the comprehension of the evolution of the production volume and value of the inland waters aquacultures of *Oncorhynchus mykiss*, as well as identifying optimal policies and possible measures of mitigating the consequences, political, social, and environmental factors in the whole of Greece. With the escalating climate change and the ever-changing human activities, it is estimated that in the future sustainable growth, ecology and food security in Greece are likely to confront further severe challenges. Hence, further thorough research is required for the development of inland water aquaculture.

References

ELSTAT. (2017). Retrieved November 4, 2019, from http://www.statistics.gr/el/statistics/-/publication/SPA06/-

European Commission. (2015). European Maritime and Fisheries Fund (EMFF) Greece. Retrieved October 17, 2019, from https://ec.europa.eu/oceans-and-fisheries/index_en.

European Commission. (2017). Aquaculture | Fisheries. Retrieved October 17, 2019, from https://ec.europa.eu/fisheries/cfp/aquaculture_en.

FAO. (2019). GLOBEFISH Highlights, Issue 1/2019 | GLOBEFISH | Food and Agriculture Organization of the United Nations. Retrieved October 17, 2019, from http://www.fao.org/in-action/globefish/publications/details-publication/en/c/ 1190222/.

Guillen, J., Asche, F., Carvalho, N., Fernández Polanco, J. M., Llorente, I., Nielsen, R., … Villasante, S. (2019). Aquaculture subsidies in the European Union: Evolution, impact and future potential for growth. Marine Policy. https://doi.org/10.1016/j.marpol. 2019.02.045.

Lacy, S. N., Meza, F. J., & Marquet, P. A. (2017). Can environmental impact assessments alone conserve freshwater fish biota? Review of the Chilean experience. Environmental Impact Assessment Review, 63, 87–94. https://doi.org/10.1016/J.EIAR.2016.12.006.

Ministry of Rural Development and Food. (2020). Retrieved January 31, 2020, from http://www.minagric.gr/index.php/el/.

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A SMALL SCALE STUDY ON FISHERIES - DOLPHINS INTERACTIONS IN THE KORINTHIAKOS GULF, EASTERN MEDITERRANEAN SEA

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Abstract
Studies on dolphins and commercial fisheries have been subject to little attention regarding the ecological aspects of such interaction. Although relative studies on the field have been shown that this interaction may be problematic for both parties, to the best of our knowledge this study is a first attempt to gather valuable data in the Korinthiakos Gulf, central Greece. Since dolphins and fishermen operate within the same ecological niches for their survival, the main area of conflict being nutritious fish. This preliminary study attempts to draw to argue the symbiosis between humans and dolphins.
Keywords: *Fisheries - dolphins interactions, Korinthiakos Gulf, eastern Mediterranean Sea, fishermen*
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1. Introduction

Nowadays, dolphins, as an iconic symbol for conservation (Banaru *et al.* 2013), are more frequently brought into conflict with anthropogenic stressors, such is fisheries. Interactions between commercial fisheries and dolphins are subject to animal welfare concerns (Dolman & Brakes 2018). Moreover, climate changes may shift abundance and distribution of food availability, resulting in an alteration in site-fidelity and home range of dolphins in a given area. The latter, in conjunction with high fishing effort, reveal that these interactions generally intensify in times of overexploitation of resources from the part of the fisheries (Pardalis et al. 2021). Such food-resource niche overlap suggests a rather over the top competition, with severe potential ecological and economic losses that can arise from dolphin populations decline (Milani et al. 2019). Thus, dolphins' conservation has a social and economic value that needs to be aknowledged by the society and stake holders. This study focuses on the Korinthiakos Gulf, analyzing a few basic parameters that potentially could play an important role in providing culturally relevant solutions toward sustainable development.

2. Material and Methods

For this purpose, five (5) small-scale fishermen from different places of the county of Korinthia were interviewed to collect data. Two of them operate within the Korinthiakos Gulf and the Saronic Gulf as well (Figure 1). Emphasis was put on their interactions with dolphins but also they answered questions concerning the fish stock. Certain questions guided the conversation and allowed the interviewers to ensure flow but did not limit the interviewees' answers. Data of this semi-structured interview are anonymized. All fishers were shown an identification catalogue of the dolphin species known (provided by ARION-Cetacean Rescue and Rehabilitation Research Center) and were requested to distinguish the ones interacting with their fishing activities.

3. Results

Questionnaires revealed that there is an increase in the population of dolphins over the last 3-4 years. The identified species as per fishermen are *Delphinus delphis, Stenella coeruleoalba* and *Tursiops truncatus.* One fisherman recognized *Globicephala melas* eating fish from the net. Dolphins' attacks usually occur in groups of 2-5 and only in full nets. That is because they may find it easier to feed on trapped fish even if this practice can be sometimes lethal. They usually get injured or captivated by carelessness in their attempt to grab fishes from the nets. Dolphins attack a random part of the net without having a specific pattern. As for the net destruction, there are two types.

More specific, dolphins either create round holes in the net almost the size of their body, or «v» shaped holes and drags the fish or enters the net, or they pull a part/line of the net destroying it all. This is called «latini» among fishermen. Moreover, 3 out of 5 fishermen had not kill any dolphin by accident or purpose. They try to avoid them by fishing in bigger depth because damage to fishing gear is not affordable. These attacks make them buy new nets every 3 months instead of 6. One positive impact is that sometimes dolphins help fishermen by gathering fish around their nets.

Figure1. Red dots in the map indicate the study area and the place of residence of the 5 small-scale fishermen.

4. Discussion and Conclusions

This survey revealed that dolphins usually eat in a specific order after their attack to the net, one by one, and that most of them appear when the phases of the moon change. Fishermen who operate within the Korinthiakos and the Saronikos Gulf agree that the dolphins of the Saronikos Gulf are less friendly, cause problems in higher frequency, even though they belong to the same species. During the interview we attempted to gather data for the fish stock as well. There is a lack of reliable and comparable data on fish stock which cannot be analyzed or categorized. Fisheries can have both positive and negative impacts on cetaceans, such as dolphins, but a lack of adequate data imposes limits and enables us to act properly. All fishermen highlight the need for orientation and updating in this field, blaming the state for indifference to their situation. However, their love for the aquatic environment and their willingness to improve their interactions with dolphins allows hope for the future. As a result, further research is needed to reach conclusions thus, further investigation and appropriate management plans are extremely important.

References

Banaru D., Mellon-Duval C., Roos D., Bigot J.L., Souplet A., Jadaud A., Beaubrun P., Fromentin J.M. (2013). Trophic structure in the Gulf of Lions marine ecosystem (north-western Mediterranean Sea) and fishing impacts. Journal of Marine Systems 111, 45–68.

Dolman S.J., Brakes P. (2018). Sustainable Fisheries Management and the Welfare of Bycaught and Entangled Cetaceans. Frontiers in Veterinary Science 5, 287.

Milani C., Vella A., Vidoris P., Christidis A., Kamidis N., Leykaditou E. (2019). Interactions between fisheries and cetaceans in the Thracian sea (Greece) and management proposals. Fisheries Management and Ecology 26, 374-388

Pardalis S., Komnenou A., Exadactylos A., Gkafas GA. (2021). Small scale fisheries, dolphins and societal challenges: A case study in the city of Volos, Greece. Conservation 1, 81-90.

MORPHOMETRIC CHARACTERISTICS OF *Dentex maroccanus* IN THE AEGEAN SEA

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Abstract
Dentex maroccanus is one of the least studied species of the family Sparidae. In this work, the relationship of 29 morphometric characters with size was examined, information necessary in stock identification and feeding studies. The relationship of all characters with total length was statistically significant. The linear model fitted the data well, and an increase of each character with length was obvious in all cases.
Key words: *Dentex maroccanus,* morphometrics*, Aegean*
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1. Introduction

Morocco Dentex, *Dentex maroccanus* (Valenciennes, 1830) is a demersal species, member of the family Sparidae. Its distribution extends in the Eastern Atlantic, from the Bay of Biscay to the Gulf of Guinea, the Strait of Gibraltar and the Southern and Eastern Mediterranean (Fisher *et al*. 1987). A few studies on the age, growth, reproduction, fecundity and the spatial distribution of *D. maroccanus* have been conducted in Mediterranean to date (Lamrini & Bouymajjane 2002, Maravelias *et al*. 2007, Mohdeb & Kara 2014, Gul 2014, Taylan *et al*. 2018). Only one published work exists on the species morphology concerning the mean value of 12 morphometric characters (Bayhan *et al*. 2016)

The objective of this work is a preliminary study of the relationship of 29 morphometric characteristics with the total length of the species in the south Aegean Sea. Morphometric measurements are important because they can provide, along with genetics, information on the stock identification of the species and they enhance our knowledge on the species ecology and diet.

2. Materials and methods

Data were reported from samples collected in the South Aegean Sea during September 2014 - May 2015 within the framework of EPILEXIS project. A total of 416 specimens were examined, ranging from 92 to 233 mm Total Length, caught at depths between 68 and 255 m. Fish were frozen immediately after capture. In the laboratory, morphometric measurements to the closest mm using electronic calliper were recorded; these included Total Length, Caudal Peduncle, Pre-anal Length, Snout, Pre-dorsal Length, Dorsal Length, Anal Length, Pectoral Length, Pelvic Length, Body Depth, Caudal Height, Caudal Fin Distance, Body Perimeter, Head Length, Pre-pectoral Length, Post-orbital Length, Eye diameter, Eye height, Inter-orbital Distance, Upper-jaw Length, Lower jaw Length, Head height eye, Head height mid-eye, Lower jaw mouth distance, Between Eyed Width, Mouth Depth, Mouth width, Protrusion upper lip Length and the Eye Lens Size. The relationship for each morphometric character with the TL was studied using the Statgraphics statistical software.

3. Results and Discussion

The relationship between TL and the 29 morphometric characters studied was found to be statistically significant (<0.005) in all cases. Data fitted well linear regression models for all morphometric characters. The slope was also significant in all cases indicating that all morphometric characters increased with increasing TL. Examples of these relationships are given in Figure 1 for the between eyes width (BEW) and the pre-anal length (PrAL) relationships.

Further study to identify differences in these relationships between sex are planned in the future since Byahan et al. (2016) found that some morphometric characters are more pronounced for females than males. The relation of some of the above-mentioned morphometric characters with the feeding habits of the species could also be of interest for further studies.

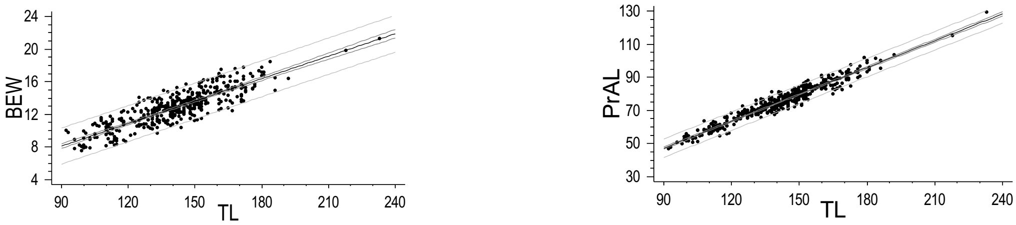

Figure 1. Relationship of the between eyes width (BEW) (left) and the pre-anal length (PrAL) (right) with total length. All measurements in mm.

Acknowledgements
The authors would like to acknowledge the HCMR personnel and the crew of the commercial vessel "Takis-Mimis" for the collection of the data sampled within the frame of the project ELIPEXIS (ΕΠΑΛ 2007-2013, ΜΕΤΡΟ 3.5, Pilotic planning, Code Action 185365).

References

Bayhan, B., Heral O., Taskavak E., Topkara E.T., Kara A. (2016) Morphometric characteristics of the Morocco *Dentex Dentex dentex*, Valenciennes, 1830, in the Izmir Bay. Rapp. Comm. Int. Mer Medit. 41. 335.

Fisher W., Schneider M., Beauchot M.-L. (1987). Fao d' Identification des Espèces pour les Besoins de la Pêche. Organisation des Nations Unies pour l'alimentation et l'agriculture, Rome, pp.1529.

Gul, G. (2014). Age, growth, and reproduction of *Dentex maroccanus* (Actinopterygii: Perciformes: Sparidae) in the Saros Bay (north Aegean Sea). Acta Ichthyologica et Piscatoria 44(4), .295-300.

Lamrini A., Bouymajjane A. (2002). Biologie de *Dentex maroccanus* (Valenciennes, 1830) dans la région de Safi, in *"Actes de l'Iav Hassan II."*22(1), Actes Editions, 11-18.

Maravelias C.D., Tsitsika E.V., Papaconstantinou C. (2007). Evidence of Morocco dentex (*Dentex maroccanus*) distribution in the NE Mediterranean and relationships with environmental factors determined by Generalized Additive Modelling. *Fisheries Oceanography*, 16(3), 294-302.

Mohdeb R. & Kara M. (2014). Age, growth and reproduction of the Morocco dentex *Dentex maroccanus* of the eastern coast of Algeria. Journal of the Marine Biological Association of the United Kingdom, 95(6), 1261-1270.

Taylan B., Bayhan B., Heral O. (2018). Fecundity of Morocco Dentex *Dentex maroccanus* Valenciennes, 1830 Distributed in Izmir Bay (Central Aegean Sea of Turkey). Turkish Journal of Agriculture, Food Science and Technology,6(5), 624.

AGE AND GROWTH OF *Micromesistius poutassou* IN NORTH AEGEAN USING OTOLITH MORPHOMETRIC OBSERVATIONS

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Abstract
Between April 2018 and December 2019, 487 samples of *M. poutassou* were collected from commercial bottom otter trawl operating in North Aegean Sea. Fish length-weight and otolith length and weight were measured. Length and weight relationship, $W=a*L^b$, did not reveal significant differences between sex. Growth parameters for combined sex were estimated, L_{inf}=268.365, K=1.02124, t_0= -0.22063. Fish age was determined by counting otolith annual rings, aged from 0 to 5 years. Furthermore, Otolith Weight (OW) and Otolith Length (OL) were obtained and correlated with fish age; OW= 0.009748 +0.046557*Age and OL curve's coefficients were L_{inf}=14.45892, K=0.46677, t_0= -1.80876.
Keywords: *Micromesistius poutassou, otolith, age reading, morphometric characteristics*

1. Introduction

Blue Whiting *(Micromesistius poutassou,* Risso 1827*)* is a pelagic gadoid with wide distribution, found in European waters from Barents Sea up to the Mediterranean Sea. In Greece, although it is a highly commercial species, landed in high numbers (1014 t of Blue Whiting have been landed in 2019, the third fish in landings for bottom otter trawls, after hake and red mullet, data: HELSTAT) its biology-growth is understudied. Even less surveys on Blue Whiting have been conducted in Northern Aegean.

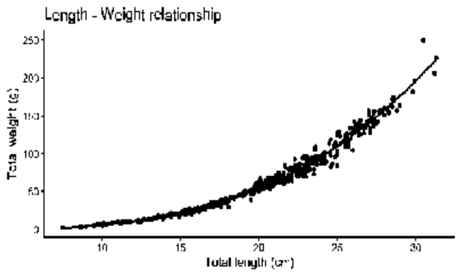
The determination of fish growth parameters is of great importance, since the relevant estimations are essential inputs for analytical stock assessment methods. Usually, age for Blue Whiting is calculated based on the detection of annual development ring on the otoliths; however, in Blue Whiting this approach is often less accurate than usual, due to the three-dimensional deposition of minerals on the otolith (Goncalves *et al.* 2017). An alternative method for fish age estimation, would be the use of Otolith Weight (OW) as a proxy for age determination, through the calculation of Age-OW relationship. This method has been applied in the past for age determination in various species (but not in Blue Whiting) and it has been proven to be cheap, fast, and accurate (Cardinale *et al.* 2000). The main goal of this study was the estimation of Blue Whiting age based on annual ring detection as well as based on otolith morphometric measures, such as Otolith Weight and Otolith Length.

2. Materials and Methods

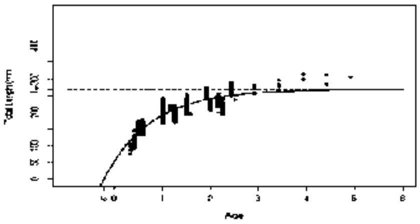
487 sample of Blue whiting were collected from commercial bottom otter trawls between April 2018 and December 2019. The samples were collected from Thracian Sea, at depths of 80-450 m (mainly 200-450 m). Fish Total Length (TL in cm) and Total Weight (TW in g) were measured in the laboratory. The otoliths were extracted, cleaned, and photographed using a NIKON Digital Sight DS–L2 system. Fish age was determined based on the annual development rings. Otolith Length (OW in mm) and Otolith Weight (OW, in g, to the nearest 0,0001 g) were also determined. The relationships TL-TW, TL-OL Age-OW were calculated within R programming environment using the packages car, MASS and FSA.

3. Results & Discussion

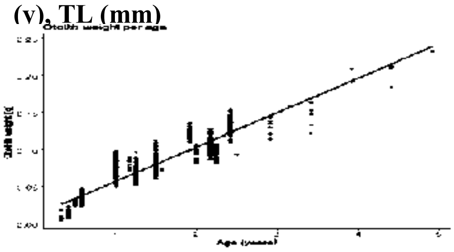
The coefficients a and b of the relationship $TW=a*TL^b$, were a=0.004, b=3.155. TW-TL relationship was not different between the two sexes (Anova test, F=0.03, p=0.87, r^2=0.991). Blue whiting allometry was positive (t-test to compare b parameter with 3, t=11.54, p<0.0001). The outcomes were comparable to a study from Portugal (Doering-Arjes *et al.* 2008), while positive allometry for Blue Whiting has also been found in study from Patraikos-Korinthiakos gulf in Greece (Παπακωνσταντίνου, Κ. *et al.*1988).



Graph. 1 Scatter plot for the relationship $TW=a*TL^b$ for Blue Whiting in Thracian Sea. X axes is Total Length (cm), and Y axis is Total Weight (g).



Graph 2. Von Bertalanffy growth curve, M. poutassou, N. Aegean. Age (y), TL (mm)



Graph 3. Otolith weight (g)-Age(y). M. poutassou, N. Aegean.

Graph 4. Otolith Length (mm), Age (y), M. poutassou, N. Aegean.

The determination of the first annual otolith ring, one of the most important issues to be solved for otolith age readers (ICES, 2013), was found to be formed within November, and the first year TL was estimated to be between 171-240mm. Von bertalanffy's coefficients were calculated L_{inf}=26,83cm, K=1,021, t_0= -0.22. L_{inf} is relatively low, compared to a study in the Adriatic (L_{inf}=31.9), since most of our sample lengths were relatively short, ranging from 170mm to 269mm. Nevertheless, growth rate in our study was greater (K=1.026) compared to (K=0.086) (Froglia & Gramitto 1981).

Mean OL per age were calculated and compared to the in Goncalves et al. (2017). For age 0 mean OL was found equal to 8.21 mm (CI=7.97-8.44mm), for age 1 mean OL=10.73mm (CI=10.57-10.88mm), for age 2 mean OL=11.78mm (CI=11.65-11.9mm), while in 3+ class mean OL=13.92mm (CI=13.22-14.62mm). Mean OL per age (for ages 0-2) were found to be lower than the corresponding values of the in Goncalves et al. (2017), (One-sample t-test: age 0 comparison to 8.98 mm, t=-6.46, $p<0.0001$, age 1 comparison to 11.87 mm, t=-14.6, $p<0.0001$ and age 2, comparison to 13.27 mm, t=-23.4, $p<0.0001$). The linear relationship between OL-TL was also calculated and found equal to OL=1.663+ 0.41*TL (r^2=0.97). The corresponding relationship in Goncalves et al. (2017) was OL=2.58+0.13*TL.

Otolith Weight has been used in the past as a proxy for determining the age in various species of the Gadidae family (Cardinale & Arrhenius 2004). To our knowledge, Age-OW has never been calculated for Blue Whiting before. The relationship has be found to be linear OW= 0.009748 +0.046557*Age (r^2=0.862). The correlation found is strong, accounting for a helpful tool in age reading

4. Conclusions

Blue Whiting at the end of its first year of life has a TL between 171-240 mm, Otolith Weight between 0.05 - 0.097 g and Otolith Length between 9.1 - 11.1 mm.

The Age-Otolith Weight relationship has been estimated for the first time, and although some OW overlap between age classes has been detected, it could be proved useful for age determination in the species.

More information about sex, sampling date, OW, OL could be of help, since the appearance of false annual rings in Blue Whiting is very common.

Data presented is part of the National Fisheries Data Collection Programme (EPSAD), Ministry of Rural Development and Food, Fisheries Research Institute of the Greek Agricultural Organization-Dimitra. (Cofounded Programme by the EU and the OP "Fisheries & Maritime" 2014-2020)

References

Cardinale, M., Arrhenious, F. & Johnsson, B., 2000. Potential use of otolith weight for the determination of age-structure of Baltic cod (Gadusmorhua) and plaice (Pleuronectes platessa). *Fisheries Research,* Volume 45, pp. 239-252.

Cardinale, M. & Arrhenius, F., 2004. Using otolith weight to estimate the age of haddock (Melanogrammus aeglefinus): a tree model application.. *Journal of Applied Ichthyology,* 20(6), p. 470–475.

Doering-Arjes, P., Cardinale, M. & Mosegaard, H., 2008. Estimating population age structure using morphometrics: atest with known-age Atlantic cod (Gadus morhua) individuals. *Canadian Journal Fishery Aquatic Sciencs,* Volume 65, pp. 2342-2350.

Froglia, C. & Gramitto, E. M., 1981. Observations on growth of Micromesistius poutassou (Risso) (Pisces, Gadidae) in the Central Adriatic Sea. *Rapp. Comm. int. Mer Medit.,* 27(5).

Goncalves, P., Avila deMelo, A.M., Murta, A.G. & Cabral, H., 2017. Blue whiting otolith pair's symmetry side effect. *International Journal of Fisheries and Aquatic Studies,* 5(3), pp. 06-09.

ICES, 2013. *Report of the Workshop on the Age Reading of Blue Whiting,,* Bergen, Norway: ICES.

Παπακωνσταντίνου, Κ. et al., 1988. *Δυναμική Βενθοπελαγικών Ιχθυοπληθυσμών στον Κορινθιακό, Πατραϊκό Κόλπο και Ιόνιο Πέλαγος,* Αθήνα: Εθνικό Κέντρο Θαλάσσιων Ερευνών.

DIET COMPOSITION OF *Dentex maroccanus* IN THE AEGEAN SEA

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Abstract
The stomach contents of 416 individuals of *D. marrocanus*, caught by bottom trawl surveys, were examined. Out of all specimens, 67 had non-empty stomachs that allowed the diet composition analysis. The diet of the species consisted of 18 different prey taxa. The dominant prey items unidentified were Decapods, Gastropods, and brachyurans, which showed the benthic character in the feeding habits of the species.
Key words: *Dentex marrocanus*, *diet, Aegean Sea*
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1. Introduction

Diet and stomach content analysis studies constitute an essential component for understanding, exploring, and contrasting trophic relationships between organisms, as well as population and community dynamics (Baker *et al*., 2013). Consequently, these studies are essential and fundamental in the Marine Ecology and Fisheries Biology fields providing useful information for fisheries management and ecosystem status (Sivadas & Bhaskaran 2009). Morocco dentex, *Dentex maroccanus*, is a demersal species of the family Sparidae of considerable commercial importance, inhabiting a depth range of 20-500 m and favoring shallower waters with higher salinity (Maravelias *et al*. 2007). *D. maroccanus* is distributed in the Eastern Atlantic, from the Bay of Biscay to the Gulf of Guinea, in the Strait of Gibraltar and the Southern and Eastern Mediterranean (Fisher *et al*. 1987).

The feeding habits of *D. maroccanus* have generally not been studied in detail so far. This paper is dedicated to presenting preliminary qualitative and quantitative results on the diet of *D. maroccanus* in the South Aegean Sea for the first time.

2. Materials and Methods

Sampling was carried out with a commercial bottom trawl in the South Aegean Sea (Cyclades islands) during September 2014 - May 2015 within the framework of EPILEXIS project. A total of 416 specimens were collected, ranging from 99 to 186 mm TL (Total Length) and caught at depths between 68 and 255 m. Fishing was conducted during daytime. Fish were frozen immediately after capture and dissected in the laboratory, where the total weight (TW) (precision 0.001 g), the total length (TL) to the closest mm and the sex were recorded. Stomach content was analyzed with a stereomicroscope to identify prey items, which were counted and weighted. Prey items were identified to the lowest possible taxonomic level. Individuals with inverted stomachs were excluded. The relative abundance (%N) of a prey to the total number of prey items and the frequency of prey occurrence (%F) were estimated (Hyslop 1980).

3. Results and Discussion

The diet composition of *D. maroccanus* was examined from the stomach contents of 416 specimens. Whilst the total number of specimens exceeded 400 individuals, the number of non-empty stomachs that were available for diet analysis was quite low, totaling to 67. The diet of the species consisted of 18 different prey categories, belonging to 4 major taxa (Polychaetes, Crustaceans, Mollusks, Osteichthyes). In particular, Decapods (unidentified) were found in greater occurrence and abundance (%N = 32, %F = 27), followed by Gastropods (%N = 13, %F = 13) and brachyurans (%N = 9, %F = 10).

The results of the present study revealed the benthic and carnivorous character in the diet of *D. maroccanus,* which is in agreement with the results reported by (Bayhan *et al*. 2017) for the species caught in the Izmir Bay. Further studies investigating the feeding strategy and feeding habits of the species in more detail are in progress.

Acknowledgements
The authors would like to acknowledge the HCMR personnel and the crew of the commercial vessel "Takis-Mimis" for the collection of the data sampled within the frame of the project ELIPEXIS (ΕΠΑΛ 2007-2013, Measure 3.5, Pilotic Planning, Code Action 185365).

References

Baker, R., Buckland, A., Sheaves, M. (2013). Fish gut content analysis: robust measures of diet composition. Fish and Fisheries, 15(1), 170-177.
Bayhan, B, Tuncay Murat S, Oğulcan H. (2017). Diet composition of the Morocco dentex: Dentex maroccanus Valenciennes, 1830 (Teleostei: Sparidae) in the central Turkish Aegean Sea. Oceanological and Hydrobiological Studies 46 (2), 133-139.
Fisher W., Schneider M., Beauchot M.-L. (1987). Fao d' Identification des Espèces pour les Besoins de la Pêche. Organisation des Nations Unies pour l'alimentation et l'agriculture, Rome, pp.1529.
Hyslop, E. J. (1980). Stomach contents analysis – a review of the methods and their application. Journal of Fish Biology 17, 411– 429.
Maravelias C.D., Tsitsika E.V., Papaconstantinou C. (2007). Evidence of Morocco dentex (*Dentex maroccanus*) distribution in the NE Mediterranean and relationships with environmental factors determined by Generalized Additive Modelling. Fisheries Oceanography 16 (3), 294-302.
Sivadas M, Bhaskaran M. (2009). Stomach content analysis of the Indian mackerel Rastrelliger kanagurta (Cuvier) from Calicut, Kerala. Indian Journal of Fisheries, 56 (2), 143-146.

OTOLITH MORPHOMETRY RELATIONSHIPS OF *Pagellus erythrinus* (L. 1758) IN THE GREEK SEAS

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Abstract
The common pandora, *P. erythrinus* (Linnaeus, 1758), is a commercial fish of the Sparidae family. In this study, the relationship of the otolith morphometric parameters with the body size was examined. A total of 1038 individuals were collected from the Aegean and Ionian Seas. Strong correlation was found between body size and each morphometric parameter. Otolith morphometric parameters differed significantly between the two areas.
Keywords: *Pagellus erythrinus, otoliths, otolith morphometry, South Aegean, lonian Sea*
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1. Introduction

Otoliths are small opaque structures composed of calcium carbonate in an organic matrix and have distinctive shape, which varies widely among fish species (Hunt, 1992). Studies on otolith morphology have recently increased in importance with the development of digital techniques, image analysis systems and shape analysis methods (Bostanci *et al.* 2016). The study on otolith morphometric characters in fishes is important for the stock identification and fishery management due to its unique advantages including low cost and relatively high efficiency (Begg and Brown 2000).

The common pandora, *Pagellus erythrinus* (Linnaeus, 1758), which belongs to the family Sparidae, is a valuable species for aquaculture and fisheries (Bauchot *et al.* 1992). The species has a relatively wide distribution, inhabiting the Black and Mediterranean Seas and from Norway to Angola (Fischer et al., 1987). *P. erythrinus* is an important resource with high commercial value (Fischer *et al.* 1987). The scope of this study is to estimate the relationship between fish size and otolith morphometrics and to detect any differences on *P. erythrinus* otolith morphometry between the two studied areas; S. Aegean and Ionian Sea.

2. Materials and Methods

A total of 1038 specimens of *P. erythrinus* were collected, between 2018 and 2020 within the MEDITS survey; 483 from the S. Aegean (total length TL: 35 to 360 mm) and 555 from the Ionian Sea (total length TL: 46 to 435 mm). The otoliths were extracted, cleaned and photographed. The morphometric parameters of the left otolith were measured using the Image Analysis Pro Plus System software. The following morphometric parameters were measured for each otolith: area (OA), perimeter (P), diameter max (DM) and width (OW), roundness (R) and circularity (C). The relationship of each morphometric parameter with the TL was examined using simple linear regression. The analysis of ANOVA was carried out, in order to detect differences on each morphometric parameter between the two areas.

3. Results & Discussion

There was a strong correlation between the TL and the parameters OA, DM, OW and P (Table 1), indicating that all morphometric parameters increase with increasing TL. No correlation was found between the TL and the parameters R and C. Otolith morphometric parameters of *P. erythrinus* significantly differed between the S. Aegean and the Ionian Seas and many factors could lead to such differences e.g. temperature, depth, food availability, salinity, growth rate and the effect of abiotic factors (Lombarte & Lleonard 1993). This study could be considered as a baseline for further studies about the otolith morphology and stock identification of the species.

Table 1. Parameters of regression analysis between total length (TL) and several otolith morphometric parameters. OA, area; DM, Diameter max; OW, width; P, perimeter; a, intercept; b, slope; N, number of specimens; *, significant difference (p-value<0.05).

TL/OA	**N**	**TL (mm)**	**OA (mm)**	**a**	**b**	**R^2 (%)**	**ANOVA**
Aegean	440	35-360	2,109-79,173	-12.182	0,251	87,7	*
Ionian	500	46-435	2,743-78,470	-9.708	0,234	74,5	
TL/DM	**N**	**TL (mm)**	**DM (mm)**	**a**	**b**	**R^2 (%)**	**ANOVA**
Aegean	475	35-360	1,638-11,849	1.628	0,031	93	*
Ionian	552	46-435	2,141-11,209	1.352	0,034	92,2	
TL/ OW	**N**	**TL (mm)**	**OW (mm)**	**a**	**b**	**R^2 (%)**	**ANOVA**
Aegean	478	35-360	1,038-9,178	0.811	0,023	95,5	*
Ionian	553	46-435	1,340-8,560	0.630	0,025	95,4	
TL/P	**N**	**TL (mm)**	**P (mm)**	**a**	**b**	**R^2 (%)**	**ANOVA**
Aegean	479	35-360	4,409-37,976	3.526	0,094	95,7	*
Ionian	552	46-435	5,904-33,890	2.905	0,101	95,2	

Acknowledgements

The present work was carried out based on samples of the National Data Collection Framework program of Greece.

4. References

Bauchot M.-L., Hureau J.-C. (1990). Sparidae. p. 790-812. In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 2.

Begg G.A., Brown R.W. (2000). Stock identification of haddock *Meleanogrammus aeglefinus* on Georges Bank base don otolith shape analysis. Transactions of the American Fisheries Society, 129, 935-945.

Bostanci D., Yilmaz M., Yedier S., Kurucu G., Kontas S., Darçin M., Polat N. (2016). Sagittal Otolith Morphology of Sharpsnout Seabream *Diplodus puntazzo* (Walbaum, 1792) in the Aegean Sea. International Journal of Morphology, 34(2), 484–488.

Fischer W., Schneider M., Bauchot M.L. (1987). Méditerranée et Mer Noire - Zone de Pêche 37. Volume 2: 1369.

Hunt J. (1992). Morphological characteristics of otoliths for selected fish in the Northwest Atlantic. Journal of Northwest Atlantic Fishery Science, 13, 63-75. https://doi.org/10.2960/J.V13.A5

Lombarte A., Lleonart J. (1993). Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes, 37, 297-306.

DISTRIBUTION PATTERN of *Dentex maroccanus* IN RELATION TO ENVIRONMENTAL FACTORS IN THE AEGEAN SEA

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Abstract
Environmental factors play an important role in the distribution pattern of demersal fish. In this work, the relationship of *Dentex maroccanus* distribution pattern with temperature, depth and season is studied in the south Aegean Sea. The three environmental factors were found statistically significantly related with the abundance of the species. The main pattern for the logarithmic transformation of the abundance showed an increasing trend with temperature ranging between 12.0 and 17.6 ºC, and a decreasing trend with depth ranging between 68 and 316 m. Among the two examined seasons, the mean abundance was higher in summer than in autumn.

Key words: *Dentex maroccanus, abundance, distribution, temperature, depth, season, Aegean.*
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1. Introduction`

Morocco Dentex, *Dentex maroccanus* (Valenciennes, 1830), which belongs to the family Sparidae, is a marine demersal species of low commercial value. It is distributed in the Eastern Atlantic, from the Bay of Biscay to the Gulf of Guinea and in the Mediterranean, mainly in the southern part (Fisher *et al*. 1987). Its bathymetrical distribution throughout the Mediterranean extends down to 500 m (Gul *et al*. 2014). Maravelias *et al*. (2007) studied the spatial distribution of *D. maroccanus* in relation to various environmental factors and found that this species inhabits mainly depths between 50–70 m, with depth and longitude been the primary determinants of its distribution.

It is well known that environmental factors play an important role in the distribution and abundance patterns of demersal fish (Damalas *et al*. 2010). The objective of this study was to provide additional information on the *D. maroccanus* distribution pattern with environmental variables such as temperature, depth and season in the south Aegean Sea.

2. Materials and methods

Data were collected during two experimental fishing surveys conducted in the South Aegean Sea during autumn 2014 and summer 2015 in depths ranging from 50 to 320 m. In total, 84 hauls, of 1 hour duration each, were carried out each period using a hired commercial trawler equipped with a bottom trawl of a very small mesh-sized codend (20 mm). Bottom temperature (from 12 to 18 ºC) was recorded in each haul using a minilog attached in the mouth of the trawl. General linear models were used to detect the relationship between the abundance of the species with two continuous factors (depth and temperature) and a categorical one (season). The logarithmic transformation of the abundance (lnN+1) was used for the analysis, which was performed using the Statgraphics statistical software.

3. Results and Discussion

General linear models showed that the relationship of the abundance of *D. maroccanus* with season, depth and temperature was statistically significant ($p<0.005$). The species was found in 108 of the hauls sampled, during both seasons, and in depths ranging between 68 and 316 m with bottom temperature from 12.0 to 17.6 ºC. The relationship of the abundance with depth and temperature showed a decreasing and increasing trend, respectively (Figure 1). The mean value of abundance was found much higher in summer than in autumn (Figure 2). Our results coincided with those of Maravelias et al. (2007) indicating strong relation of the species distribution with environmental factors. R^2 of the model used was 58% indicating that the linear model was not the most adequate to represent these relationships. The use of a non-linear model, as Maravelias et al. (2007) found, might represent better the variability of the data, an issue that will be studied in a future work.

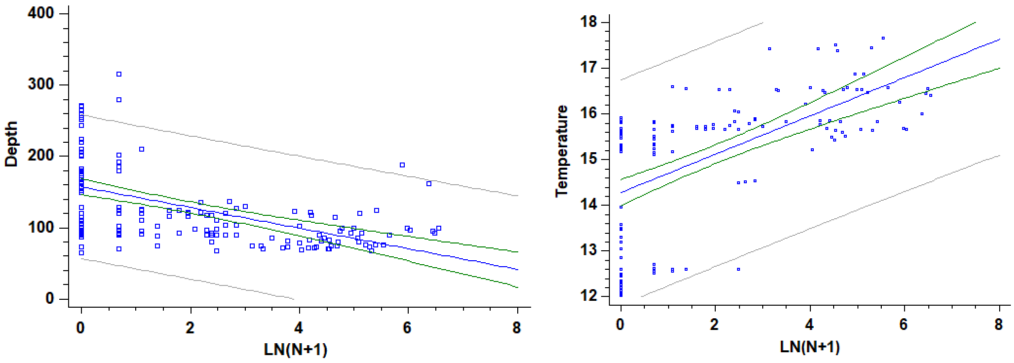

Figure 1. **Relationship between the logarithmic transformation of the abundance [LN(N+1)] of *Dentex maroccanus* with depth, m (left) or temperature, C° (right). N: individuals/h.**

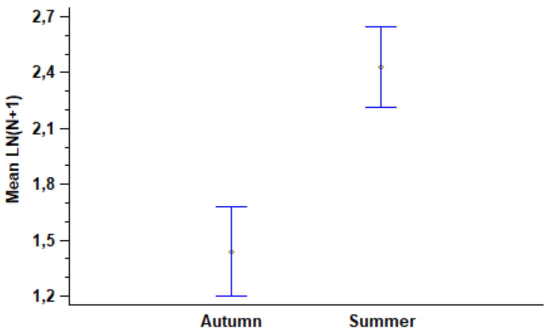

Figure 2. **Mean abundance [LN(N+1)] of *Dentex maroccanus* in each sampling period.**

4. References

Damalas D., Maravelias C.D., Katsanevakis S., Karageorgis A. P., Papaconstantinou C. (2010). Seasonal abundance of non-commercial demersal fish in the eastern Mediterranean Sea in relation to hydrographic and sediment characteristics. Estuarine, Coastal and Shelf Science 89, 107–118.

Fisher W., Schneider M., Beauchot M.-L. (1987). FAO Fishes d' Identification des Espè1ces pour les Besoins de la Pêche. Organisation des Nations Unies pour l'alimentation et l'agriculture, Rome, pp.1529.

Gul, G. Ismen A., Arslan M. (2014). Age, growth, and reproduction of *Dentex maroccanus* (Actinopterygii: Perciformes: Sparidae) in the Saros Bay (north Aegean Sea). Acta Ichthyologica et Piscatoria 44(4) .295-300.

Maravelias C.D., Tsitsika E.V., Papaconstantinou C. (2007). Evidence of Morocco dentex (*Dentex maroccanus*) distribution in the NE Mediterranean and relationships with environmental factors determined by Generalized Additive Modelling. *Fisheries Oceanography*, 16(3), 294-302.

MORPHOMETRIC MEASUREMENTS OF *Serranus hepatus* OTOLITHS IN THE GREEK SEAS

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Abstract
Serranus hepatus is a species of the Serranidae family for which information on its otolith morphometrics in the Mediterranean is generally lacking. In the present study, the relationship of ten morphometric characteristics of the otoliths of this species with body size was examined in samples collected from the Aegean and the Ionian Seas (Eastern Mediterranean Sea). High correlation was found between body size and the otolith characters (radius, area, diameter, width and perimeter). Differences between the two areas were detected for the characters of otolith area and diameter.
Keywords: *Serranus hepatus, otolith morphometrics*
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1. Introduction

Serranus hepatus (L., 1758), the brown comber, is a small-sized demersal fish which belongs to the family of Serranidae. It occurs in the eastern Atlantic coasts and in the Mediterranean and Black Seas. It inhabits sandy and muddy bottoms with seagrass and rocks at depths ranging from 5 to 100 m (Fischer *et al*. 1987). It is mainly caught by bottom trawl and discarded at sea, in contrast to *Serranus cabrilla* and *Serranus scriba,* which both are commercial species of low value. This may explain why there are not so many studies about its biology and ecology (Dulcic *et al.,* 2007).

Otolith morphology is used in fish stock identification studies as it is a low-cost technique. Altin & Ayyildiz (2017) and Bilge *et al.* (2018) studied the relation between some body and otolith characters of this species caught at the northeastern and south-eastern Aegean Sea (Turkish waters), respectively. The aim of the present work was to study for the first time the otolith morphometrics of *S. hepatus* in the south-western Aegean and the Ionian Seas (Greek waters) and define potential differences between the two areas, which can be considered as a base for further studies on the otolith morphology of *S. hepatus* and the distinction of the stocks of this species in this part of the Mediterranean.

2. Materials and Methods

Data on the otolith morphometrics were collected from 299 individuals of *S. hepatus* caught by bottom trawl; 149 in the southwestern Aegean Sea (total length TL: 57 - 117 mm) and 150 in the Gulf of Kerkyra, Ionian Sea (total length TL: 66 - 98 mm). For each specimen, the left and the right sagitta otolith were removed, cleaned, photographed and measured using the Image-ProPlus software (Version 4.5.0.29). Only the right otolith was used for the measurements. The following otolith morphometric parameters were recorded: Radius (R); Area (A); Diameter (D); Width (W); Perimeter (P); Roundness, according to Ponton (2006) and Circularity, according to Tuset *et al*. (2003). Additionally, the following shape factors were calculated: Form Factor, Rectangularity, and Ellipticity according to Tuset *et al*. (2003). The relationship of each morphometric parameter with the TL was examined using simple linear regression. Furthermore, the differences in regression lines were checked through Analysis of Variance. Different linear regressions for the otolith characters were used for each area when significant differences were found between them (P value<0.05). Broken and damaged otoliths were not included in the analysis.

3. Results and Discussion

Statistically significant correlation was found between TL and the examined otolith characters (Table 1). There was no correlation between TL and the factors Roundness, Circularity, Form Factor, Rectangularity and Ellipticity. Our study is in accordance with the findings of Altin & Ayyildiz (2017); Bilge *et al.* (2018), who also indicated a high correlation in the relationships between total length and otolith measurements in *S. hepatus*.

Table 1. Parameters of Regression analysis between total length (TL) and various otolith morphometric measurements per area; R, radius; A, area; D, diameter; W, width; P, perimeter; a, intercept; b, slope; S.E, standard error; R^2, coefficients of determination; *, significant difference (P-value<0.05).

Regression	a	b	R^2	S.E	P-value for the comparison of b between areas
Aegean **TL/R**	0.64	0.02	72	0.02	0.33
Ionian **TL/R**	0.57	0.02	62	0.02	
Aegean **TL/A**	-3.14	0.11	71	0.15	0.03*
Ionian **TL/A**	-4.82	0.14	74	0.12	
Aegean **TL/D**	1.59	0.03	71	0.04	0.03*
Ionian **TL/D**	1.17	0.04	70	0.03	
Aegean **TL/W**	0.39	0.02	70	0.03	0.18
Ionian **TL/W**	0.24	0.02	63	0.02	
Aegean **TL/P**	2.62	0.09	75	0.12	0.08
Ionian **TL/P**	1.81	0.11	76	0.09	

In this study, significant differences between the two areas were exhibited for the relationships between TL with A and TL with D (Table 1). The differences found in the morphology of the otoliths of *S. hepatus* between the two locations should be attributed to differences in the environmental conditions and particular abiotic factors, such as food availability, salinity, temperature as well as depth prevailing in the 2 different seas, factores which is known that may affect otolith morphometry (Campana & Casselman1993, Lombarte & Lleonart 1993; Capoccioni *et al*. 2011).

Acknowledgements
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4. References

Altin A., Ayyildiz H. (2018). Relationships between total length and otolith measurements for 36 fish species from Gökçeada Island, Turkey. Journal of Applied Ichthyology, 34(1), 136–141.

Bilge G., Yapıcı S., Filiz H. (2018). Relationships Between Fish and Otolith Dimensions for *Serranus hepatus* (Linnaeus, 1758) from the Southern Aegean Sea. Natural and Engineering Sciences, 3(2), 128–132.

Campana S.E., Casselman J.M. (1993). Stock Discrimination Using Otolith Shape Analysis. Canadian Journal of Fisheries and Aquatic Sciences. 50(5), 1062-1083.

Capoccioni F., Costa C., Aguzzi J., Menesatti P., Lombarte A., Ciccotti E. (2011). Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (*Anguilla anguilla*, L.) local stocks. Journal of Experimental Marine.

Dulčić J., Matić-Skoko S., Paladin A., Kraljević M. (2007). Age, growth and mortality of brown comber, *Serranus hepatus* (Linnaeus, 1758) (Pisces: Serranidae), in the eastern Adriatic (Croatian coast). Journal of Applied Ichthyology, 23, 195-197.

Fischer W., Schneider M., Bauchot M.L. (1987). Méditerranée et Mer Noire - Zone de Pêche 37. Volume 2: 1318

Lombarte A., Lleonart J. (1993). Otolith size changes related with body growth, habitat depth and temperature. Environ Biol Fish 37, 297–306.

Ponton D. (2006). Is geometric morphometrics efficient for comparing otolith shape of different fish species? Journal of Morphology 267, 750–757.

Tuset V.M., Lozano I.J., Gonzĺez J.A., Pertusa J.F., García-Díaz M.M. (2003). Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). Journal of Applied Ichthyology, 19(2), 88–93.

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SPATIOTEMPORAL MONITORING OF SEA SURFACE TEMPERATURE IN A CENTRAL AEGEAN MPA

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Abstract
This work examines the spatial and temporal variability of sea surface temperature (SST) in a marine protected area (MPA), using remotely sensed data. The National Marine Park of Alonissos, Northern Sporades, was selected as a study area. Satellite data from MODIS (Moderate Resolution Imaging Spectroradiometer), NASA's Terra satellite, were used, covering a period of twenty years. Both day and night monthly SST satellite images were examined and the slope of temperature was extracted for each pixel of the study area. The results showed that there is an increasing trend in SST throughout the study area with local variations.
Keywords: *remote sensing, MODIS, Terra, marine protected areas, Natura 2000, Geographical Information Systems (GIS)*
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1. Introduction

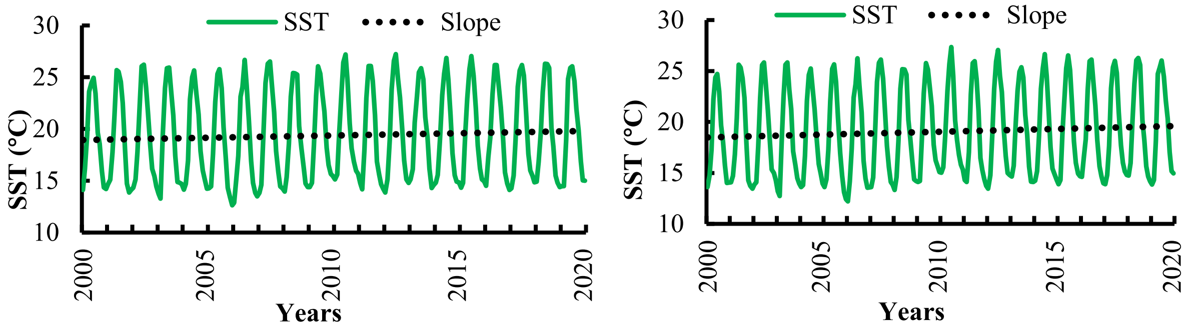
According to the definition given by the International Union for Conservation of Nature – (IUCN), as a protected area is defined «a recognized and clearly defined geographical area for where a conservation commitment exists and managed through legal or other effective means, in order to achieve long-term conservation and nature protection, with its related ecosystem services and cultural values» (Day *et al.* 2019). Protected areas are a key element in conserving biodiversity, contributing to human survival. Protected areas are at the heart of nature conservation efforts and the services provided - food, clean water and protection from the effects of natural disasters. The global network of protected areas is estimated to store at least 15% of the global terrestrial carbon where it appears to be unquestionably the important role that protected areas play in the phenomenon of climate change (Bush 2018). This definition also applies to marine protected areas (MPAs), which is defined as an area designated and effectively managed to protect marine ecosystems, their processes and functions, species and habitats. MPAs can contribute to the restoration and replenishment of natural resources as well as to social, economic and cultural enrichment (Reuchlin & McKenzie 2015). Monitoring geophysical parameters of such areas is a difficult task. Remote sensing could assist the examination of the environmental condition in a marine environment. The present study is based on satellite imagery to map the per pixel and total trends of SST in a Greek MPA, from daily and night monthly images.

2. Material and Methods

SST data from the sensor MODIS (Moderate Resolution Imaging Spectroradiometer), of the Terra satellite were obtained from the official website of National Aeronautics and Space Administration (NASA) and Ocean Color Web (https://oceancolor.gsfc.nasa.gov/cgi/l3). These data are managed by the Ocean Biology Processing Group (OBPG) of the Goddard Space Flight Center (GSFC), constituting a research laboratory of NASA. MODIS combines satisfactory spectral, spatial, temporal and radiometric resolution and provides high quality data from products of geophysical parameters (Minnett *et al.* 2004), such as SST. The data products at level 3 SMI (Standard Mapped Image) were downloaded in netCDF (network Common Data Form). The readily available products are based on the wavelengths 11μm and 12μm, for the daytime images (corresponding to 31 and 32 MODIS channels), while 3μm and 4μm used for nighttime images (corresponding to 20, 22 and 23 MODIS channels), with a spatial resolution of 4km (Kilpatrick *et al.* 2015). From 1/3/2000 to 31/2/2020, a total of 480 Level-3 (SMI) images (240 for SST daytime and 240 for SST nighttime, each one corresponding to a month for the 20-year period), were used for the estimation of the average SST. Data were retrieved for the 128 points, enclosed by the polygon corresponding to the study area, excluding any terrestrial section, as only SST is used. The SeaDAS and QGIS software were used.

3. Results and discussion

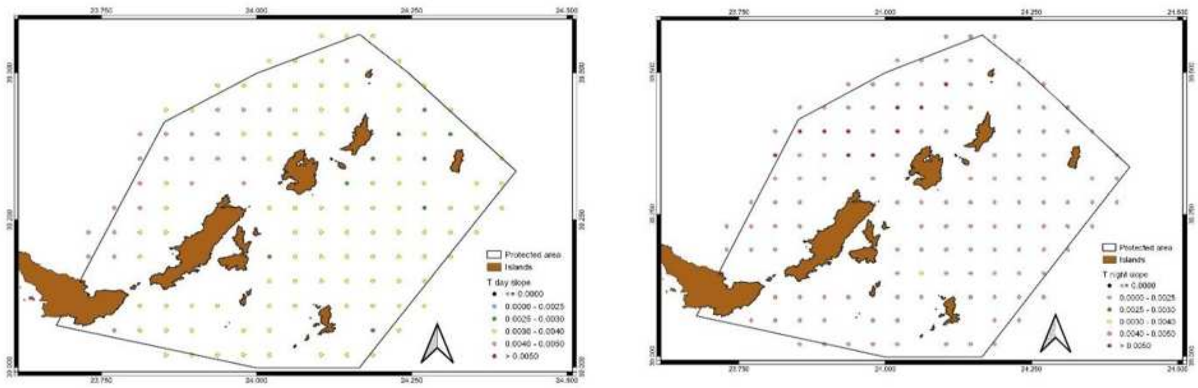
For the average SST variation in daytime and nighttime, there is no significant change throughout the 20-year time series. For the whole time series, the highest average monthly SST value is observed at 27.35 °C, in 2010 during nighttime while the lowest SST at 12.21 °C in 2006 during nighttime (Figure 1).



a) b)

Figure 1. Trend Line Graph showing the average a) daytime and b) nighttime SST variation

For the whole time series, the highest increase of the SST slope is observed in the western and northwestern part of the National Park. The linear trends for the whole 20-year time series are 0.029 ± 0.007 and 0.030 ± 0.007 °C/yr during daytime and nighttime, respectively (Figure 2). All the trends provided are statistically significant at the 95% level ($p \leq 0.05$).



a) b)

Figure 2. Spatiotemporal SST slope distribution from 2000 to 2020 during a) daytime and b) nighttime

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References

Day J., Dudley N., Hockings M., Holmes G., Laffoley D., Stolton S., Wells S., Wenzel L. (2019). Guidelines for applying the IUCN protected area management categories to marine protected areas. 2nd edition, Best Practice Protected Area Guidelines Series. Gland, Switzerland.

Bush M.J. (2018). Climate Change Adaptation in Small Island Developing States; John Wiley & Sons Ltd: Oxford, pp. 240.

Reuchlin H.E., McKenzie E. (2015). Marine protected areas: Smart investments in ocean health. WWF, Gland, Switzerland, pp. 20.

Minnett P.J., Brown O.B., Evans R.H., Key E.L., Kearns E.J., Kilpatrick K., Kumar A., Maillet K.A., Szczodrak G. (2004). Sea-surface temperature measurements from the Moderate-Resolution Imaging Spectroradiometer (MODIS) on Aqua and Terra. Proceedings of 2004 IEEE International Geoscience and Remote Sensing Symposium Proceedings: Science for Society: Exploring and Managing a Changing Planet (IGARSS 2004), vol. 7, pp. 4576–4579.

Kilpatrick K.A., Podestá G., Walsh S., Williams E., Halliwell V., Szczodrak M., Brown O.B., Minnett P.J., Evans R. (2015). A decade of sea surface temperature from MODIS. Remote Sensing of Environment 165, 27–41.

SUNSCREENS AS EMERGING MARINE POLLUTANTS: ENVIRONMENTAL CONCERNS AND IMPLICATIONS FOR THE MEDITERRANEAN SEA

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Abstract
The global upward rise in the use of sunscreen products is related to high temperatures, high levels of ultraviolet radiation and recent trends in the tourism market. UV filters, contained in sunscreen products are extensively released in the aquatic environment and especially in the sea. They are considered a new group of emerging pollutants, with potentially serious effects on the environment, marine organisms and human health, as endocrine disruptors, although their ecotoxicological footprint has not yet been fully investigated. This paper provides an overview of the occurrence and toxicity of sunscreen compounds in the marine environment, with environmental and health effects concerns underlined for the Mediterranean countries, considered as global tourist destinations.
Keywords: *Sunscreens, UV filters, emerging pollutants, toxicity, marine environment*
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1. Introduction

During the recent years, specialists advise the systematic, even daily, use of sunscreen products, with UV filters, in order to protect human skin from the adverse effects that could be caused by solar radiation. UV Filters are considered new "emerging pollutants" with possible adverse effects on marine environment (Lapworth *et al*. 2012, Thomaidi *et al*. 2015). Long-term exposure of humans to emerging pollutants increases the probability of cancer, reproduction problems, diseases of endocrin and immune system, neurodevelopmental problems, and learning disabilities. Moreover, due to their bioaccumulation potential in the fatty tissues during lifetime, they are source of human internal exposure (Borg *et al*. 2013). A literature review was performed regarding the levels and toxicity of sunscreen compounds in the marine environment. Implications for the Mediterranean Sea due to the increasing release of these emerging pollutants are highlighted.

2. Occurrence and toxicity of sunscreens in seawater and sediments

Coastal touristic activity is the fastest developed sector of tourism industry. The number of tourists has increased from 463 millions in 1992 to 763 millions in 2004, in global scale, and in 2022 this number is expected to reach 1,50 billions (Honey & Krantz 2007). Mediterranean Sea is, by far, the most popular touristic destination, receiving more than 330 million tourists annually. Therefore, Mediterranean countries such as Greece, Italy, Croatia, Spain, Turkey, Morocco, Tunisia, Cyprus, are exposed to intense touristic pressure, with important effects on their ecosystems. Solar radiation in these countries especially during the summer months makes sunscreens use necessary for human health protection, while indirect release via wastewater also occurs. Table 1 collectively presents sunscreen compounds occurrence data reported in the literature in marine waters and sediments and Table 2 provides their toxicity data for marine organisms. According to the results of this overview, the UV filters 3-(4′-methylobenzylideno)-d-1-camphor (4-MBC), benzophenone-3 (BP3), octinoxate (OMC) and octocrylene (OC) are those detected mainly in marine organisms. BP-3 seems to be more toxic, as its toxic effect was initiated at lower concentration levels in comparison to the rest filters. Next more toxic compounds are 4-MBC, OMC and OC. BP-3 is toxic not only to smaller organisms, such as algae, but also to larger marine organisms such as turtles, fish etc. According to research by Calafat *et al*. (2008) BP-3 was detected in 96,8% of urine samples from 2517 adults in USA. Concentration range was 0,4 to 21700 mg L^{-1}, with average 22,9 mg L^{-1}, while only in 30 volunteers BP-3 was not detectable. Schlumpf *et al*. (2008) reported the presence of BP-3, 4-MBC, OMC, OC and other filters in breastmilk of 34 women. 27 of these women reported use of some type of personal care product containing UV filters, which were detected in 26 of 27 breastmilk samples. These findings reflect the widespread occurrence of these compounds, as well as the possible effects of direct and indirect human exposure to them via the environment.

3. Implications for the Mediterranean Sea-concluding remarks

The extensive release of sunscreens in the Mediterranean, a closed system with limiter water exchange with the ocean and high retention time of surface waters, poses significant risk for the marine environment. Future projections of international tourist arrivals report an average increase to 2,6 millions annually by 2030

(UNWTO, 2017). In this context further research is necessary in order to fully understand the ecotoxicological effects and bioaccumulation of sunscreen filters in the marine environment, as well as to develop new alternative environmentally friendly products.

Table 1. Occurrence of sunscreen compounds in marine waters and sediments

Substance	Type of sample	Average concentration in one sampling point	Range of average concentrations in many sampling points	Area	Reference
Benzophenone-3 (BP-3)	Seawater	1,395 mg L^{-1}		Virgin Islands, USA	Downs et al. (2016)
Benzophenone-3 (BP-3)	Seawater	19,2 μg L^{-1}		Hawaii, USA	Downs et al. (2016)
Benzophenone-3 (BP-3)	Seawater	692 μg L^{-1}		Galicia, Spain	Vila et al. (2016)
Benzophenone-3 (BP-3)	Seawater	5429 ng L^{-1}		Hong Kong, China	Tsui et al. (2014)
Benzophenone-3 (BP-3)	Seawater	3316,7 ng L^{-1}		Gran Canaria, Spain	Sánchez- et al. (2014)
Benzophenone-3 (BP-3)	Seawater		10 – 2013 ng L^{-1}	South Carolina, USA	Cadena-Aizaga et al. (2020)
Benzophenone-3 (BP-3)	Seawater		10 – 1540 ng L^{-1}	Holland	Cadena-Aizaga et al. (2020)
Benzophenone-3 (BP-3)	Seawater		9 – 1258 ng L^{-1}	Japan	Cadena-Aizaga et al. (2020)
Benzophenone-3 (BP-3)	Seawater		18,8 – 1233 ng L^{-1}	Taiwan	Cadena-Aizaga et al. (2020)
Benzophenone-3 (BP-3)	Seawater		13 – 439,9 ng L^{-1}	Norway	Kotnik et al. (2014)
Benzophenone-3 (BP-3)	Seawater		96 – 380 ng L^{-1}	Slovenia	Langford et al. (2018)
Benzophenone-3 (BP-3)	Seawater		< 300 ng L^{-1}	Portugal	Cadena-Aizaga et al. (2020)
Octinoxate (OMC)	Seawater	4043 ng L^{-1}		China	Tsui et al. (2014)
Octinoxate (OMC)	Seawater	1200 ng L^{-1}		Spain	Vila et al. (2016)
Octinoxate (OMC)	Seawater	1080 ng L^{-1}		Japan	Sankoda et al. (2015)
Octinoxate (OMC)	Sediment	456 ng g^{-1}		China	Huang et al. (2016)
Octocrylene (OC)	Seawater	171 μg L^{-1}		Spain	Vila et al. (2016)
Octocrylene (OC)	Seawater	7301 ng L^{-1}		Norway	Langford et al. (2018)
Octocrylene (OC)	Sediment	670 ng g^{-1}		Gran Canaria, Spain	Sankoda et al. (2015)
Octocrylene (OC)	Sediment	551 ng g^{-1}		China	Nakata et al. (2009)
bimethyl-PABA	Seawater		0,03 – 1187 ng L^{-1}	China/Japan	Tsui et al. (2014)
bimethyl-PABA	Seawater	5,8 ng L^{-1}		Gran Canaria, Spain	Bratkovics et al. (2013)
bimethyl-PABA	Sediment	150 ng g^{-1}		China	Tsui et al. (2014)
bimethyl-PABA	Sediment		0,8 – 13,9 ng g^{-1}	Northern Pacific/Norway	Langford et al. (2018)

Table 2. Toxicity of sunscreens on marine organisms

Substance	Marine organism	Effective detected concentration	Reference
4-MBC	Fish- lipid tissue	123 ng g	Balmer et al. (2004)
4-MBC	Microalgae *Isochryris galbana*	EC$_{50}$: 74,72 ng mL^{-1}	Paredes et al. (2011)
4-MBC	Protozoan *Tetrahymena thermophile*	EC$_{50}$: 5,1 mg L^{-1}	Paredes et al. (2011)
4-MBC	Invertebrate *Siriella armata*	EC$_{50}$: 192,63 μg L^{-1}	Scmitt et al. (2008)
4-MBC	*Paracentrotus lividus* (sea urchin)	EC$_{50}$: 853,74 μg L^{-1}	Scmitt et al. (2008)
4-MBC	*Mytilus galloprovincialis* (mussel)	EC$_{50}$: 199,43 μg L^{-1}	Scmitt et al. (2008)
BP-3	Microalgae *Isochryris galbana*	EC$_{50}$: 13,87 ng mL^{-1}	Paredes et al. (2011)
BP-3	Protozoan *Tetrahymena thermophile*	EC$_{50}$: 7,5 mg L^{-1}	Paredes et al. (2011)
BP-3	*Danio rerio* (zebrafish)	< 1 mg L^{-1}	Sánchez et al. (2015)
BP-3	Fish *Oryzias latipe*	5,2 mg L^{-1}	Fivenson et al. (2021)
BP-3	Fish *Mugil liza*	3,1 mg L^{-1}	Molins-Delgado et al. (2017)
BP-3	Turtle *Caretta- caretta*	28,43 μg mL^{-1}	Cocci et al. (2020)
BP-3	Coral reefs	10 - 300 μg L^{-1}	Schneider & Lim (2019)
BP-4	Microalgae *Isochryris galbana*	EC$_{50}$: 171,45 ng mL^{-1}	Paredes et al. (2011)
OMC	Invertebrate *Siriella armata*	EC$_{50}$: 199,43 μg L^{-1}	Scmitt et al. (2008)
OMC	*Mytilus galloprovincialis* (mussel)	EC$_{50}$: 31118 μg L^{-1}	Scmitt et al. (2008)
OC	Dolphin- *Pontoporia blainvillei*	782 ng g^{-1}	Gago-Ferrero et al. (2013)

References (selected)

Borg D., Lund B.O., Lindquist N.G., Håkansson H. (2013). Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances in the Swedish population. Environ. Int. 59, 112-23.

Honey M., Krantz D. (2007). Global Trends in Coastal Tourism, Marine Program World Wildlife Fund. Center on Ecotourism and Sustainable Development, Washington, DC.

Lapworth D.J., Baran N., Stuart M.E., Ward R.S. (2012). Emerging Organic Contaminants in Groundwater: A Review of Sources, Fate and Occurrence. Environmental Pollution 163, 287-303.

Thomaidi V., Stasinakis A., Borova V., Thomaidis N. (2015). Is there a risk for the aquatic environment due to the existence of emerging organic contaminant in treated domestic waste waters. J. Hazard. Mater. 283, 740-747.

UNWTO (2017). UNWTO Tourism Highlights: 2017 Edition. https://www.e-unwto.org/doi/book/10.18111/9789284419029

PROFILES OF TOXIC METALS IN EASTERN MEDITERRANEAN MARINE ENVIRONMENTS

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Abstract
The occurrence of toxic metals such as Cd, Hg, Pb, in marine environments has been well documented by sampling and analysis during the last decades. Marine water and sediments consist the final "receivers" of these toxic substances that can bioaccumulate in organisms and eventually pose serious environmental and human health risks. The continued use and release of this group of toxic substances contributes to the degradation of marine environmental quality. Mediterranean Sea is a semi-enclosed system of particular importance, and subject to considerable anthropogenic pressures including toxic metals pollution. This overview focuses especially on Eastern Mediterranean region, presenting recent relevant research regarding the levels of metals in marine environmental samples in different countries and comparatively discussing their profiles and trends.
Keywords: *Toxic metals, seawater, marine sediments, Eastern Mediterranean Sea*
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1. Introduction

Toxic metals such as Cd, Hg, Pb are important marine pollutants that can degrade the quality of seawater and sediment and pose serious health risks to marine organisms and eventually humans via bioaccumulation. Their toxicity to marine organisms can vary depending on the simultaneous existence of different metals in marine environment that can have synergistic toxic effects. Bioaccumulation potentials depend on the metal and on the organism. Neurophysiological defects, cell alterations that can lead to mutations, teratogenesis and carcinogenesis possibility, effects on enzymic and hormonal activities, on growth and reproductive abilities of organisms are among the toxic effects of metals (Govind & Madhuri, 2014; Amundsen *et al*. 1997). Metals enter marine organisms mainly via the trophic chain, where they bioaccumulate and thereafter can lead to biomagnification and can be transferred to human organisms via seafood consumption (Per-Arne *et al*. 1997; Roméo *et al*. 1999). The aim of this paper is to present an overview of the occurrence of the most important toxic metals investigated during the recent years, in marine environmental samples (seawater, sediments, selected marine organisms) from the Eastern Mediterranean Sea. Their profiles in samples from different locations/countries are comparatively presented and discussed. The results highlight their ubiquitous presence in the marine environment, and the need to control their levels.

2. Occurrence of toxic metals in Eastern Mediterranean Sea

The profiles of toxic metals in coastal marine environments of Egypt, Cyprus, Turkey and Greece were investigated via recently published scientific literature. The gulfs of Eastern Mediterranean were found to be subject of "moderate" degree of ecological degradation. Older research (2004) based on ecological indicators (bivalves) had shown higher levels of Cu, Fe, and Mn in Egyptian coasts compared to Red Sea, possibly due to heavier industrial activity in the area. Following research (2009) in Egyptian coasts, also showed a moderate degree of pollution from Ni and Cu. More recent literature in Alexandria coasts, reported that Zn, Cu, Cd and Ni showed 'low to moderate enrichment', while Pb 'moderate to high enrichment' in sediments. Abu-Qir was characterized as the most polluted area among those investigated. Recent research in marine sediments of Northern Cyprus coasts reported "insignificant enrichment" of toxic metals. In Turkish seawater samples (2020) from the area of Izmir, concentrations of toxic metals followed the order Cd < Pb < Cu< Cr < Zn < Fe, and were significantly lower compared to previous estimations, while in Nicomedia Gulf (Marmara Sea) the levels of some metals (Zn, Cu, Cr, and Ni) exceeded geological background concentrations with relatively high factors (1.2 - 4). In Greece marine sediment samples from Thermaikos and Saronikos Gulf as well as from the coastal area of Stratoni, Chalkidiki, have shown the most intense anthropogenic toxic metal pollution (Table 1).

Concluding remarks

On the basis of the recent research literature reviewed in this paper, regarding the coastal areas of Turkey, Cyprus, Greece and Egypt, it can be concluded that the gulfs of the Eastern Mediterranean Sea are currently subject to a "medium" degree of pollution from a range of anthropogenic heavy metals.

Table 1. Levels of toxic metals in sediments (μg/g) of the Eastern Mediterranean Sea.

Country/Area	Values	As	Cd	Cu	Cr	Pb	Zn	Mn	Fe	Ni	Ref
Egypt *Alexandria*	Average	-	0.29	27.00	23.81	32.55	46.47	143.90	14.85	33.18	Khaled *et al*. 2020
West part	Range	-	0.52-0.92	26.5-33.3	16.3-34.2	20.7-35.6	26.3-112.1	32.3-108.9	709-1434	31.7-43.6	Ahdy & Khaled 2009
	Average	-	0.72	30.30	24,1	27,8	59,7	58	1123	38,8	
	TEL[3]	-	0.99	31.6	43.4	35.8	121	-	-	22,7	
	PEL[1]	-	4.98	146	111	128	459	-	-	48.6	
Greece *Stratoni. Chalkidiki (N. Aegean Sea)*	Range	320-4096	-	31-206	-	567-1698	936-4078	3835-25982	-	-	Pappa *et al*. 2016
	Average	1378	-	143	-	1146	1863	10150	-	-	
	PEL[1]	33	-	149	-	128	459	-	-	-	
	SEL[2]	33	-	110	-	250	820	1100	-	-	
Thermaikos Gulf (N. Aegean Sea)	Range	-	0.2-13	21.3-180.1	65.5-173.5	29.4-195.4	48.6-538.2	141.5-546.8	0.83-3.49	41.8-171.3	Christophoridis *et al*. 2019
	Average	-	2.51	77.23	115.40	84.19	218	314.2	2.03	82.45	
	Background	-	0.3	39	90	23	120	870	4,8	68	
	TEL[3]	-	0.7	18.7	52.3	30.2	124	-	-	15.9	
	PEL[1]	-	4.2	108	160	112	271	-	-	42.8	
Saronikos Gulf (W Aegean Sea)	Range	2 -179	-	7 -365	19 -544	5 -374	15 -982	62 -5736	-	9 - 392	Karageorgis *et al*. 2020
	Average	19	-	52	152	69	169	447	-	77	
	Background (Keratsini)	29	-	26	230	21	63	496	-	164	
	Background (Elefsis)	19	-	14	136	21	59	379	-	115	
Amvrakikos Gulf (Ionian Sea)	Average	3.0	0.2	25.0	180	18	60	-	-	90	Vasileiadou *et al*. 2016
	TEL[3]	5.9	1.0	40.0	37.3	35	140	-	-	18	
Cyprus	Range (S. part)	-	-	9–127	55–144	1.1–24	29–128	112–99	-	44–245	Kontaş *et al*. 2015
	Range (N. part)	-	-	11–49	15–93	11 -22.0	26–72	287–715	-	11–37	Abbasi & Mirekhtiary, 2020
	TEL[3]	-	-	18.7	52.3	30.2	124	-	-	15.9	
	PEL[1]	-	-	108	160	112	271	-	-	42.8	
Turkey *Izmir Gulf (E. Aegean Sea)*	Range	-	0.0043-0.199	1.95-15.30	14.45-32.80	2.51-5.45	28.20-36.60	-	5460-10700	-	Tas & Sunlu, 2019
Nicomedia Bay (Marmara Sea)	Range	-	0.10-1.0	50-105	41-121	10.0-34	124-363	-	2.58-5.89	-	Tan & Aslan, 2019

[1]PEL: Probable Effect Level, [2]SEL: Severe Effect Level, [3]TEL Threshold effect level

References (selected)

Abbasi A., Mirekhtiary F. (2020). Heavy metals and natural radioactivity concentration in sediments of the Mediterranean Sea coast. Marine Pollution Bulletin 154, 111041.

Christophoridis C., Bourliva A., Evgenakis E., Papadopoulou L., Fytianos K. (2019). Effects of anthropogenic activities on the levels of heavy metals in marine surface sediments of the Thessaloniki Bay, Northern Greece: Spatial distribution, sources and contamination assessment. Microchemical Journal 149, 104001.

Ahdy H.H.H., Khaled A. (2009). Heavy Metals Contamination in Sediments of the Western Part of Egyptian Mediterranean Sea. Australian Journal of Basic and Applied Sciences 3(4), 3330-3336.

Karageorgis A.P., Botsou F., Kaberi H., Iliakis, S. (2020). Geochemistry of major and trace elements in surface sediments of the Saronikos Gulf (Greece): Assessment of contamination between 1999 and 2018. Science of The Total Environment 717, 137046.

Khaled A., El Sayed A.E. Hamed, Addy, H., Ahmend H.O. et al (2021). Spatial distribution and potential risk assessment of heavy metals in sediment along Alexandria Coast, Mediterranean Sea, Egypt. The Egyptian Journal of Aquatic Research, 47, 137-43.

Kontaş A., Uluturhan E., Akçalı İ., Darılmaz E., Altay O. (2015). Spatial Distribution Patterns, Sources of Heavy Metals, and Relation to Ecological Risk of Surface Sediments of the Cyprus Northern Shelf (Eastern Mediterranean). Environmental Forensics 16(3), 264-2743.

Pappa F.K., Tsabaris C., Ioannidou A., Patiris D.L., Kaberi H., Pashalidis I., Eleftheriou G., Androulakaki E.G., Vlastou R. (2016). Radioactivity and metal concentrations in marine sediments associated with mining activities in Ierissos Gulf, North Aegean Sea, Greece. Applied Radiation and Isotopes 116, 22–33.

Tan I., Aslan, E. (2020). Metal pollution status and ecological risk assessment in marine sediments of the inner Izmit Bay. Regional Studies in Marine Science 33, 100850.

Tas E.G., Sunlu, U. (2019). Heavy Metal Concentrations in Razor Clam (Solen marginatus, Pulteney, 1799) and Sediments from Izmir Bay, Aegean Sea, Turkey. Turkish Journal of Agriculture - Food Science and Technology, 7(2), 306-313.

Vasileiadou K., Pavloudi C., Kalantzi I., Apostolaki E.T., Chatzigeorgiou G., Chatzinikolaou E., Pafilis E., Papageorgiou N., Fanini L., Konstas S., Fragopoulou N., Arvanitidis C. (2016). Environmental variability and heavy metal concentrations from five lagoons in the Ionian Sea (Amvrakikos Gulf, W Greece). Biodivers Data J. 4, e8233.

WIND WAVE ANALYSIS OF COASTAL WATERS USING THE OCEANLYZ TOOLBOX

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Abstract
Wind wave analysis in depth limited coastal waters is a very complicated issue and many different parameters are needed in order to conclude an accurate and high-quality output. In literature there are many well established methods in order to process and analyze measured wind wave data. This study concerns wind wave analysis in coastal waters of the Mytilene harbor, Lesvos Island, using Oceanlyz toolbox and GNU Octave software. This toolbox contains MATLAB functions for the estimation of wave properties in time domain analysis (zero-crossing method) and frequency domain analysis (spectral analysis method). The water level data analysis concerns the Mytilene harbor, in 2m depth with a sampling frequency f_s= 2Hz for the period October 15th-17th 2015. Some indicative output data of Mytilene harbor wind wave analysis is: H_{m0}=0.15m, f_p=0.15Hz, T_p=6.73s and f_{sep}=0.09Hz.
Keywords: *Wind wave analysis, time and frequency domain analysis, Oceanlyz toolbox, GNU Octave*
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1. Introduction

Wind wave analysis and wave modelling in general concern very interesting issues of common interest for coastal engineers, geoscientists and technicians. In the literature there are plenty of methods described in order to process measured wave data and estimate wave parameters. These methods concern both depth-limited (shallow) and deep waters. In order to conclude in accurate wave output additional steps are needed to properly implement these methods. The main goal of this study is to describe the steps required in order to do the wave analysis for coastal waters of Lesvos Island and at the same time to present the output of this analysis. The first step is to evaluate recorded data and prepare them for wave data analysis. The two methods used in this study are time domain analysis (zero-crossing method) and frequency domain analysis (spectral analysis method), emphasizing in the second one. With spectral analysis method the main wave properties are calculated as well as both separation frequency f_{sep} and the tail correction frequency f_{tail}. The whole process is done with Oceanlyz toolbox using GNU Octave software. The final step is to "run" Matlab functions and then to analyze and discuss the output which is the wave properties. The analysis include comparison both of two methods in terms of accuracy. At the end, a discussion is done in comparison with the results in other areas of the world (Louisiana, USA).

2. Material and Methods

Oceanlyz is derived from Ocean Analyze and it contains a number of Matlab functions aimed at wave analysis using time and frequency domain methods. The output of Oceanlyz is wave properties such as zero-moment wave height H_{m0}, peak wave period T_p, peak wave frequency f_p, Power Spectral Density, Separation Sea/Swell wave frequency f_{sep}.

The procedure is to convert water pressure data measured from a pressure transducer into water surface elevation data, separate low and high frequency data, partition of Wind Sea from Swell energy and finally estimate the peak wave frequency from the weighted integral of the wave power spectrum. Data are measured from a level logger which is placed in 2m depth in Mytilene harbor with a sampling frequency f_s= 2Hz and the measuring period is 2 days. Water level (m) data are converted into pressure data (N/m^2) in order to input them correctly to the GNU Octave software. A proper selection of pressure data is needed in order to have accurate input data. After data evaluation and confirmation, next step is the preparation of data for wave data analysis using zero-crossing method (time domain analysis) and spectral analysis method (frequency domain analysis). In time domain analysis method assuming the wave heights follow the Rayleigh distribution, the wave properties can be calculated using specific equations (Dean & Dalrymple 1991; Holthuijsen 2007; McCormick 2009). The second method used in this study to assess the data is the spectral analysis method. In this method the measured data are transformed from the time domain to the frequency domain by using the Fast Fourier Transform (*FFT*) algorithm. From this method the wave spectrum is calculated (power spectral density (Figure 1a), Fourier series of the surface elevation $\eta(t)$, the water surface elevation power spectral density $S_{\eta\eta}$ and the peak wave frequency f_p, which is the frequency associated with the maximum value of the $S_{\eta\eta}$. Pressure data correction for dynamic pressure attenuation in depth is done automatically calculated from Oceanlyz toolbox using $f_{maxpcorr}$ function. Finally, it is crucial to cut-off high frequency recorded data because they are mostly noise, with an insignificant signal-to-noise ratio. To do so the f_{tail} correction is used to remove this part of high frequencies from the spectrum and this part of spectrum tail is replaced by the so-TMA spectrum tail in intermediate and shallow water (Bouws *et al*. 1985).

3. Results and Discussion

After "running" Oceanlyz toolbox code for both methods, the wave properties are calculated. The output derived from both methods are approximately same. This indicates also the accuracy of each method. For Mytilene harbor the output of wind wave analysis is the Power Spectral Density (Figure 1a): H_{m0}=0.15m (Figure 1c), f_p=0.15Hz (Figure 1b), T_p=6.73s and f_{sep}=0.09Hz (Figure 1d). Power Spectrum Density diagram is representative of wind wave analysis. Wind sea and swell wave energies are shown in Figure 1d. $f>f_{sep}$ are wind waves and $f<f_{sep}$ are swell waves. Frequency of tail correction f_{tail} is shown at the end of the curve as well as the replacement of high frequencies by the diagnostic tail (TMA spectrum). The higher wave energy which concerns peak wave frequency f_p. is shown in Figure 1d.

Comparing the output of this study with those of Louisiana estuaries, we will notice similar values (H_{m0}=0.21m, f_p=0.14Hz, T_p=7.11s). The output data in Mytilene harbor are representative of the region and the season, concerning the meteorological conditions of this area. Weather and climate conditions for the specific measuring period can differentiate whole wind wave spectrum for each area. In general, Oceanlyz is a reliable tool and it can output accurate wind wave properties, particularly in depth-limited water bodies, as in the current study.

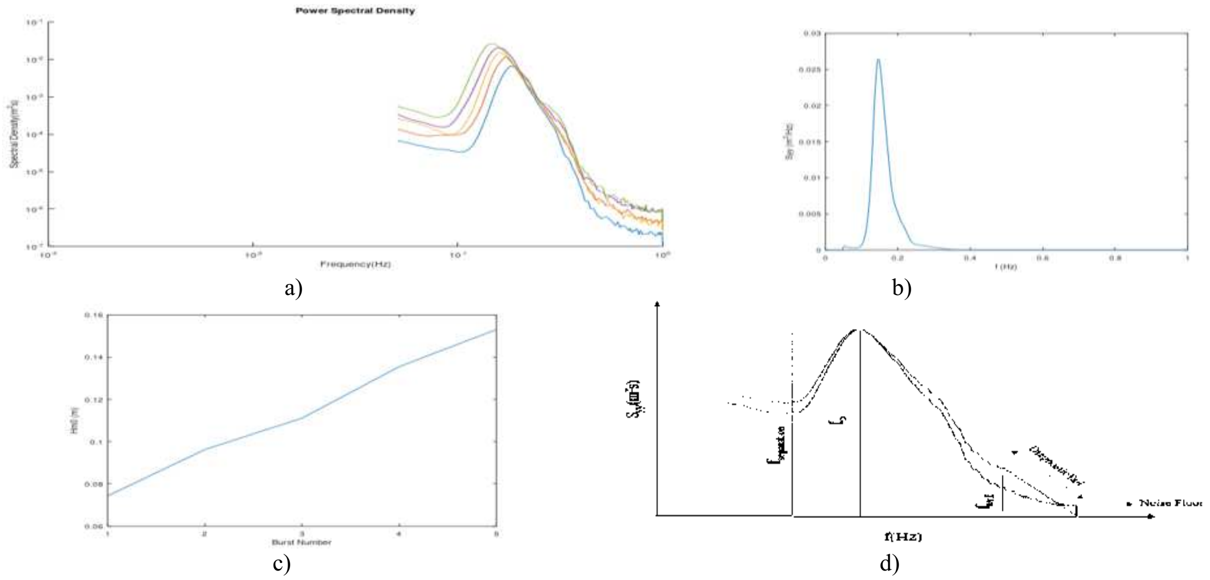

Figure 1. a) Power Spectral Density, b) $S_{\eta\eta}$ of highest wave, c) H_{m0} versus burst number, d) $S_{\eta\eta}$ versus f

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References

Bouws E., Günther H., Rosenthal W., Vincent C.L. (1985). Similarity of the wind wave spectrum in finite depth water: 1. spectral form. J. Geophys. Res.: Oceans 90 (C1), 975–986.

Dean R.G., Dalrymple R.A. (1991). Water wave mechanics for engineers and scientists. Advanced Series on Ocean Engineering: Volume 2. World Scientific Publishing, pp. 368.

Holthuijsen L.H. (2007). Waves in Oceanic and Coastal Waters. Cambridge University Press, pp. 404.

Karimpour A., Chen Q. (2017). Wind Wave Analysis in Depth Limited Water Using OCEANLYZ, a MATLAB toolbox. Computers & Geosciences 106, 181-189

McCormick M.E. (2009). Ocean Engineering Mechanics: With Applications. Cambridge University Press doi:10.1017/CBO9780511812309

CONSTRUCTION OF LOW-COST HYDROPHONES USING OFF-THE-SHELF COMPONENTS

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Abstract

This work reports on our effort to develop custom-built, low-cost hydrophones using components and tools that can be readily found in the general market. Two hydrophone variants were built, and a Sensor Technology SQ26-08 scientific hydrophone was used to obtain reference concurrent recordings. The raw materials and final products are summarily presented herein, as well as initial results from field tests conducted at Mytilene, Lesvos, Greece. Home-made hydrophones cannot replace their scientific counterparts, but are essential and affordable tools for acoustic education and research.

Keywords: *Passive acoustic technology, hydrophone, low-cost, scientific outreach*

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1. Introduction

Passive acoustics provide a breadth of information about the ocean and the animals living therein, however the specialised equipment needed is expensive and therefore inaccessible for schools or research teams with limited financial resources. The aim of this work is to build hydrophones using cheap components that can be constructed inhouse with no need for electrical or mechanical expertise. Previous similar projects and initiatives were used as a basis (e.g. Romero Vivas & León López 2010; Babb *et al*. 2018), modified to account for the availability of materials in the Greek market and the ease of assembly.

2. Materials and Methods

The design of the custom-made hydrophones utilised a piezoelectric element that costs about 1.5 €, engulfed in a PVC cap filled with 2-part epoxy adhesive. Two variants were built, using a different sized piezo (ø35 and ø27 mm) to study the possible differences in frequency response. The materials for building the hydrophones and the final prototypes are shown in Figure 1a and 1b, respecively.

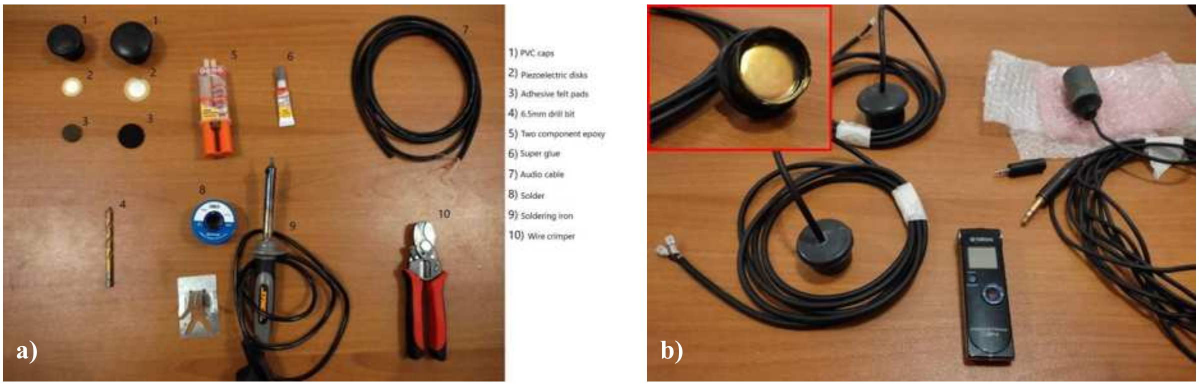

Figure 1. a) Materials and tools used for constructing the PVC cap hydrophones. b) Assembled PVC cap prototypes constructed in this study, alongside the SQ26-08 reference hydrophone and POCKETRAK C24 recorder; the inset image shows the face of the ø35 mm variant.

Field recordings were obtained at two sites, i.e. inside the port of Mytilene, Lesvos, and at a nearby location where controlled passes of a speedboat were performed at a distance of 30 m and 130 m from the hydrophones. For all experiments, the PVC cap prototypes were connected to a POCKETRAK C24 recorder, while a laptop computer was used to record the data from the SQ26-08 hydrophone (sampling frequency: 44.1 kHz/16-bit for all devices). The hydrophones were deployed at 1 m depth, and the overall recording pipeline was not calibrated.

3. Results and Discussion

The field recordings showed that the PVC cap prototypes functioned properly, but were considerably less sensitive than the SQ26-08 hydrophone (Figures 2 and 3). While the engine noise of a stationary commercial vessel in the port of Mytilene was better logged by the 27 mm variant (Figure 2c) when compared to the 35 mm one (Figure 2a), both prototypes seem to be unsuitable for monitoring low-frequency boat traffic. Contrary, Figure 3 shows that speedboat engine noise is adequately recorded, particularly at the mid-frequency bands (5–10 kHz). The next phase of this initiative should focus on investigating the directionality of the PVC cap prototypes and on their calibration, in order to obtain quantitative measures of their performance across different frequency bands. Despite their evident deficiencies, this effort shows that home-made hydrophones could offer a decent alternative to commercial solutions, especially for education purposes or science projects with limited resources.

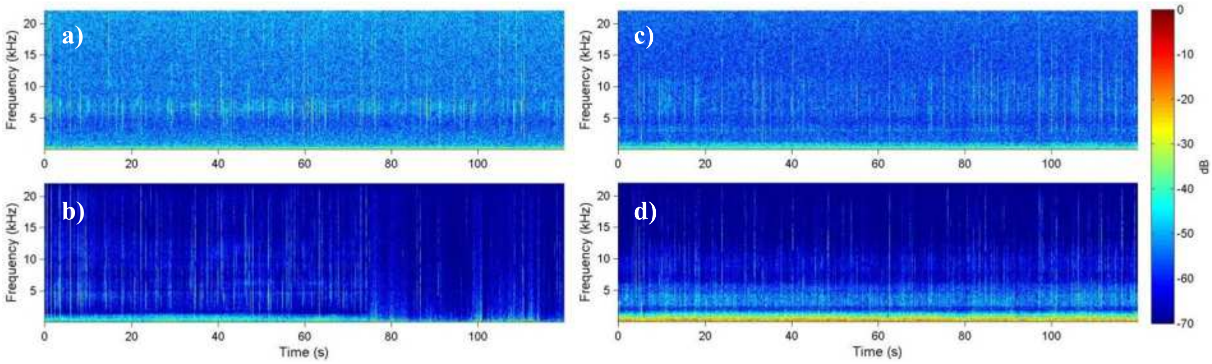

Figure 2. Uncalibrated spectrograms at the port of Mytilene; a) and c) are the 35 and 27 mm variants, respectively, while b) and d) are the respective concurrent recordings from the SQ26-08 hydrophone.

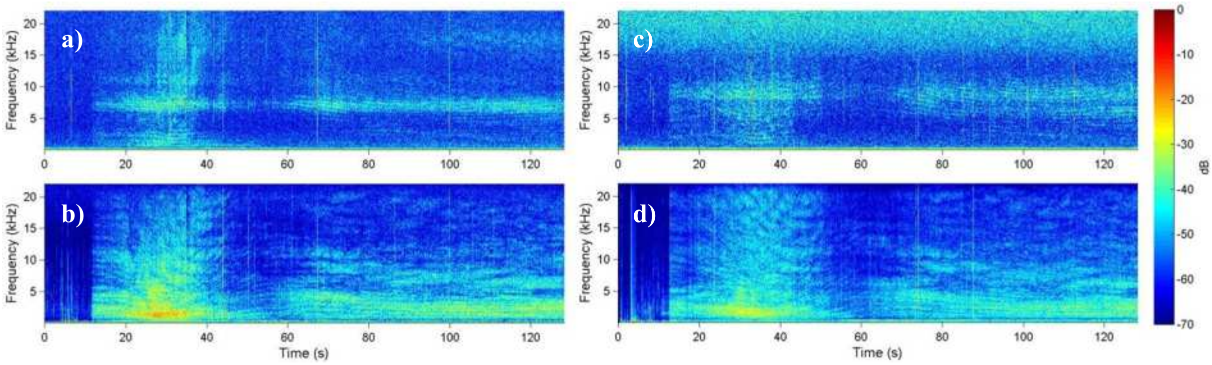

Figure 3. Uncalibrated spectrograms of speedboat noise. The time-stamps 20–40 s and 90–120 s correspond to a hydrophone-to-speedboat distance of 30 m and 130 m, respectively. a) and c) are the 35 and 27 mm variants, while b) and d) are the respective concurrent recordings from the SQ26-08.

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References

Babb I.G., Scowcroft G., Gingras A. (2018). Marine Technologies for Teachers and Students (MaTTS): A continuum of professional development and instruction in ocean science and technology. Marine Technology Society Journal 52(1), 33–46.

Romero Vivas E., León López B. (2011). Construction, calibration, and field test of a home-made, low-cost hydrophone system for cetacean acoustic research. Proceedings of the 160th Meeting of the Acoustical Society of America, vol. 11, 010001.

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CLIMATIC STABILITY OF THE MEDITERRANEAN MARINE PROTECTED NETWORK

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Abstract
There is limited evidence on what extent climate change would alter the efficiency of marine protected areas (MPAs). Here, we used metric of analog-based velocity of climate change to assess the climatic resilience of marine protected surfaces across the Mediterranean Sea for 120 years (i.e., 1950-2069). First, we investigated the variability of climate velocity between different 30-year periods (i.e., 1950-1979, 1980-2009, 2010-2039 and 2039-2069) based on Sea Surface Temperature data (SST). The increasing, but not spatially consistent, patterns of climate change velocities over successive 30-year periods delineated a highly heterogeneous and dynamic climatic space, while more than 90% of the protected marine surface might become less stable through time.
Keywords: *Climate change, climatic stability, marine protected areas, velocity of climate change*
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1. Introduction

As Earth's climate changes, numerous species should move to track evolving conditions, prompting a rearrangement of biodiversity (Moritz & Agudo 2013). Such a rearrangement has serious consequences for protected areas (PAs; Araujo *et al.* 2004). Given that PAs are designed to aim the persistence of species and ecosystems under stable conditions of climate (Lemieux & Scott 2005), so with the impact of climate change they may lose their effectiveness in protection of these components (Pressey *et al*. 2007). As a result, in the near future many species may lose their suitable climatic conditions in protected areas (Araújo *et al.* 2011) and this creates gaps in their effective protection (Velásquez-Tibatá *et al*. 2013).

2. Materials and Methods

Polygons of MPAs in the Mediterranean Sea were downloaded from the World Database on Protected Areas (UNEP-WCMC, IUCN, 2019). We selected only MPAs which are exclusively marine or coastal (i.e., 1383) and maintained their marine surface. We spatially dissolved boundaries of MPAs to avoid overestimation of the marine protected surfaces (resulting to 441 marine areas).

We used daily data of historical simulations (1950-2010) and future projections (2010-2069) of SST along the Mediterranean Sea at 0.0625° (~7 km) spatial resolution. Future climate projections were taken from the regional ocean-atmosphere coupled model COSMONEMO_MFS, generated by CMCC (Cavicchia *et al.* 2015), archived by the Fifth Assessment Report (AR5) and under climatic scenario RCP8.5. The 120 years (i.e., 1950-2069) were divided into four 30-year periods (i.e., 1950-1979, 1980-2009, 2010-2039, 2040-2069), and for each of them we created 9 bioclimatic variables related with temperature (Hijmans *et al.* 2005).

Climate-analog velocity across the Mediterranean Sea was estimated following a multivariate approach based on the first two axes of principal component analysis (PCA) of the multiple bioclimatic variables (Hamann *et al.* 2015). Next, we applied the nearest-neighbor algorithm, using R package yaImpute (Crookston & Finley 2008). This search is applied to each grid cell in a current location for which the algorithm identifies the nearest cell in a future location that has a similar climatic value as the focal. In order to be able to assess the climatic stability of the marine surface enclosed within MPAs in the Mediterranean Sea, we generated a climatic stability (CS) index: $CS = \frac{1}{n*m}\sum_{i=1}^{n}\sum_{j=1}^{m}\frac{1}{1+v_{i,j}}$; where n is the number of pairs from 1950 to 2069 of the consecutive 30-year studied periods, m is the number of cells of a protected surface, and v is the velocity value of the cell j estimated for a given pair (i of n) of the consecutive 30-year periods. The index ranges from 0 (unstable areas: cells with high climate velocity) to 1 (stable ones: cells with low climate velocity).

3. Results and Discussion

The values of climate-analog velocities within the marine protected surfaces for the study periods ranged from 0 to 97.52 km/yr (mean ~3.63 km/yr). Values of the CS index fluctuated from 0 to 0.82 (Figure 1). About 63% of all the protected surfaces exhibited very low and low climatic stability (i.e., 29.7% and 32.43%, respectively). About 33% of the protected surfaces (n = 147 out of 441) presented medium levels of climatic stability. Less than 5% of marine protected surfaces displayed high CS values (4.3%, n = 19 out of 441). Our analysis highlights the increased vulnerability of more than half of the existing marine protected areas throughout the Mediterranean Sea. This strengthens the main belief that the existing network of MPAs is designed ignoring the continuously altering climatic conditions and their resilience under climate change is at stake (Bruno *et al.* 2018).

Figure 1. Climatic stability (CS) index of the surfaces under protection across the Mediterranean basin based on the values of the velocity for all the cells within each polygon over all the 30-year periods. CS index has been classified into five equal classes, indicating very low (i.e., cells with high climate velocity; brown polygons) to very high (i.e., cells with low climate velocity; beige polygons) stability.

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References

Araújo M.B., Alagador D., Cabeza M., Nogués-Bravo D., Thuiller W. (2011). Climate change threatens European conservation areas. Ecology Letters 14, 484–492.

Araújo M.B., Cabeza M., Thuiller W., Hannah L., Williams P.H. (2004). Would climate change drive species out of reserves? An assessment of existing reserve-selection methods. Global Change Biology 10, 1618–1626.

Bruno J.F., Bates A.E., Cacciapaglia C., Pike E.P., Amstrup S.C., *et al.* (2018). Climate change threatens the world's marine protected areas. Nature Climate Change 8, 499–503.

Crookston N.L., Finley A.O. (2008). yaImpute: an R package for k-NN imputation. Journal of Statistical Software 23, 1–16.

Hamann A., Roberts D.R., Barber Q.E., Carroll C., Nielsen S.E. (2015). Velocity of climate change algorithms for guiding conservation and management. Global Change Biology 21, 997–1004.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. (2005). Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965–1978.

Lemieux C.J., Scott D.J. (2005). Climate change, biodiversity conservation and protected area planning in Canada. Canadian Geographer 49, 384–397.

Moritz C., Agudo R. (2013). The future of species under climate change: resilience or decline? Science 341, 504–508.

Pressey R.L., Cabeza M., Watts M.E., Cowling R.M., Wilson K.A. (2007). Conservation planning in a changing world. Trends in Ecology & Evolution 22, 583–592.

UNEP-WCMC, IUCN. (2019). Protected Planet: The World Database on Protected Areas (accessed: 9-10-2019).

Velásquez-Tibatá J., Salaman P., Graham C.H. (2013). Effects of climate change on species distribution, community structure, and conservation of birds in protected areas in Colombia. Regional Environmental Change 13, 235–248.

ASSESSING UNCERTAINTY IN CLIMATIC NICHE MODELS FOR JUVENILE SEA TURTLES IN THE MEDITERRANEAN SEA

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Abstract
Spatial identification of important habitats of charismatic marine megafauna represents a challenging task for conservation. Climatic niche models could facilitate the tracking of their important areas, still, there exist challenges in uncertainty inherited from input data and modelling processes. Here, we assessed the influence that input data, predictors, and models could exert in the produced outcomes of climatic niche models, taking as an example the juvenile loggerheads of the Mediterranean region.
Keywords: *Important marine areas, bioclimatic modelling, uncertainty, marine turtles, subadults*
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1. Introduction

Delineation of the important habitats of charismatic marine megafauna is necessary to enhance our conservation capacity. However, for species like sea turtles that have a long-life span, a complex life history and a highly migratory nature, this represents a rather complicated task (Casale *et al*. 2018). Especially for juveniles, acquisition of information is scarce and challenging due to their small size and them spending their entire life at sea. Climatic niche models could support such efforts, as they outline climatically suitable areas for species, based on environmental conditions on their presence records (New & Araujo 2006). However, many sources of uncertainty exist in input data, algorithms and model complexity (Beale & Lennon 2012). Here, we explored alternative selections in input data, variables and models to assess different outcomes in the estimated distribution patterns of juvenile loggerheads, a critical life stage for which knowledge is rather limited, under present and future conditions in the Mediterranean Sea.

2. Materials and Methods

We compiled in a digital database all existing published satellite data of 51 foraging juveniles (by July 2020) for the Mediterranean region, by reviewing the relevant literature. Based on this information and sea surface temperature (SST) data, we applied climatic niche models to explore suitable marine habitats under current (1991-2020) and future conditions (2051-2080). To explore differences in the performance and output of the models, we examined different datasets on climatic data, variables, and algorithms.

Using climatic datasets of historical and projected mean daily SST data from the Centro Euro-Mediterraneo sui Cambiamenti Climatici (CMCC) (Cavicchia *et al*. 2015), we built 9 bioclimatic variables (Hijmans *et al*. 2011), including all possible combinations of uncorrelated pairs in the models. We also ran a PCA analysis to construct alternative models and compare resulting outputs. For future climatic conditions, we used SST data based on projections of the Fifth Assessment Report of the IPCC and the rcp8.5 emission scenario. Next, we explored whether the selection of pseudoabsence points using a standard distance away from presence points influenced the predictive ability of the models. We extracted 10 random sets of pseudoabsences in cells of no presence records, to avoid sampling bias. To explore whether different climatic datasets would affect outputs, we also compared models built on climatic data derived from the regional oceanic-atmospheric model NEMOMED8 (Sevault *et al*. 2009) at a resolution of 0.125^{o} (CMCC: 0.0625^{o}). Finally, we explored the differences arising from the use of ensemble models that combine outputs of different algorithms, herein Generalized Linear Models, Generalized Additive Models, Random Forest and Multivariate Adaptive Regression Spline, and the use of single algorithms. All model comparisons were evaluated by the statistical indices Area Under the Curve (AUC) and True Skill Statistic (TSS), as well as the image of the output distribution.

3. Results and Discussion

The predictive performance of models with different combinations of bioclimatic variables and the PCA model was quite similar, with the first group of models performing slightly better (mean AUC: 0.918, mean TSS: 0.760) than the latter (AUC: 0.908, TSS: 0.747; Figure 1). Under present conditions, all models' distributions resembled. However, in future, the PCA model predicted maintenance of suitable areas in the west Mediterranean, whereas the other models predicted loss of large areas there, and new suitable areas for the region of Levantine. The application of PCA analysis helped towards avoiding arbitrariness in variable selection, through incorporating the majority of data variability. The outputs generated by using PCA axes as inputs, highlighted areas which were consistent when compared with sites known to host juvenile loggerheads, while models with bioclimatic variables seemed to inflate results for the future distributions.

The selection of pseudoabsence points is a critical part of the analysis with many alternatives proposed (Iturbide *et al*. 2015); herein, their selection using a certain distance improved the output and performance of the model. For the PCA model, AUC increased from 0.721 (TSS: 0.397) to 0.908 (TSS: 0.747), and for the models with bioclimatic variables, AUC increased from 0.741 (TSS: 0.428) to 0.918 (TSS: 0.760). However, strategies selecting pseudoabsences depend highly on the number of presence records (Barbet-Massin *et al*. 2012).

Alternative climatic data differentiated outputs at specific sites (e.g., North Adriatic Sea), mirroring differences inherited by data processing in climate modelling (Zumwald *et al*. 2020), however the bigger picture remained the same. Finally, present and future distributions of the ensemble and single models resembled, yet, the Random Forest algorithm produced a more inflated distribution for both present and future. The use of ensemble ensemble approaches in modelling is highly proposed as it ensures the best predictability and takes into account simple as well as complicated models in the final output of climatic suitability (New & Araujo 2006). This way, overfitting of a model is avoided and the combination of algorithms can counterbalance the uncertainty of the selection of a single model.

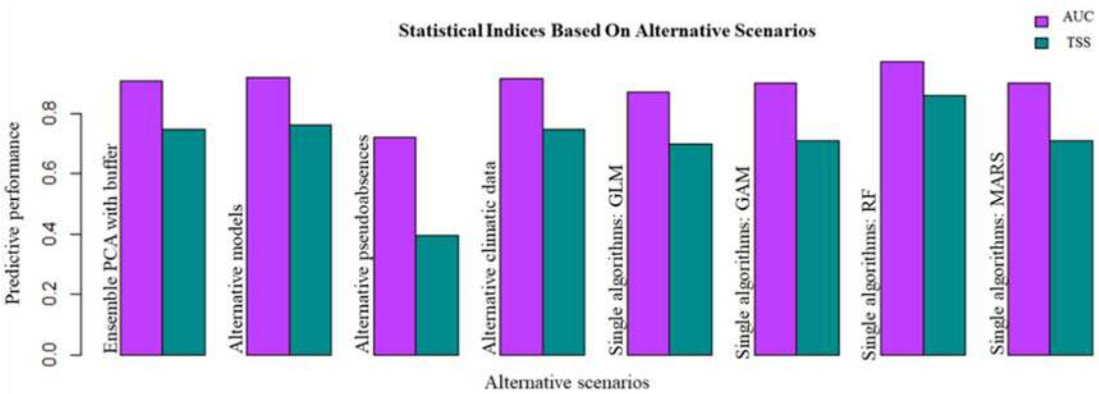

Figure 1. Predictive performance of AUC and TSS based on scenarios of input data, variables, and models.

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References

Barbet-Massin M., Jiguet F., Albert C.H., Thuiller W. (2012). Selecting pseudo-absences for species distribution models: how, where and how many? Methods in Ecology and Evolution 3, 327–338.

Beale C.M., Lennon J.J. (2012). Incorporating uncertainty in predictive species distribution modelling. Philosophical Transactions of the Royal Society B 367, 247–258.

Casale P., Broderick A.C., Camiñas J.A., Cardona L., Carreras C., *et al.* (2018). Mediterranean sea turtles: current knowledge and priorities for conservation and research. Endangered Species Research 36, 229–267.

Cavicchia L., Gualdi S., Sanna A., Oddo P. (2015). The Regional Ocean-Atmosphere Coupled Model COSMO-NEMO_MFS. CMCC Research Papers, RP0254, 1–22.

Hijmans A.R.J., Phillips S., Leathwick J., Elith J. (2011). Package 'dismo'. Available online at: http://cran.r-project.org/web/packages/dismo/index.html.

Iturbide M., Bedia J., Herrera S., Pinto M., Manuel J. (2015). A framework for species distribution modelling with improved pseudo-absence generation. Ecological Modelling 312, 166–174.

New M., Araujo M.B. (2006). Ensemble forecasting of species distributions. Trends in Ecology and Evolution 22, 42–47.

Sevault F., Somot S., Beuvier J., Cnrm M. (2009). A regional version of the NEMO ocean engine on the Mediterranean Sea: NEMOMED8 user's guide.

Zumwald M., Knüsel B., Baumberger C., Bresch D.N., Hadorn G.H. (2020). Understanding and assessing uncertainty of observational climate datasets for model evaluation using ensembles. WIREs Climate Change 11, 1–19.

FALSE KILLER WHALES (*Pseudorca crassidens*) PRESENCE IN GREECE, EAST MEDITERRANEAN: OLD AND NEW DEVELOPMENTS

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Abstract
The Mediterranean Sea is home to 28 species of marine mammals, out of which 13 species of cetaceans are documented in Greek waters, including the false killer whale (*Pseudorca crassidens*). Although the species has been reported since 1992 in Greece, only few sightings of *P. crassidens* have been documented in Eastern Mediterranean, so far. In July 31st 2019, a pod of 20 false killer whales was photographically documented in Chalkidiki Peninsula (NE Aegean Sea), whilst, the latest report was in June 20th 2021 in Porto Germeno, Corinthian Gulf. This recent report of false killer whale ~~sightings~~ ~~makes~~ up the 6th report in Greek waters and, overall, the 9th in the Eastern Mediterranean. So far, the majority of sightings reported in Greek waters occurred in North Aegean Sea. However, in 2020, the species was also observed in Turkish waters, indicating that false killer whales may use a wider area of the Northern Aegean Sea as potential range area. Regardless the low numbers of sightings, false killer whale appears to be sporadically present in Greek waters over the last 19 years. This study is a combination of literature review records and recent *in situ* observations in Greece by the ARION-Cetacean Rescue and Rehabilitation Research Centre.
Keywords: *Pseudorca crassidens, Aegean Sea, East Mediterranean.*
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1. Introduction

The Mediterranean Sea is home to 28 species of marine mammals out of which, 14 species are documented in Greek waters (Frantzis *et al*. 2001; Komnenou *et al.* 2001; Kapiris *et al.* 2018). Nine species reside permanently, whilst five species are occasionally recorded. Seven species of marine mammals in Greek waters are enlisted in the IUCN Red List as "near threatened", "endangered", "critically endangered" or "vulnerable", including the false killer whale (*Pseudorca crassidens*) (Frantzis *et al*. 2003).

False killer whale's distribution is worldwide. The species prefers tropical to warm-temperate waters and several adjacent seas. The species is commonly detected in deep oceanic waters, but often sighted over continental shelves and coastal waters (Notarbartolo di Sciara & Birkun 2010). In the Eastern Mediterranean, *P. crassidens* has been reported all year round mainly in Greece, Cyprus, and Turkey (Frantzis *et al*. 2003; Öztürk & Öztürk 1998). However, the sightings are rare, pointing out that the species is a "visitor" to these waters (Notarbartolo di Sciara & Birkun 2010). False killer whale is listed in the Greek cetacean fauna since the early 90s, but sightings of the species in Greece over the last 19 years are rare (ARION Cetacean Databank, Frantzis *et al*. 2003). In the North Aegean Sea (Greece) reports started in 1999, making these sightings the first to confirm the presence of the species in this area of Greece. The aim of this paper is to review the presence of the species in Eastern Mediterranean, focusing on Greek waters via sightings and strandings data, but also report the latest *in situ* observations from North Aegean Sea.

2. Materials and Methods

Sightings and strandings data covering the time period 1991-2021 in Greece were retrieved from the cetacean databank of ARION and from literature review of scientific journals, as well as from boat-based transect-line and opportunistic surveys that were conducted in Greece by ARION Cetacean Rescue and Rehabilitation Research Centre and Pelagos Cetacean Research Institute. The surveys took place mainly in North Aegean Sea, Thracian Sea, and the Hellenic Trench between June-September yearly, since 1996.

3. Results and Discussion

Overall, 9 reports of *P. crassidens* occurred in the Mediterranean Sea, out of which 6 were reported in Greek waters. The first report in the North Aegean Sea (Greece) was in 1999, followed by sightings in 2003 and

2019 (Table 1). The most recent sighting of *P. crassidens* occurred in June 20th 2021, in Porto Germeno at the Corinthian Gulf. The sighted animal was an adult individual of approximately 4.5-5 m in length and in good condition. The individual exhibited curious behaviour towards the boat when the engine was stopped and stayed in close proximity for 30 minutes. This rare report of false killer whale (*P. crassidens*) makes up the 6th report in Greek waters and, overall, the 9th in the Eastern Mediterranean.

Table 1: Overview of *P. crassidens* presence in Eastern Mediterranean Sea.

Country	Area	Year	Status	Details/ Reference
Greece	Chios strait	1992	Sighted	7 individuals/ Frantzis *et al*. 2003
Greece	Argolikos Gulf, Peloponnese	1993	Stranded	1 individual/ Frantzis *et al.* 2003
Greece	Kavala, North Aegean Sea	1999	Sighted	1 individual / ARION Cetacean Databank
Greece	Athos, North Aegean Sea	2003	Sighted	3 individuals/ ARION Cetacean Databank
Greece	Chalkidiki Peninsula	2019	Sighted	Pod of 20 individuals, interspecies interaction with *Grampus griseus* & *Tursiops truncatus* Thomas Perrier/ ARION Cetacean Databank
Greece	Porto Germeno, Corinthiakos Gulf	2021	Sighted	1 individual/ ARION Cetacean Databank
Turkey	Izmir	1995	Stranded	1 individual/ Öztürk & Öztürk 1998
Turkey	Gökçeada Island	2020	Sighted	8 individuals/ Dede *et al*. 2020
Cyprus	South of Cyprus	2013	Sighted	2 individuals/ Ryan *et al*. 2013

Over these 19 years, *P. crassidens* has been observed in several locations in Greece. However, in North Aegean Sea, it was reported in either single-species or in interspecies interactions with confirmed populations of Risso's dolphins (*Grampus griseus*) and bottlenose dolphins (*Tursiops truncatus*) (IUCN Red List: Least Concerned and Vulnerable, respectively), pointing out the importance of the marine areas and habitats of these waters for marine mammals. Combined with the presence of the endangered species harbour porpoise (*Phocoena phocoena*) and other characteristics of the area (bathymetry, physical oceanography, marine geomorphology), the North Aegean Sea may meet the criteria for establishing a potential future Marine Mammal Protection Area.

False killer whales in Greece have been recorded all year round, indicating that the species may not exhibit a particular seasonality pattern. However, systematic research is needed in order to study in depth *P. crassidens* population dynamics, mortality, and health issues in Greek waters.

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References

Dede A., Tonay A.M., Gönülal O., Öztürk A.A. (2020). First sighting of false killer whales (*Pseudorca crassidens*) in the northern Aegean Sea. Journal of the Black Sea/Mediterranean Environment 1, 106–111.

Frantzis A., Alexiadou P., Paximadis P., Politi E., Gannier A., Corsini-Foka M. (2003). Current knowledge of the cetacean fauna of the Greek Seas. Journal of Cetacean Research and Management 3, 219–232.

Jefferson T.A., Curry B.E. (2015). The humpback dolphins: A brief introduction to the genus Sousa. In: "Humpback Dolphins (Sousa spp.): Current Status and Conservation, Part 1. Advances in Marine Biology", Jefferson T.A., Curry B.E. (eds.). Elsevier/Academic Press, p. 1–16.

Kapiris K., Kapakos Y., Christidis G., Drougas A., Kofidou E., Komnenou A. (2018). Status of marine mammals' and reptiles strandings in the Greek Coasts between 2014-2015. 13th European Wildlife Disease Association Conference (EWDA), August 27-31, 2018 Larissa, Thessaly, Greece.

Komnenou A., Drougas A. (2001). First Aid Techniques for Cetaceans. CITES Management Authority-Ministry of Agriculture, ISBN 960-87156-0-1, Athens, Greece, 70 pp.

Notarbartolo di Sciara G., Birkun A. Jr. (2010). Conserving whales and dolphins and porpoises in the Mediterranean and Black Seas: An ACCOBAMS status report (2010), ACCOBAMS. Monaco, p. 212.

Öztürk B. Öztürk A.A. (1998). Cetacean strandings in the Aegean and Mediterranean coast of Turkey. Rapp. Comm. int. Mer Médit. 35, p. 476.

Paracentrotus lividus AS BIOCONTROLLER OF INVASIVE SPECIES *Rugulopteryx okamurae*. A LABORATORY APPROACH

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Abstract
Rugulopteryx okamurae is an invasive nonindigenous marine species (NIS) macroalgae present in the benthic communities of the Strait of Gibraltar. *R. okamurae* has quickly colonized most of the coast of this Strait, triggering ecological, economic, and social consequences. Sea urchins are generalist herbivores with potential to provide resistance to invasive seaweed species. The objective of this study is to know if the generalist herbivore sea urchin consumes the invasive macroalgae *R. okamurae*, as well as the grazing preferences when this NIS macroalgae is offered together with the native species *Ulva* spp. In this study, two experiments were carried out. In a first experiment, sea urchins were evaluated by groups for two months, and thereafter the same specimens were used to analyse individually preferences and feeding rates in a second experiment. Daily food consumption (DFC) rates showed that sea urchins accepted the alien seaweed *R. okamurae* as food under laboratory conditions, exhibiting higher DFC values when animals where cultured individually. Regarding individual variability, although, in general, sea urchins showed preferences for *Ulva* when both algae were supplied at the same time, we found specimens that presented preference for *R. okamurae* throughout the entire experimental period. These results open an interesting research line addressed to select sea urchins families with preferences for grazing this alien species.
Key words: *Rugulopteryx okamurae*, *sea urchins*, *alien seaweeds*, *biocontrol*
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1. Introduction

Rugulopteryx okamurae is an invasive nonindigenous marine species (NIS) macroalgae present in the benthic communities of the Strait of Gibraltar. It was detected for the first time in the coast of the Strait of Gibraltar in 2015 (Altamirano-Jeschke *et al*. 2016), and was previously cited on the coastal lagoon of Thau in the French Mediterranean coast (Verlaque *et al*. 2009). *R. okamurae* has quickly colonized most of the coast of this Strait, triggering ecological, economic, and social consequences. The high propagation capacity of this species threatens other areas along the entire southern and eastern Iberian Peninsula, including areas of great conservation interest.

The Enemy Release Hypothesis (ERH) is one of the most accepted hypotheses to explain invasions. ERH assumes that an introduced species is subjected to little regulation by natural enemies, resulting in a rapid increase in their abundance. Regarding consumers, the purple sea urchin is one of the most common benthic herbivores in south Atlantic and Mediterranean coast (Gago *et al*. 2003). Sea urchins are generalist herbivores with strong ecological roles in structuring benthic macrophyte communities (Hay & Steinberg 1992). Moreover, sea urchins are good candidates to provide resistance to invasion. The objective of this study is to know if the generalist herbivore sea urchin consumes the invasive macroalgae *R. okamurae*, as well as the grazing preferences when this NIS macroalgae is offered together with the native species of *Ulva spp*. To this end, two experiments were carried out. In a first experiment, sea urchins were evaluated by groups for two months, and thereafter the same specimens were used to analyse individually preferences and feeding rates.

2. Material and Methods

Seaweeds were collected in their habitats, transported to the experimental facilities, and kept in separated aerated seaweed tanks for acclimation, until the start of the experiment. Individuals of *P. lividus* were collected one month before the start of the first experiment (sea urchins in group) in southern Spain (La Herradura, Granada). All individuals were starved 48 h to standardize the initial hunger condition.

For the groups experiment, three treatments were established: i) sea urchins fed *R. okamurae* (RUGU); ii) sea urchins fed *Ulva spp*. (ULVA); and iii) sea urchins fed with both macroalgae in equal amounts (BOTH). In all cases, three replicates were set by treatment (13 animals by replicate). In the second experiment (individually) we set two experimental treatments (30 replicates by treatment): i) sea urchins fed *R. okamurae* (RUGUindiv); and ii) sea urchins fed *Ulva spp*. and *R. okamurae* in the same amounts (BOTHindiv). For both experiments, offered seaweeds were pre-weighed (wet weight; excess of water was blotted off with blotting paper), placed in each box at the same time and allowed to be grazed for 7 days. A week later, the remaining algae was weighed to evaluate biomass differences due to herbivory and a new pre-weighted algae was offered. To evaluate loss of macroalgae biomass, three control by algae and experiment were established. To calculate algae consume for sea urchin groups (Group Trial) or individual box (Individual Trial) we used the Daily Food Consumption rates (DFC):

$$DFC\ (mg_{\ macroalgae} \times g^{-1}_{\ sea\ urchin} \times day^{-1}) = (Fg - Fr - Fd) / (W \times t)$$

, where Fg was the DW (mg) of a given macroalgae, Fr was the DW (mg) of the remaining macroalgae and Fd was the DW of the macroalgae lost by degradation. W was the wet weight (g) of the sea urchin and t was the time in days.

3. Results and Discussion

Daily food consumption rates showed that sea urchins accepted the alien seaweed *R. okamurae* as food under laboratory conditions, exhibiting higher DFC values when animals where cultured individually. The previous adaptation to laboratory conditions feeding *R. okamurae* during the first experiment, as well as the possible interaction with other sea urchins in culture cages could explain these results. In general, values of DFC were similar to those reported by Cardoso *et al*. (2020), but lower to those found by Castilla-Gavilán *et al*. (2019). Regarding individual variability, although, in general, sea urchins showed preferences for *Ulva* when both algae were supplied at the same time, we found specimens that presented preference by *R. okamurae* throughout the entire experimental period. These results open an interesting research line addressed to select sea urchins families with preferences for grazing this alien species, and therefore can act as biocontroller.

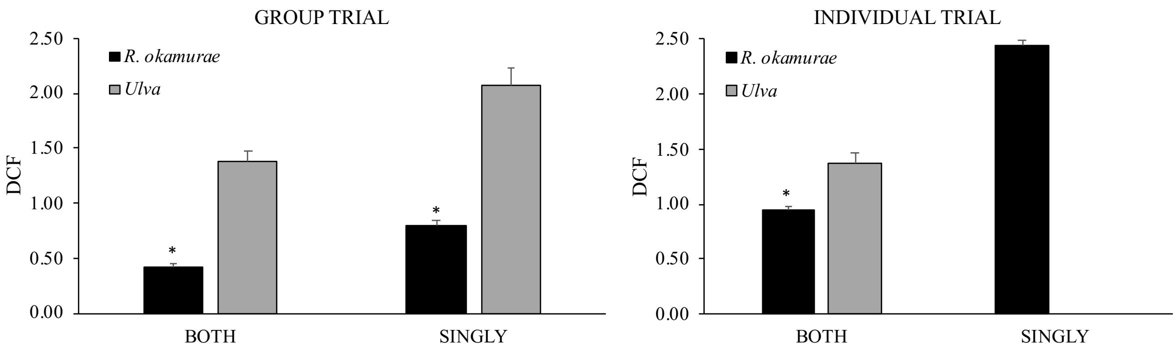

Figure 1. Daily food consumption rates (DFC; mean ± SE) of seaweeds *R. okamurae* (black bars) and *Ulva* (grey bars) in group and individual trials. Algae were supplied alone (singly) or both together in the same amounts (both). Asterisks indicate significant differences between seaweeds ($p < 0.05$).

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References

Altamirano-Jeschke M., De la Rosa J., Martínez J. (2016). Arribazones de la especie exótica *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim (Dictyotales, Ochrophyta) en el Estrecho de Gibraltar: primera cita para el Atlántico y España. Algas 52:20.

Castilla-Gavilán M., Cognie B., Ragueneau E., Turpin V., Decottignies P. (2019) Evaluation of dried macrophytes as an alternative diet for the rearing of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture Research 50, 1762–1769.

Gago J., Range P., Luis O. (2003). Growth, reproductive biology and habitat selection of the sea urchin *Paracentrotus lividus* in the coastal waters of Cascais, Portugal. In: "Echinoderm Research", Féral J-P., David B. (eds). A.A. Balkema Publishers, p. 269–276.

Hay M.E., Steinberg P.D. (1992). The chemical ecology of plant-herbivore interactions in marine versus terrestrial communities. In: "Herbivores: Their Interactions with Secondary Plant Metabolites (2nd edition)", Rosenthal G.A., Berenbaum M.R (eds). Academic press, p. 371–413.

Verlaque M., Steen F., De Clerck O. (2009). Rugulopteryx (Dictyotales, Phaeophyceae), a genus recently introduced to the Mediterranean. Phycologia 48, 536–542.

Cardoso A., Arenas F., Sousa-Pinto I., Barreiro A., Franco J. (2020) Sea urchin grazing preferences on native and non-native macroalgae. Ecological Indicators 111, 106046.

ALIEN AND RARELY REPORTED MOBILE SPECIES IN MARINE CAVES OF THE AEGEAN SEA, GREECE

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Abstract
Although the distribution patterns of marine alien species in the Mediterranean Sea are well studied, little is known about their presence in marine caves. Thus, a protocol for the recording of mobile species in marine caves was applied for the first time in 12 marine caves in 9 Greek islands. Both the abundance and species richness of mobile fauna was recorded in 3-min visual surveys at distinct ecological zones (i.e. entrance, semidark and dark zones) of the studied caves. All species observed out of transect were also documented. A total of 14 alien and cryptogenic species were recorded. Non-indigenous species were mostly fish, reaching up to 10% of the total fish abundance and comprising 4 to 46% of recorded fish individuals in the studied caves. Two invertebrate species of Indo-Pacific origin were reported for the first time from Greek waters. In addition, several cryptobenthic native species – rarely reported in the Eastern Mediterranean Sea – were observed, including new records from Greek waters and the regional marine cave fauna.
Key words: *Non-indigenous species, cryptobenthic fish, benthic invertebrates, biodiversity, sea caves*
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1. Introduction

Over the last decades, more than 200 alien species have been recorded in the Aegean Sea (Katsanevakis *et al*. 2020a). Most of them are known from shallow rocky bottoms and artificial substrates (e.g. marinas and harbors), while there is little information about inaccessible dark environments such as sea caves (Gerovasileiou *et al*. 2016). More than 600 sea caves have been recorded on the Aegean rocky coasts (Sini *et al*. 2017). Through the Research Program "Aliens in the Aegean – a sea under siege (ALAS)" (Katsanevakis *et al*. 2020b) we aim to investigate alien species establishment in different types of marine ecosystems including marine caves.

2. Materials and Methods

During the summer months of 2020, a total of 12 marine caves, located in 9 Greek islands, were surveyed for the first time for their mobile fauna. In each cave, two scientific divers recorded both the abundance and species richness of mobile taxa in 3-min visual surveys at three distinct ecological zones (i.e. entrance, semidark and dark zones). In addition, all mobile taxa observed during the whole dive (at least 90 minutes) were documented in order to produce a species inventory for the studied caves.

3. Results and Discussion

In total, more than 80 mobile species were recorded, including 14 alien and/or cryptogenic and 9 rarely reported native species. Most alien species were fish (6), comprising 10% of the total fish abundance in the studied caves. The Indo-Pacific sweeper fish *Pempheris rhomboidea* Kossmann & Räuber, 1877 exhibited the highest abundance, reaching over 200 individuals at the dark interior and more than 200 juveniles at the entrance of the southeasternmost studied cave in Kastellorizo Island, indicating its successful establishment (Figure 1A). The lionfish *Pterois miles* (Bennett, 1828) was recorded at all cave zones while the red soldier fish *Sargocentron rubrum* (Forsskål, 1775) appeared strictly at dark zones. Two alien species were recorded for the first time in Greek waters. The Indo-Pacific shrimp *Urocaridella pulchella* Yokes & Galil, 2006 was recorded in three sites, with an individual photographed carrying eggs in a cave of the Cyclades Islands (Figure 1B). In addition, two individuals of the non-indigenous nudibranch *Plocamopherus ocellatus* Rüppell & Leuckart, 1828 were spotted out of transect in the Blue Cave of Kastellorizo Island. All the other alien and cryptogenic species were reported at the cave entrance or semidark zone [i.e. *Diadema setosum* (Leske, 1778), *Enchelycore anatina* (Lowe, 1838), *Siganus luridus* (Rüppell, 1829), *Siganus rivulatus* Forsskål & Niebuhr, 1775, *Torquigener flavimaculosus* Hardy & Randall, 1983, *Parupeneus forsskali* (Fourmanoir & Guézé, 1976), *Carupa tenuipes* Dana, 1852, *Cerithium scabridum* Philippi, 1848, *Ergalatax junionae* Houart, 2008]. Alien fish comprised 4 to 46% of recorded fish individuals in the southeastern Aegean caves. In addition, the rarely seen shrimp *Brachycarpus biunguiculatus* (H. Lucas, 1846) and eight rare small-sized cryptobenthic fish were recorded out of transect, namely *Corcyrogobius liechtensteini* (Kolombatović, 1891), *Didogobius splechtnai* Ahnelt and Patzner, 1995, *Gammogobius steinitzi* Bath, 1971 (Figure 1C), *Thorogobius ephippiatus* (Lowe, 1839), *Microlipophrys nigriceps* (Vinciguerra, 1883), *Tripterygion melanurum* (Guichenot, 1850), *Grammonus ater* (Risso, 1810) and *Lepadogaster* cf. *lepadogaster* (Bonnaterre, 1788). The species *D. splechtnai* and *G. steinitzi* were reported for the first and second time from Greek waters, respectively.

Figure 1. Individuals of *Pempheris rhomboidea* (A), *Urocaridella pulchella* carrying eggs (B), and the cryptobenthic fish *Gammogobius steinitzi* (C). Photos by Michail Ragkousis and Markos Digenis.

The distribution pattern of alien species recorded in marine caves seems to fit well with their known distribution from other marine habitats in the Aegean Sea (Katsanevakis *et al*. 2020a). Through this study, the regional marine cave fauna has been significantly updated, including new records of alien and native species from Greek waters (Digenis *et al*. 2020; Ragkousis *et al*. 2020; 2021). Further analysis of the collected data as well as additional samplings are expected to provide better estimates of alien species impacts on marine cave ecosystems of the Aegean and the Eastern Mediterranean Sea.

Acknowledgements

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References

Digenis M., Ragkousis M., Vasileiadou K., Gerovasileiou V., Katsanevakis S. (2021). New records of the Indo-Pacific shrimp *Urocaridella pulchella* Yokes & Galil, 2006 from the Eastern Mediterranean Sea. BioInvasions Records 10, 295–303.

Gerovasileiou V., Voultsiadou E., Issaris Y., Zenetos A. (2016) Alien biodiversity in Mediterranean marine caves. Marine Ecology 37, 239–256.

Katsanevakis S., Zenetos A., Corsini-Foka M., Tsiamis K. (2020a) Biological Invasions in the Aegean Sea: Temporal Trends, Pathways and Impacts, In: "The Aegean Sea Environment: The Natural System", Anagnostou C.L., Kostianoy A.G., Mariolakos I.D., Panayotidis P., Soilemezidou M., Tsaltas G. (eds). Springer Nature, p. 1–13.

Katsanevakis S., Tsirintanis K., Sini M., Gerovasileiou V., Koukourouvli N. (2020b) Aliens in the Aegean – a sea under siege (ALAS). Research Ideas and Outcomes 6, e53057.

Ragkousis M., Abdelali N., Azzurro E., Badreddine A., Bariche M., Bitar G., *et al*. (2020). New Alien Mediterranean Biodiversity Records (October 2020). Mediterranean Marine Science 21, 631–652.

Ragkousis M., Digenis M., Kovačić M., Katsanevakis S., Gerovasileiou V. (2021). Rarely reported cryptobenthic fish in marine caves of the Eastern Mediterranean Sea. Journal of Marine Science and Engineering 9, 557.

Sini M., Katsanevakis S., Koukourouvli N., Gerovasileiou V., Dailianis T., *et al*. (2017) Assembling ecological pieces to reconstruct the conservation puzzle of the Aegean Sea. Frontiers in Marine Science 4, 347.

OCCURRENCE OF *Trachipterus trachypterus* AND *Zu cristatus* IN THE GREEK SEAS: CONTRIBUTION OF CITIZEN SCIENCE PROJECTS IN RARE SPECIES MONITORING

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Abstract
The occurrence of two ribbonfish species, *Zu cristatus* (Bonelli, 1819) and *Trachipterus trachypterus* (Gmelin, 1789), in the Greek seas was examined through contributions made to the citizen-science project «Is it Alien to you… Share it!!!». In total, 8 sightings, 2 for *Z. cristatus* and 6 for *T. trachypterus*, were reported in various locations of the Aegean and Ionian Seas. In particular, *Z. cristatus* was only recorded in the North and central Aegean, while *T. trachypterus* was reported in both the West Aegean Sea and the Ionian Sea.

Keywords: *Citizen science, ribbonfish, rare species, Trachipterus trachypterus*, *Zu cristatus*

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1. Introduction

The representatives of the family Trachipteridae constitute some of the most rarely encountered inhabitants of the Mediterranean mesopelagic communities (Macali *et al.* 2020). *Trachipterus trachypterus* (Gmelin, 1789) and *Zu cristatus* (Bonelli, 1819) are found in tropical and subtropical waters worldwide, with the latter displaying a circumglobal distribution. Adults of both species are thought to feed off midwater fish and squid (Borme & Voltolina 2006).

The scarcity of data on the biology and ecology of these species accentuates the need for monitoring organisms of similar ecological traits and niche. This study aims to add to the literature for the known occurrence of these species in the Greek seas.

2. Materials and Methods

The occurrence of these two species was recorded in the context of the citizen science project «Is it Alien to you… Share it!!!» which has the primary aim to monitor the expansion and establishment of marine alien and rare species in Greece and neighboring countries in the Eastern Mediterranean (Naasan Aga Spyridopoulou *et al.* 2020). These records and their relevant data were collected from social media platforms, through contributions from citizen-scientists, mostly professional and recreational fishermen. All data were collected with the consent of their respective owner. The photographed specimens were identified as being *T. trachypterus* or *Z. cristatus* by relevant taxonomic experts.

3. Results and Discussion

In total, two specimens of *Z. cristatus* and six specimens of *T. trachypterus* were recorded. Most of these records were reported quite recently, within the time frame of 2013-2020. All coordinates for the sightings are given in decimal degrees, with the Latitude and Longitude being the first and second values, respectively.

Regarding the records of occurrence for the species in this study, *T. trachypterus* (Figure 1) was mainly distributed in the Aegean Sea, in Maliakos Gulf (38.884557, 22.557513 and 38.900940, 22.593101) and Pagasitikos Gulf (39.271071, 23.164670), around the Attica peninsula (37.807358, 24.063139), as well as in the Ionian Sea in Kerkyra Island (39.640804, 19.903049 and 39.723785, 19.896125). *Zu cristatus* (Figure 2) was recorded solely in the Aegean Sea, specifically in the central Aegean, amidst the Dodecanese Islands (37.033568, 27.060899) and in the North Aegean Sea, in Lesvos Island (39.176013, 26.509893).

Figure 1. Specimens of *Trachipterus trachypterus* sighted by the project's citizen-scientists. Photo provider: (1) preferred to stay anonymous; (2) Panagiotis Mpahot; (3) downloadcorfu (YouTube acc); (4) Nick Vassileiou; (5) Giorgos Pet; (6) Giannis Vlahopoulos.

Figure 2. Specimens of *Zu cristatus* sighted by the project's citizen-scientists. Photo provider: (1) Christos Pikoulellis; (2) Philippos Pantelis.

The observations of this work enrich the existing literature on these species by expanding the known occurrence of both *T. trachypterus* (new records in Maliakos Gulf, Pagasitikos Gulf, Attica peninsula) and *Z. cristatus* (new records in Dodecanese Islands, Lesvos Island) for the Greek Seas (Papaconstantinou 2014), and highlight the importance of citizen-science projects and long-running programs in studying marine species.

Acknowledgements

The authors would like to express their gratitude to all the citizen-scientists who contributed to this study by providing valuable data on species sightings.

References

Borme D., Voltolina F. (2006). On the occurrence of ribbon fish *Trachipterus trachypterus* (Gmelin, 1789) in the Gulf of Trieste (northern Adriatic Sea). Annales: Series Historia Naturalis 16(2), 181–188.

Macali A., Semenov A., Paladini F., Dinoi A., Bergami E., Tiralongo F. (2020). Relative influence of environmental factors on biodiversity and behavioural traits of a rare mesopelagic fish, *Trachipterus trachypterus* (Gmelin, 1789), in a in a continental shelf front of the Mediterranean Sea. Journal of Marine Science and Engineering 8(581), 1–13.

Naasan Aga Spyridopoulou R., Langeneck J., Boyziotis D., Giovos I., Kleitou P., Kalogirou S. (2020). Filling the gap of data-limited fish species in the eastern Mediterranean Sea: A contribution by citizen science. Journal of Marine Science and Engineering 8(2), 107.

Papaconstantinou C. (2014). Fauna Graeciae: An updated checklist of the fishes in the Hellenic Seas. Hellenic Centre for Marine Research (HCMR), Athens, pp. 340.

OCCURRENCE OF *Trachipterus trachypterus* AND *Zu cristatus* IN THE GREEK SEAS: CONTRIBUTION OF CITIZEN SCIENCE PROJECTS IN RARE SPECIES MONITORING

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Abstract
In this study, the antioxidant activity of extracts from strains of genera *Tetraselmis* and *Dunaliella* was evaluated. Mild solvent systems and ultrasound assisted extraction was applied. The extracts of both strains showed a relatively high anti-oxidant capacity, warranting further investigation towards their potential use in the food, cosmetics and pharmaceutical industries.
Keywords: *Dunaliella, Tetraselmis, DPPH, IC50, local strains, microalgae cultivation*
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1. Introduction

The genera *Tetraselmis* and *Dunaliella* are wide-spread in aquatic environments, including a variety of species living in marine and hypersaline waters. Strains belonging to these genera have been shown to have antioxidant properties and they are of particular interest to aquaculture, as well as to food, cosmetics and pharmaceutical industries (e.g. Zamani *et al.* 2019; Sedjati *et al.* 2020). Herein, we present the first results of the investigation of two microalgal strains (AthU-Al Mes_17 and AthU-Al Dun_32) isolated from Greek coastal areas, with regards to their culture kinetics and anti-oxidant capacity (DPPH and IC50), in the framework of the PHYCOSMETIC-GR project.

2. Materials and Methods

The studied strains are preserved in the AthU-Al (Athens University Algae) strain bank of the National and Kapodistrian University of Athens (NKUA). The strain *Tetraselmis verrucosa* f. *rubens* AthU-Al Mes_17 was isolated from the Mesolonghi salt pans, while the strain *Dunaliella salina* AthU-Al Dun_32 was isolated from the salt pans of Megalon Emvolon and they are preserved in 40‰ and 60‰ salinity respectively. Both strains were cultivated in artificial salt water using Walne's medium (CCAP 2002). The cultures were preserved in a culture chamber (temperature: 21-23 ^{o}C; relative air humidity: ~35%; light intensity: 42 μmoles photons m^{-2} s^{-1}; light period: 12/12 h), in aerated Erlenmeyer flasks to a final volume of 2L in triplicates, and they were sampled daily for the estimation of culture kinetics. Samples for dry weight and pigment content estimation as well as anti-oxidant capacity trials were taken on the post-exponential phase. Cell counts were performed using a Neubauer haemocytometer. Dry weight was estimated after filtration on pre-weighed glass microfiber (GF/C) filters, drying and measurement in a 5-digit scale.

Freeze dried biomass was extracted using the ultrasonic reaction system XO-SM50. The solvents used were water, ethnol:water (75:25) and ethyl acetate:water (75:25). The extraction parameters were biomass: solvent ratio 1:20 g/ mL solvent. The extraction probe was inserted at 2 cm above the beaker bottom and the system operated at 25 kHz frequency, at 250 Watt and temperature 50 ^{o}C for a total duration of 15 min. The free-radical scavenging activity was evaluated with the use of stable radical 2.2-diphenyl-1-picrylhydrazyl (DPPH) reagent (Stramarkou *et al*. 2017). Briefly, extracts of 6 mg and 1 mL of methanol were prepared and vortexed. A fresh radical DPPH solution, which was used as blank, was prepared each day by adding 0.003 g of DPPH powder (Sigma Aldrich) with 100 mL of methanol and was magnetically stirred for 15 min at room temperature. The ONDA UV-21 (Giorgio Bormac, Italy) spectrophotometer was used to measure the absorbance at 515 nm of 3.9 mL of blank DPPH solution at 25 °C. Afterwards, an aliquot of 0.1 mL of the extracts was added in the cuvette and the absorbance was measured again after at 60 min. The percentage of the DPPH inhibition was calculated as described from Eq. 1:

$$\%\text{DPPH Inhibition} = 1 - ([\text{DPPH}\bullet]t{=}60 / [\text{DPPH}\bullet]t{=}0) \times 100\% \qquad (1)$$

DPPH measurements were conducted in triplicates.

3. Results and Discussion

The growth curves for each strain (average of three trials) are shown in Figure 1. Daily fluctuation of culture density (cells/mL) and specific growth rate (μ) are shown up to the post-exponential phase. Maximum specific growth rate (μmax) is also shown. Both strains reached maximum growth rate on day 3 of culture. In the post-exponential phase, the dry weight of the cultures was 0.493±0.03 mg/mL and 0.175±0.01 mg/mL for Mes_17 and Dun_32 respectively.

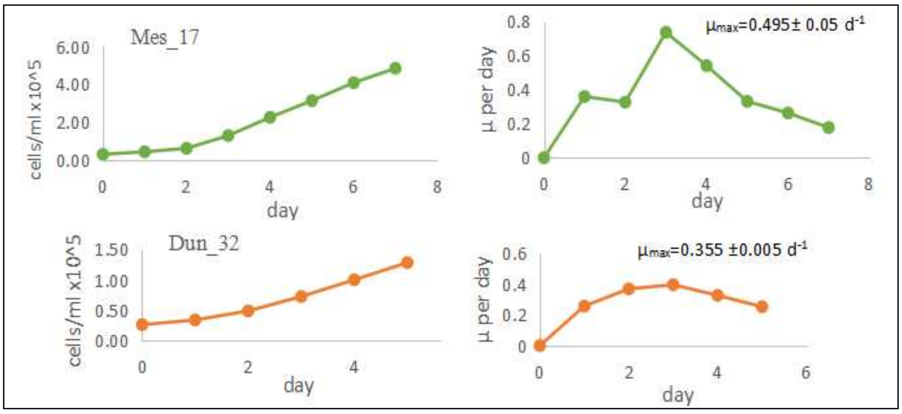

Figure 1. Growth curves of the strains *Tetrasemis* AthU-Al Mes_17 (up) and *Dunaliella* AthU-Al Dun_32 (down).

The free-radical scavenging activity of the strain extracts is shown in Table 1. Both strains show sufficient antioxidant activity with the strain Mes_17 showing significant antioxidant activity in ethyl acetate:water extract, while Dun_32 showing sufficient antioxidant activity even in water extract and overall better antioxidant capacity. The studied strains show significant antioxidant capacity indicating them as promising strains to be exploited in various applications such as cosmetics and food supplements.

Table 1. Free-radical scavenging activity of Mes-17 and Dun-32 extracts.

Strain	Solvent System	%Scavenging activity	IC50 (mg/ml)
Mes-17	water	15.67	20.62
	Ethanol/water	39.16	7.89
	Ethyl acetate/water	26.11	11.46
Dun-32	water	20.32	16.22
	Ethanol/water	32.69	8.36
	Ethyl acetate/water	29.12	9.22

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References

CCAP (2002). Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory. https://www.ccap.ac.uk/wp-content/uploads/MR_Walnes.pdf (accessed: 22-07-21).

Sedjati S., Pringgenies D., Fajri M. (2020). Determination of the pigment content and antioxidant activity of the marine microalga *Tetraselmis suecica*. Jordan Journal of Biological Sciences, 13(1), 55–58.

Stramarkou M., Papadaki S., Kyriakopoulou K., Krokida M. (2017). Effect of drying and extraction conditions on the recovery of bioactive compounds from *Chlorella vulgaris*. Journal of Applied Phycology 29(6), 2947–2960.

Zamani H., Rastegari B., Varamini M. (2019). Antioxidant and anti-cancer activity of *Dunaliella salina* extract and oral drug delivery potential via nano-based formulations of gum Arabic coated magnetite nanoparticles. Journal of Drug Delivery Science and Technology 54, 101278.

PLANKTONIC MALACOSTRACAN FAUNA OF THE NORTH AEGEAN SEA (NAS) REGION: A PRELIMINARY REPORT

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Abstract

Malacostracan mesozooplankton composition was examined in the NAS, during April 2019, October 2019 and June 2021, covering the period of zooplankton abundance peaks. Samples were collected from six stations by means of WP2 nets of 60 μm and 200 μm mesh size. Overall, 924 individuals were identified to the lower taxonomic level, belonging to 63 different species. Maximum values of total Malacostracan abundance were found in April 2019, followed by June 2021 and October 2019. Amphipoda were the dominant group during spring, while in summer and autumn samples Decapoda were found with higher dominances. However, further detailed examination of zooplankton communities of the area, which is under analysis, will fill the existing gaps.

Keywords: *Planktonic Malacostracan, abundance, diversity, NAS*

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1. Introduction

One of the most distinct, complex and dynamic ecological subsystems of the Mediterranean Sea is NAS. This marine subregion, with characteristics of a semi-enclosed area includes habitats of significant biodiversity and fishing grounds of high economic impact (Papapanagiotou *et al*. 2020). Malacostracan mesozooplankton constitutes an intermediate link in the flow of energy from lower to higher trophic levels, significantly contributing to the overall diet of planktivorous species (Karachle & Stergiou 2013). Information on planktonic Malacostracan taxonomy and ecology from the NAS region is scattered and fragmented. Up to date, the relevant literature includes few publications (e.g., Karachle & Stergiou 2013; Karagianni *et al*. 2019). The present study addresses the abundance and diversity to the lower taxonomical level of planktonic Malacostracan taxa of NAS, during three sampling periods, aiming at quantifying information for further investigation linked to zooplankton fauna of the region.

2. Materials and Methods

Six monitoring sites were selected in NAS, based on the hydrological complexity and the strong depth heterogeneity of the area: Kavala Gulf, Thasos Isl., Samothraki Isl., Limnos Isl., Agios Efstratios Isl., Psathoura Isl., with bottom depths 46 m, 308 m, 874 m, 283 m, 94 m and 378 m, respectively. Samplings were carried out in April 2019, October 2019, and June 2021. Initially, in April 2019 a single WP2 net (of 200 μm mesh size) was used. For the rest two samplings, two WP2 nets (of 60 μm and 200 μm mesh size) were used consecutively in each station, considered as two replicates. WP2 nets, equipped with a flowmeter, were vertically hauled at a speed of 0.5 m per sec from the bottom depth to the surface. A total of 26 samples were collected and preserved in 70% alcohol and 1% glycerol deionized water solution. Individuals were photographed and identified to the species level, when possible, after micro-stereoscopic inspection (Nikon MSZ200N) by means of specified keys. Abundances were expressed as number of individuals per volume unit (m^3) by div of the filtered water. Filtration volume was calculated as follows: *Indicated number of revolutions x 0,3 x net opening area* (m^2) *x 1000 = water volume*.

3. Results

Four main orders were found to be represented in the Malacostracan zooplankton of NAS: Amphipoda, Euphausiacea, Decapoda and Stomatopoda. Spring Malacostracan zooplankton were found to be represented by 19 species belonging to 14 families. Amphipoda, Euphausiacea and Decapoda were represented by 7, 5 and 7 species, respectively. Autumn samples diversity included 31 species belonging to 25 families. Amphipoda, Euphausiacea, Decapoda and Stomatopoda were represented by 10, 4, 16 and 1 species, respectively. Finally, summer samples were found to be represented by 26 species belonging to 14 families. Amphipoda, Euphausiacea and Decapoda were represented by 13, 7 and 6 species, respectively. Abundance values for the main planktonic Malacostracan taxa per station and season are presented in Table 1; representative species are shown in Figure 1.

Table 1. Abundances' (ind. m^{-3}) mean values and standard deviations (±SD) of Amphipoda (A), Euphausiacea (E), Decapoda (D) and Stomatopoda (S) per station.

Abundances (ind. m^{-3})										
Season	April 2019			October 2019				June 2021		
Taxa	A	E	D	A	E	D	S	A	E	D
Station				Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
Kavala Gulf	5.495	0.083	0.832	0.369 (±0.522)		0.658 (±0.782)			0.350 (±0.399)	0.933 (±0.220)
Thasos Isl.	1.202	0.083	0.015	0.061 (±0.076)	0.039 (±0.012)	0.632 (±0.319)	0.007 (±0.010)	0.037 (±0.005)	0.104 (±0.053)	
Samothraki Isl.	0.298	1.041	0.017	0.008 (±0.011)	0.059 (±0.083)	0.059 (±0.050)		0.014 (±0.020)	0.126 (±0.073)	0.014 (±0.007)
Limnos Isl.	0.312	0.346	0.017					0.036 (±0.281)	0.089 (±0.048)	0.155 (±0.094)
Agios Efstratios Isl.				0.014 (±0.019)	0.051 (±0.005)	0.110 (±0.000)		0.264 (±0.137)		1.573 (±1.044)
Psathoura Isl.				0.242 (±0.109)	0.118 (±0.000)	0.118 (±0.066)	0.004 (±0.005)	0.160 (±0.065)	0.357 (±0.076)	0.017 (±0.002)

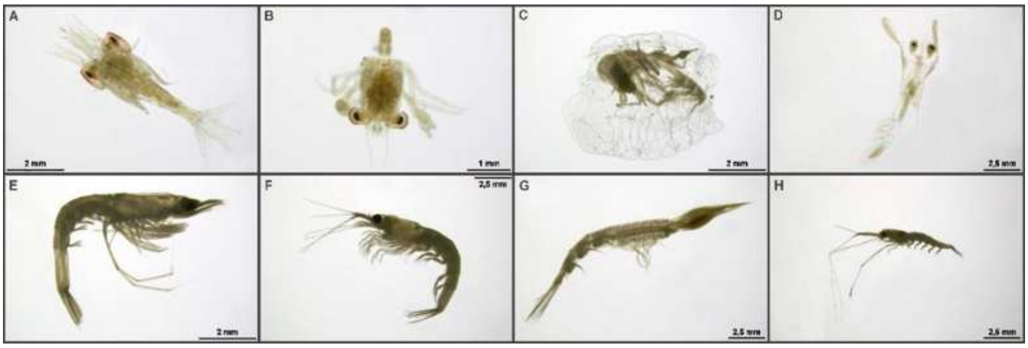

Figure 1. Representative species of Malacostracan *taxa*: (A) *Munida sp.,* (B) *Carcinus aestuarii,* (C) *Phronima sedentaria,* inhabiting a host salp, (D) *Squilla mantis,* (E) *Alpheus glaber,* (F) *Euphasia krohnii,* (G) *Streetsia challengeri* and (H) *Stylocheiron abbreviatum*.

4. Discussion

The present study provides the first assessment of planktonic Malacostracan recording at species level in the NAS region. Our results reveal that taxa abundances varied spatially and seasonally. The higher mean values of total abundances measured in April 2019, followed by October 2019 and June 2021. In spring, Amphipoda was the dominant order, while Amphipoda and Decapoda presented equally the highest species diversity, with seven species. In autumn, Decapoda were the dominant group and the most diverse. These recordings, so far, agree with the literature data (Karachle & Stergiou 2013). In summer, Decapoda were the dominant group, whereas Amphipoda presented the highest diversity (13 taxa). All the samples are characterized by low abundance's mean values compared to the relevant studies from the region (Karagianni *et al*. 2019). This could be attributed to the fact that all the samplings took place in the pelagic zone, whereas, in general, Malacostrocan larvae meet mainly in the neritic zone and are more abundant near the coastal sites. The complete absence of Mysidacea could be justified due to the mainly night occurrence of the taxon, whereas all the samplings of the present study took place from 8:00 am to 2:30 pm.

Acknowledgements

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References

Karagianni A., Artemiou A., Tsikliras A.C., Michaloudi E. (2019). Summer mesozooplankton assemblages in relation to environmental parameters in Kavala Gulf, northern Aegean Sea. Scientia Marina 83, 41–45.

Karachle P.K., Stergiou K.I. (2013). Feeding & ecomorphology of three clupeoids in the N Aegean Sea. Mediterranean Marine Science 15, 9–26.

Papapanagiotou G., Tsagarakis K., Koutsidi M., Tzanatos E. (2020) Using traits to build and explain an ecosystem model: Ecopath with Ecosim modelling of the North Aegean Sea (Eastern Mediterranean), Estuarine, Coastal and Shelf Science 236, 106614.

PRELIMINARY STUDY OF THE REPRODUCTIVE BIOLOGY OF SEA CUCUMBER *Holothuria poli* (DELLE CHIAJE, 1823) FROM CENTRAL AEGEAN SEA

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Abstract
This study is focused on the description of the histological structure of the reproductive system of *Holothuria (Roweothuria) poli*. Sea cucumbers were hand-caught from the sea bed on the Cyclades sea area off the East Mediterranean Sea, over a period of three months, May 2021 to July 2021. The analysis of the obtained data showed that from May until July the sample consisted of mature individuals, with spawning starting in July.
Keywords: *Histology*, *Holothuria poli*, *reproduction*, *sexual maturity*, *gonads*, *East Mediterranean Sea*
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1. Introduction

Sea cucumbers are benthic organisms, found from tidal to deep-sea and from polar to tropical zones of the ocean (Conand 2004). New sea cucumber fisheries are being developed rapidly in the Mediterranean Sea and then exported mostly to Asian countries, where they are consumed for their nutritional and medicinal properties (Chen 2003). The present study will investigate the reproductive cycle and gonad development in sea cucumber, *H. poli*. around Cyclades islands. Our study presents biologically meaningful data of the first months of an annual scheduled sampling and a clear picture of gametogenic histological stages.

2. Materials and Methods

2.1. Sampling locations and collection of specimens

Individuals of *H. poli* were collected from five islands around the Cyclades sea area (Paros, Naxos, Ios, Milos, Serifos). From each region, between 35 and 40 individuals were collected monthly, from May 2021 to July 2021. The sampling will last until April 2022 in the same location for annual variability analysis. This location was chosen because it has a dense population of sea cucumbers.

2.2. Histology

For histological analysis, samples were dissected and fixed in 10% neutral buffered formalin. The embedded tissues were placed in cassettes and then in the Histokinette (Leica TP 1020). Then, the paraffin blocks of tissues were prepared and placed in the Leica EG 1150H. The mold was then removed and the blocks ready for the cutting process of histological sections (Hatziioannou & Vafidis 2015). Using a microtome (SLEE MAINZ CUT 5062), 5 μm sections were taken. The next step of the procedure is the staining using hematoxylin-eosin. The covering of the histological sections was performed using Canada balsam mounting medium. The histological sections were photographed in increments using an optical microscope with an attached digital camera (Progress Plus 2.1) and by using the software Progress Capture 2.1.

3. Results

Based on the staining responses to H/E, the histological stages of gametogenesis are as follows:

Growing: Thick, long, and branched tubules. In females, tubules are pale white to orange in colour (Figure 1A). Early and mid-vitellogenic oocytes were abundant (Figure 1a).

Maturity: Female gonads are orange in colour, the number of tubules and their branches reach their maximum (Figure 1B); mature oocytes with a single nucleolus (Figure 1b,c). In males, the tubules are milky in colour (Figure 1C), the walls of the tubules are thin, and the lumen is filled with mature spermatozoa (Figure 1d,e).

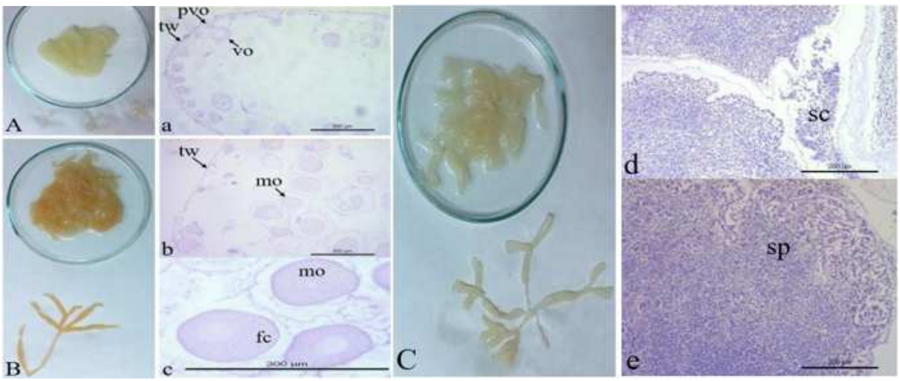

Figure 1. A. Macroscopic and microscopic description of female gonads characteristics of *Holothuria poli* at growth stage, scale bar = 200 mm. a. Details of tubules with various development stages [Early vitellogenic oocytes (vo) detached from the thin tubule wall (tw); pre-vitellogenic oocytes (pvo)]. B, C. Female (left) and male (right) gonads at mature stage, scale bar = 200 mm. b, c. Tubule [with densely packed fully mature oocytes (mo) and thin tubule walls (tw)]. Mature oocyte (om) separated from its follicle (fc). d, e. Thin tubule wall (tw) with spermatocytes (sc).

4. Discussion

H. poli presents a single annual reproductive cycle (Hamel & Mecier 1996), with maximum reproductive activity observed from the end of June 2021 and into early July 2021. Our study shows that *H. poli* in the Cyclades present a general reproduction pattern characterized by an increase in the percentage of mature individuals in June, with spawning beginning in July. At the end of the samplings, which will continue until April 2022, we will be able to give results about the reproductive pattern/gametogenesis of *H. poli* populations that live in a narrow latitudinal range. Hence, the reproductive parameters that will be obtained in the present study will provide an important basis for establishing regulatory measures for the management of sea cucumbers and preserving biodiversity in the East Mediterranean Sea.

References

Chen J. (2003) Overview of sea cucumber farming and sea ranching practices in China. SPC Beche-de-mer Information Bulletin 18, 18–23.

Conand C. (2004) Present status of world sea cucumber resources and utilisation: an international overview. Session I – Status of resources and utilization. Université de La Réunion, Saint Denis messag, La Réunion, France.

Hamel J-F., Mercier A. (1996). Gonad morphology and gametogenesis of the sea cucumber *Cucumaria frondosa*. SPC Beche-de-mer Information Bulletin 8, 22–33.

Hatziioannou M., Vafidis D. (2015). Εργαστηριακές ασκήσεις ιστολογίας. [ebook] Athens: Hellenic Academic Libraries Link. Chapter 6, Available at: http://hdl.handle.net/11419/5131

Slimane-Tamacha F., Soualili D., Mezali K. (2019). Reproductive biology of *Holothuria (Roweothuria) poli* (Holothuroidea: Echinodermata) from Oran Bay, Algeria. SPC Beche-de-mer Information Bulletin 39, 47–53.

GROWTH AND MORTALITY OF THE SEA URCHIN *Arbacia lixula* (LINNAEUS, 1758) IN PAGASITIKOS GULF (CENTRAL AEGEAN SEA)

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Abstract

Test diameter frequency data of the black sea urchin (*Arbacia lixula*) population in Pagasitikos gulf (central Greece) were used to assess growth and mortality parameters. The ELEFAN system was used to provide quantitative information on the growth of the black sea urchin. Response surfaces and K scan were used to estimate growth parameters. Total mortality estimated using the Beverton & Holt equation, was 2.31. The Von Bertalanffy growth equation was estimated as: Test diameter = 67.6 × (1-e$^{-0.47 \times Age}$)

Keywords: *Arbacia lixula, sea urchin*, *growth*, *mortality*, *Pagasitikos gulf*

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1. Introduction

The non-edible black sea urchin, *Arbacia lixula* (Linnaeus, 1758) (Echinodermata: Echinoidea), is a common echinoid in the Mediterranean sublittoral zone (Boudouresque & Verlaque 2001). Sea urchins are benthic marine echinoderms with high dispersal life history, high fecundity, and external fertilization playing a key role in nutrient recycling in intertidal areas, seagrass meadows and coral ecosystems (Alcoverro & Mariani 2002). The aim of this study was to assess growth and mortality parameters of the black sea urchin in Pagasitikos gulf, using test diameter frequency data.

2. Materials and Methods

Sampling was carried out monthly at depths between 3 and 7 meters, from December 2008 to November 2009 with scuba diving in two locations in north Pagasitikos gulf, a semi-enclosed landlocked gulf located in the northwestern part of the Aegean Sea (Figure 1A). Test diameter (T.D.) was measured with vernier calipers to the nearest mm for a total of 920 individuals. The ELEFAN system (Electronic LEngth Frequency ANalysis) was used to provide quantitative information on the growth of the black sea urchin (Brey & Pauly 1986) using a seasonally oscillating version of the von Bertalanffy Growth Formula (VBGF) (Figures 2, 3). It has been suggested (Pauly 1987) that length-frequency data (Figure 1B) are probably the most underutilized type of information available on fish and other aquatic resources. Total mortality was estimated using the Beverton & Holt (1956) equation:

$$Z = K * \frac{Linf - \bar{L}}{\bar{L} - L'},$$

which estimates the total instantaneous mortality coefficient, Z, in a steady-state population with constant exponential mortality and von Bertalanffy growth, from the mean length, in a random sample above length L′.

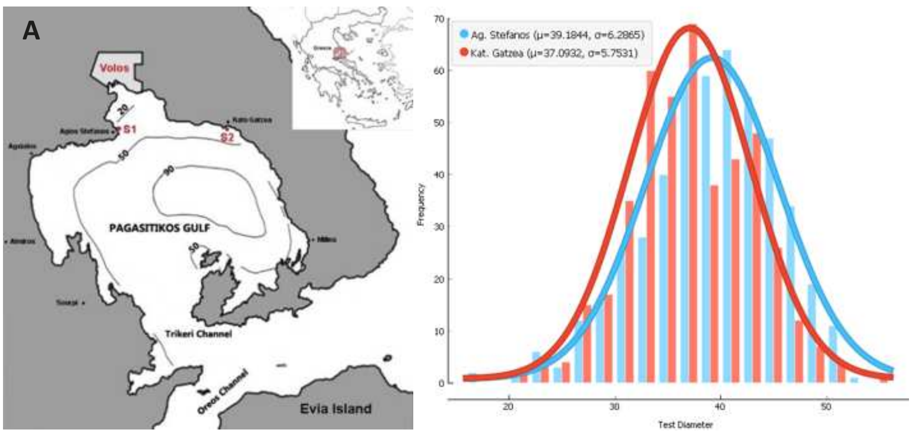

Figure 1. (A) Sampling locations: S1 – Agios Stefanos, S2 Kato Gatzea. (B) Test diameter distribution at each sampling location.

3. Results

The Von Bertalanffy growth equation for the entire population was estimated as: T.D. = $67.6 \times (1\text{-}e^{-0.47 \times Age})$. Total mortality estimation was Z = 2.31.

K\Loo	64,70	66,15	67,60	69,05	70,50	71,95	73,40
0,28	0,074	0,109	0,080	0,069	0,072	0,085	0,093
0,30	0,088	0,074	0,078	0,092	0,083	0,086	0,114
0,33	0,081	0,083	0,090	0,093	0,103	0,118	0,194
0,35	0,079	0,093	0,079	0,107	0,120	0,206	0,286
0,38	0,097	0,077	0,109	0,177	0,231	0,272	0,292
0,40	0,078	0,107	0,177	0,275	0,281	0,286	0,274
0,43	0,100	0,199	0,220	0,266	0,291	0,292	0,238
0,45	0,188	0,220	0,266	0,291	0,292	0,238	0,181
0,48	0,215	0,250	0,334	0,292	0,249	0,181	0,151
0,50	0,292	0,322	0,292	0,249	0,181	0,149	0,158

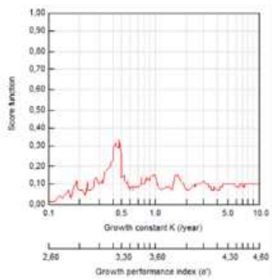

Figure 2. Response surface and K-Scan of VBGF using ELEFAN to acquire growth parameter estimations.

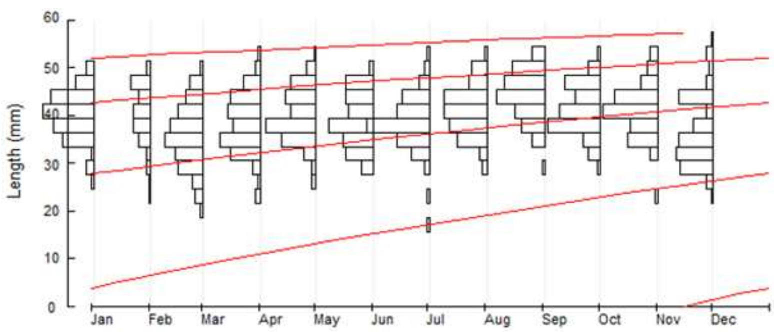

Figure 3. VBGF and length frequency plot.

4. Discussion

The von Bertalanffy model is the most used function for modelling growth, because it describes the growth pattern of many species satisfactorily (Ouréns *et al*. 2013). Food quality and quantity affects the reproductive maturation and growth of sea urchins (Fernandez & Pergent 1998). General growth function can be affected by the environmental and demographic conditions to which sea urchins are subjected. In the Mediterranean, maximum growth of the co-occurring edible species *Paracentrotus lividus* occurs between 12 and 18 degrees in spring, sometimes in autumn, and minimally in winter (Shpigel *et al*. 2004).

References

Alcoverro T., Mariani S. (2002). Effects of sea urchin grazing on seagrass (*Thalassodendron ciliatum*) beds of a Kenyan lagoon. Marine Ecology Progress Series 226, 255–263.

Beverton R.J.H., Holt S.J. (1956). A review of methods for estimating mortality rates in fish populations, with special reference to sources of bias in catch sampling. Rapports et procés-verbaux des réunions Commission internationale pour l'exploration scientifique de la Mer Méditerranée 140, 67–83.

Boudouresque C., Verlaque M. (2001). Ecology of *Paracentrotus lividus*. Developments in Aquaculture and Fisheries Science 32, 177–216.

Brey T., Pauly D. (1986). Electronic length frequency analysis: a revised and expanded user's guide to ELEFAN 0, 1 and 2. https://hdl.handle.net/20.500.12348/3361.

Fernandez C., Pergent G. (1998). Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin *Paracentrotus lividus*. Journal of Shellfish Research 17, 1571–1581.

Ouréns R., Flores L., Fernández L., Freire J. (2013). Habitat and density-dependent growth of the sea urchin *Paracentrotus lividus* in Galicia (NW Spain). Journal of Sea Research 76, 50–60.

Pauly D. (1987). A review of the ELEFAN system for analysis of length-frequency data in fish and aquatic invertebrates, p. 7-34. In: "Length-based methods in fisheries research", D. Pauly and G.R. Morgan (eds). ICLARM Conference Proceedings 13, 468 p. International Center for Living Aquatic Resources Management, Manila, Philippines, and Kuwait Institute for Scientific Research, Safat, Kuwait.

Shpigel M., McBride S.C., Marciano S., Lupatsch I. (2004). The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. Aquaculture 232, 343–355.

HISTOLOGICAL STUDY OF THE SCALDFISH, *Arnoglossus laterna* (WALBAUM, 1792)

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Abstract
This study was focused on the description of the histological structure of the Mediterranean scaldfish *Arnoglossus laterna*. Histological sections, obtained from the dissection of 10 individuals weighting on average 25 g each, were examined with light microscope and photographed. The histological analysis was performed in a number of biological systems, namely: digestive, muscular and reproductive. Their structure was compared with known histological structures of similar species. The results provide a clear image of the tissue texture.
Keywords: *Arnoglossus laterna*, *scaldfish, arnozageta, bothidae, histological study*
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1. Introduction

Scaldfish is a benthic bothid found in muddy bottoms, at depths ranging from 10 to 200 m (Jardas 1996). The species is distributed in the Atlantic from Norway to Morocco and throughout the Mediterranean Sea (Nielsen 1986). Maximum standard length recorded is 25 cm (Muus *et al.* 1999) and maximum age 8+ years (Gibson & Ezzi 1980). The body is flattened and translucent with large head and eyes.

The study of the internal morphology of animal tissue and specifically organ texture, requires a histological approach. Cell morphology facilitates the understanding of tissue structure and function in both normal and pathological situations. Although, studies have been conducted on the geographic distribution of the species, there is considerable lack of information on the histological structure of various organs. The aim of the study was to present preliminary results in order to fill the literature gap and extend our knowledge on the flatfish ecophysiology (nutrition, reproduction and defense mechanisms).

2. Materials and Methods

Ten individuals weighting on average 25 g each were dissected and fixed in 10% neutral formalin solution. Fixed tissues were further processed in the laboratory using histological examination. The middle portions of each tissue were placed in a histokinette (Leica TP 1020, Leica Microsystems GmbH, Nussloch, Germany) for dehydration (immersion in ethanol solution of increasing concentrations), clearing (immersion in xylene solutions to replace ethanol with an organic dissolvent), and embedding in liquid paraffin wax. Paraffin blocks with tissues were left for cooling (Leica EG 1150H Leica Microsystems GmbH, Nussloch, Germany); mold was removed, and blocks were mounted on a microtome (Slee Mainz Cut 5062, SLEE medical GmbH, Mainz, Germany) for sectioning (5 µm sections). Sections were stained with hematoxylin–eosin regressive staining procedure, covered with Canada balsam mounting medium, and observed under light microscop connected with a digital camera (ProgRes Plus 2.1, JENOPTIC Optical Systems GmbH, Jena, Germany). Histological sections were photographed in appropriate magnification scale using the software Progress Capture 2.1 (Hatziioannou & Vafidis 2015).

3. Results

The histological structure of four *A. laterna* organs and corresponding part number are shown in Figure 1 and Table 1.

Table 1: Elements of histological sections shown in Figure 1.

Figure 1A	Muscle tissue	1	Striated muscle fibers	Figure 1C	Liver	9	Lumen of central hepatic vein
		2	Endomysium			10	Vascular endothelium
		3	Nucleus of striated muscle cells			11	Fat vacuoles
Figure 1B	Intestine	4	Muscularis	Figure 1D	Ovary	12	Oocyte nucleus
		5	Circular muscular layer			13	Vitellogenic oocyte
		6	Longitudinal muscular layer			14	Hydrated oocyte
		7	Intestinal lumen			15	Atretic vitellogenic oocyte
		8	Blood vessel			16	Zona pellucida
						17	Membrana granulosa

Striated muscle tissue in the Mediterranean scaldfish, is arranged in separate muscle fascicles (myomeres) connected to the vertebrae through the myoseptum and are consisted of muscle fibers which are run parallel to the logitudinal body axis. The striated muscle tissue has a similar structure to the rest of the teleosts (Figure 1A). In intestine, muscularis consists of an inner circular muscular layer and an outer longitudinal muscular layer of smooth muscle fibers. Along the intestine, mucosa is covered by a simple columnar epithelium composed of brush border columnar cells (absorptive cells) and goblet cells. Below the epithelium, there is the lamina propria which appears rich perfusion (Figure 1B). In the liver (Figure 1C), the hepatic central vein, its lumen, the fat vacuoles and the vascular endothelium are visible. The ovary at atretic oocyte stage is shown in Figure 1D.

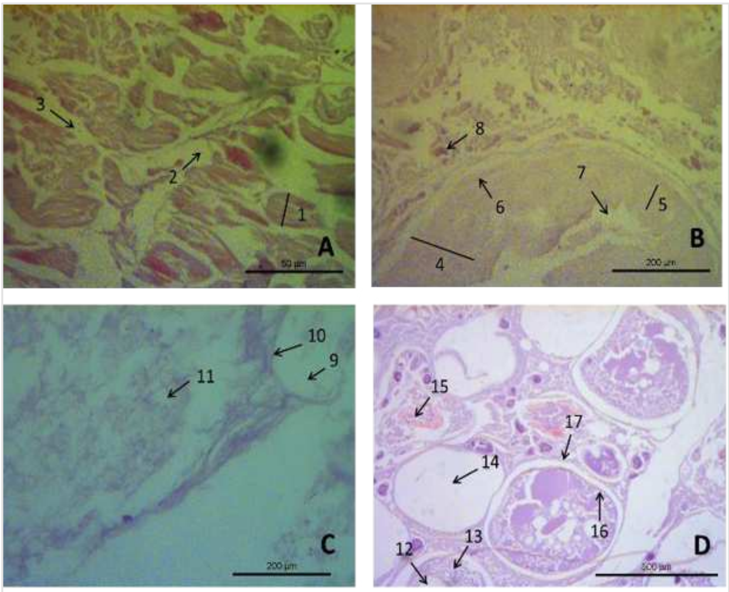

Figure 1. Histology photomicrographs of A) muscle tissue; B) intestine; C) liver; D) ovary.

4. Discussion

The study revealed that both muscular, digestive and reproductive system of *Arnoglossus laterna* have several similarities in relation to other teleosts. Reproductive biology of this species, similarly to other teleosts (flatfishes), is remarkable in that ovulation occurs probably during the larval phase (Tyler & Sumpter 1996). It is also worth noting that the ovarian development receives environmental cues via the hypothalamus pituitary axes mediated by the secretion of neural and peptide hormones (gonadotropins), which control the production of sex steroids (Tyler *et al.* 1999). Lastly, maturation is regulated by a combination of photoperiod and temperature signals (Migaud *et al.* 2010).

References

Gibson R.N., Ezzi I.A. (1980). The biology of the scaldfish, *Arnoglossus laterna* (Walbaum) on the west coast of Scotland. Journal of Fish Biology 17(5), 565–575.

Hatziioannou M., Vafidis D. (2015). Εργαστηριακές ασκήσεις ιστολογίας. [ebook] Athens: Hellenic Academic Libraries Link. Chapter 6, Available at: http://hdl.handle.net/11419/5131

Jardas I. (1996). In: Adriatic Ichthyofauna. J. Matekalo-Draganović (ed.), Školska knjiga, Zagreb, pp. 533.

Migaud H., Davie A., Taylor J.F. (2010). Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. Journal of Fish Biology 76, 27–68.

Muus B.J., Nielsen J.G., Dahlstrom P. (1999). Sea Fish. Scandinavian Fishing Year Book.

Nielsen J.G. (1986). Bothidae. In: "Fishes of the northeastern Atlantic and the Mediterranean", Whitehead P.J.P., Bauchot M.L., Huredu J.C., Nielsen J., Tortonese E. (eds). UNESCO, Paris, p. 1294–1298.

Stoskopf K. (1993). Fish Medicine. W. B. Saunders. London, UK.

Tyler C.R., Santos E.M., Prat F. (1999). Unscrambling the egg – Cellular, biochemical, molecular and endocrine advances in oogenesis. Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish, Bergen 1999, Jon Greg AS, Bergen, pp. 273–280.

Tyler C.R., Sumpter J.P. (1996). Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6, 287–318.

PRELIMINARY STUDY ON THE REPRODUCTIVE BIOLOGY OF THE EUROPEAN HAKE *Merluccius merluccius* (LINNAEUS, 1758) IN PAGASITIKOS GULF, GREECE

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Abstract
It is believed that European hake, a multiple batch spawner with indeterminate fecundity, exhibits variable maturity stages for different sexes at different stages of life cycle with earlier maturity in males. The reproductive biology in Pagasitikos gulf was assessed through histological examination. None of the male individuals examined were mature spawners and two females were mature. Spawning cycle of *Merluccius merluccius*, in Pagasitikos gulf is yet to be determined with further investigation warranted to assess its reproductive cycle.
Keywords: *Histology, Merluccius merluccius, reproduction, Pagasitikos gulf, European hake*
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1. Introduction

European hake inhabits the Atlantic coast of Europe and western North Africa, northward to Norway and Iceland, southward to Mauritania. It is abundant in the Mediterranean Sea and along the southern coast of the Black Sea (FAO 1990). The spawning period is annual and exhibits considerable variation amongst populations. In the Mediterranean Sea reproduction mainly occurs between December and June. Spawning takes place between 100 and 300m depth (FAO 1990). The European hake is a multiple batch spawner with indeterminate fecundity, associated with the asynchronous development of the oocytes (Murua *et al.* 1998). Males and females mature at different sizes, probably at different ages, with males maturing earlier than females. (Domínguez-Petit 2007). The aim of this study was to investigate the reproductive biology of the male European hake in Pagasitikos gulf.

2. Materials and Methods

Gonads were immediately fixed in 10% neutral buffered formalin solution. Fixed gonads were further processed in the laboratory using histological examination. The middle portions of each gonad tissues were placed in a histokinette (Leica TP 1020, Leica Microsystems GmbH, Nussloch, Germany) for dehydration (immersion in ethanol solution of increasing concentrations), clearing (immersion in xylene solutions to replace ethanol with an organic dissolvent), and embedding in liquid paraffin wax. Paraffin blocks with gonad tissue were left for cooling (Leica EG 1150H Leica Microsystems GmbH, Nussloch, Germany); mold was removed, and blocks were mounted on a microtome (Slee Mainz Cut 5062, SLEE medical GmbH, Mainz, Germany) for sectioning (5 μm sections). Sections were stained with hematoxylin–eosin regressive staining procedure, covered with Canada balsam mounting medium, and observed under light microscop connected with a digital camera (ProgRes Plus 2.1, JENOPTIC Optical Systems GmbH, Jena, Germany). Histological sections were photographed in appropriate magnification scale using the software Progress Capture 2.1. Different developmental gametogenic stages were assessed according to FAO (2019).

3. Results

Male microscopic maturity stages are shown in Figure 1. Stage 1: Only spermatogonia and primary spermatocytes are visible (Figure 1A). Stage 4A: Seminiferous tubules are empty, with residual spermatozoa (Figure 1B). The female microscopic maturity stage key was defined as: RECOVERING STAGE: The histological depiction of three microscopic maturity stages was carried out (Figure 2A). The most common characteristics of the immature stages were the oogonia and small primary growth oocytes (PG). MATURE SPAWNER STAGE: In this stage, germinal vesicle breakdown stage could be observed (Figure 2B). RESTING STAGE: Females at this stage do not produce more oocytes, but are characterized by a high level of atresia (Figure 2C).

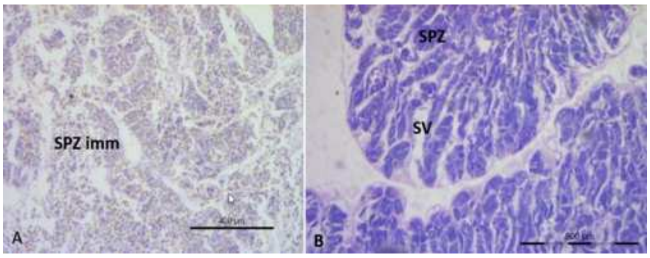

Figure 1. Male reproductive cycle for European hake. Microscopic maturity stages. A) Stage 1; B) Stage 4A; SPZ imm; immature spermatozoa, SPZ; Spermatozoa, SV; seminal vesicle; H & E stain; Scale bar A 400 µm; B, 500 µm.

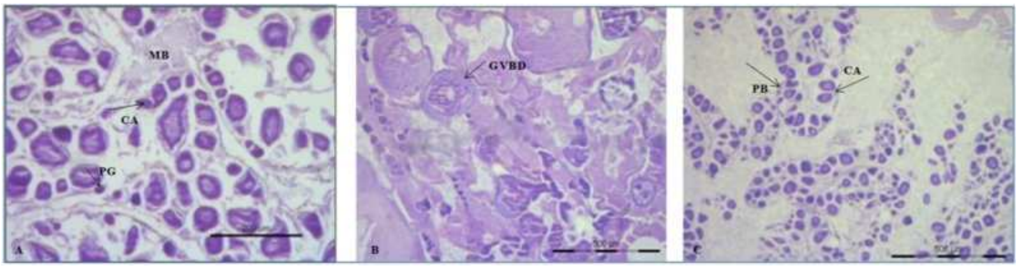

Figure 2. Histological sections of *Merluccius merluccius* ovaries, A) recovering ovary; B) mature spawner; C) resting. (MB, muscle bundle; PG, primary growth; CA, cortical alveolar oocyte; GVBD, germinal vesicle breakdown stage; H & E stain; Scale bar A 200 µm; B, C 500 µm).

4. Discussion

European hake is a highly prized commercial species with a considerable reduction in annual landings in Europe since the 1960s (Groison *et al.* 2008). There is lack of available information on the reproduction of male *Merluccius merluccius* and a better understanding on male reproductive biology could contribute towards more suitable management decisions. According to Al-Absawy (2010), the European hake in the Mediterranean Basin exhibits a well-defined spawning peak from January to April. The current preliminary study indicated that in Pagasitikos gulf, *Merluccius merluccius* presents a similar reproductive pattern, since during May three female maturity stages were observed. The completed study aims to provide further information on the reproductive biology of the species in Eastern Mediterranean Sea.

References

Al-Absawy M.A.E.-G. (2010). The Reproductive Biology and the Histological and Ultrastructural Characteristics in Ovaries of the Female Gadidae Fish *Merluccius Merluccius* from the Egyptian Mediterranean Water. *African Journal of Biotechnology* 9(17), 2544–2559.

Cohen D.M., Inada T., Iwamoto T., Scialabba N. (1990). FAO species catalogue. Vol. 10. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. FAO Fish. Synop. 125(10). Rome: FAO. 442 p.

Groison A.L., Suquet M., Cosson J., Le Coz J.R., Jolivet A., Garren F. (2008): Biological characteristics of European hake (*Merluccius merluccius*) sperm. Cybium 32, 178.

Follesa M.C., Carbonara P. (2019). Atlas of the maturity stages of Mediterranean fishery resources (Issue April).

Hatziioannou M., Vafidis D. (2015). *Εργαστηριακές ασκήσεις ιστολογίας*. [ebook] Athens: Hellenic Academic Libraries Link. Chapter 6. Available Online at: http://hdl.handle.net/11419/5131

Murua H., Motos L., Lucio P. (1998). Reproductive modality and batch fecundity of the European hake (*Merluccius Merluccius* L.) in the Bay of Biscay. Calif. Coop. Ocean. Fish. Investig. Rep. 196–203.

Domínguez-Petit R. (2007). Study on reproductive potential of *Merluccius merluccius* in the Galician shelf. Thesis Doctoral. http://hdl.handle.net/10261/4377.

GENETIC DIVERSITY PATTERNS OF MESOPELAGIC FISH IN THE GREEK SEAS

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Abstract
Mesopelagic fish comprise the most abundant group of vertebrates in the marine environment as well as in the total biosphere. Despite their unique biological and ecological traits, research on this group has not been implemented extensively. The present study investigates the intraspecific genetic diversity of three mesopelagic fishes (*Maurolicus muelleri*, *Benthosema glaciale*, *Hygophum benoiti*) in the Greek Seas. Analyses of three mitochondrial genes (COI, 12S, 16S) from 223 samples revealed a lack of phylogeographic structure for *M. muelleri* and *B. glaciale* across the studied area. However, *H. benoiti* specimens from the Corinthian Gulf differed from the rest of the populations, suggesting that the limited connection with the open sea may act as a barrier to gene flow. The shallow haplotype genealogy (high haplotype diversity and low nucleotide diversity) in association with recent demographic expansion events can be considered as the result of the recent evolutionary history of these species in the Greek seas.
Keywords: *Phylogeography, genetic structure, genetic differentiation, mitochondrial DNA*
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1. Introduction

Mesopelagic fishes are usually small sized species, which inhabit the twilight zone, usually defined as the depths between 200-1,000 m. Most species of this group undertake diurnal vertical migrations following their prey into the epipelagic zone to feed at night (Olivar *et al*. 2012). Mesopelagic fish play an integral role in marine food webs and global biochemical cycles (Irigoien *et al.* 2014). Furthermore, their large abundance makes them particularly attractive for exploitation (John *et al.* 2016). Yet, mesopelagic fauna still remains largely unknown and genetic studies for these species have been particularly scarce.

The aim of the present study is to explore the genetic diversity and phylogeographical patterns of three mesopelagic fishes (*M. muelleri*, *B. glaciale*, *H. benoiti*) in the Greek Seas.

2. Materials and Methods

Samples were collected from six locations in Greek Seas (Corinthian Gulf, Saronic Gulf, North Aegean Sea, Euboean Gulf, Cretan Sea, Ionian Sea) and from two locations of the Spanish Mediterranean, during various scientific expeditions carried out between November 2018 and October 2020. Mitochondrial genes cytochrome c oxidase subunit 1 (COI), 16S rDNA and 12S rDNA were partially sequenced and analysed. Molecular diversity indices for the concatenated dataset including the number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π) and the number of private haplotypes (Hpr) were estimated for each sampled population using the software DNAsp v.5. The relationships between concatenated haplotypes were depicted with median-joining networks which were constructed in the software PopART. To test departures from neutrality Fu's F test and Tajima's D test were employed, both implemented in ARLEQUIN v.3.5.2.2.

3. Results and Discussion

Haplotype networks exhibited star-like shapes, which in combination with the observed high haplotype diversity and low nucleotide diversity values (Tables 1-3) indicate a signature of recent population expansion. Negative and statistically significant values of Tajima's D and Fu's F (Figure 1) further suggest recent demographic expansion for the three mesopelagic species.

According to the median-joining networks, no phylogeographic pattern was found among *M. muelleri* (Figure 1A) or *B. glaciale* (Figure 1B) populations. On the contrary, *H. benoiti* individuals from the Corinthian Gulf were differentiated from the rest of the studied populations. This was evident by the dominance of a haplotype in the samples from the Corinthian Gulf, which was different from the main haplotype shared by the individuals from the Saronic Gulf and the North Aegean Sea (Figure 1C).

The Corinthian Gulf is semi-closed with limited connection to the open sea and has unique hydrological and topographical characteristics. Therefore, gene flow between the Corinthian Gulf and the open sea could also be limited. One possible explanation of the fact that genetic differentiation was evident only in one of the three species may be that *H. benoiti* potentially has unique traits compared to the other species, limiting the distance of passive transfer by the currents. Strong currents and wind-driven circulation has been reported in the Corinthian Gulf (Drakopoulos & Lascaratos, 1998), which may affect the distribution of *H. benoiti* in the area.

Table 1: Molecular diversity indices for *M. muelleri* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	14	10 (7)	0.923	0.118
Saronic G.	14	5 (2)	0.505	0.047
N. Aegean	14	9 (3)	0.879	0.099
Euboean G.	14	3 (1)	0.385	0.027
Cretan Sea	14	10 (6)	0.923	0.120
Ionian Sea	14	4 (2)	0.495	0.043
Spain (GSA01)	12	6 (2)	0.682	0.066
Spain (GSA06)	15	9 (5)	0.8	0.096
Total	111	36	0.722	0.078

N: number of samples, Nh: number of haplotypes, Nph: number of private haplotypes, Hd: haplotype diversity, π: nucleotide diversity

Table 2: Molecular diversity indices for *B. glaciale* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	14	9 (8)	0.835	0.131
Saronic G.	14	8 (6)	0.769	0.102
N. Aegean	14	12 (9)	0.967	0.231
Euboean G.	14	9 (7)	0.879	0.122
Cretan Sea	14	8 (6)	0.824	0.113
Total	6	39	0.848	0.133

Table 3: Molecular diversity indices for *H. benoiti* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	10	3 (2)	0.511	0.036
Saronic G.	10	5 (3)	0.533	0.052
N. Aegean	14	6 (5)	0.505	0.047
Total	34	12	0.761	0.042

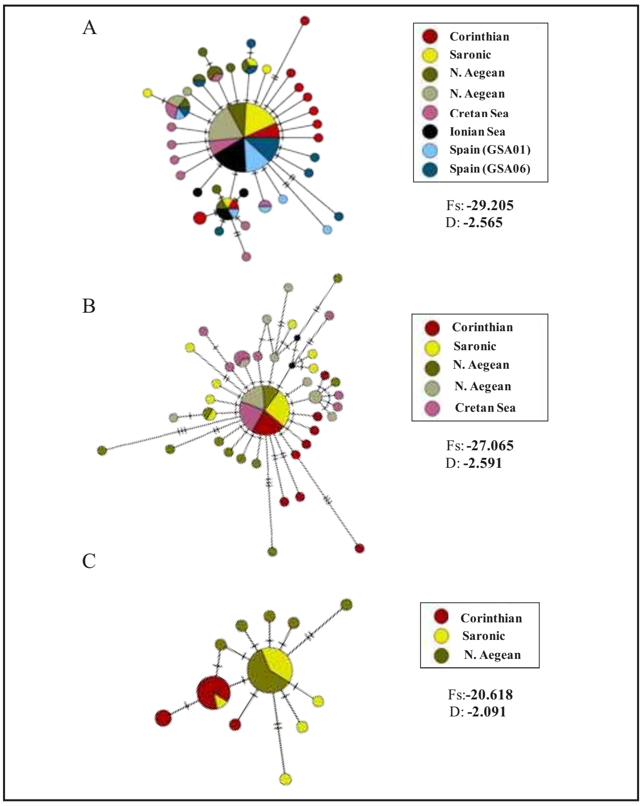

Figure 1: Median-joining haplotype networks and neutrality test results of *M. muelleri* (A), *B. glaciale* (B) and *H. benoiti* (C). Characters in bold indicate statistically significant values (p<0.01).

Acknowledgements

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References

Drakopoulos P.G., Lascaratos A. (1998). A preliminary study on the internal tides of the gulfs of Patras and Korinthos, Greece. Continental Shelf Research 18(12), 1517–1529.

Irigoien X., Klevjer T.A., Røstad A., Martinez U., Boyra G., Acuña J.L., Bode A., Echevarria F., Gonzalez-Gordillo J.I., Hernandez-Leon S., Agusti S., Aksnes D.L., Duarte C.M., Kaartvedt S. (2014). Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nature Communications 5, 3271.

John M.A.S., Borja A., Chust G., Heath M., Grigorov I., Mariani P., Martin A.P., Santos R.S. (2016). A Dark Hole in Our Understanding of Marine Ecosystems and Their Services : Perspectives from the Mesopelagic Community. Frontiers in Marine Science 3, 1–6.

Olivar M.P., Bernal A., Molí B., Peña M., Balbín R., Castellón A., Miquel J., Massutí E. (2012). Vertical distribution, diversity and assemblages of mesopelagic fishes in the western Mediterranean. Deep-Sea Research Part I: Oceanographic Research Papers 62, 53–69.

FIRST INVENTORY OF SEA CAVES AROUND CYPRUS

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Abstract
Mediterranean sea caves harbour a high variety of rare, endemic, protected and commercially important species. The most recent census identified over 700 sea caves in the eastern Mediterranean, however no comprehensive survey had been undertaken around Cyprus at that time. In this study, we present the results of the first detailed inventory of sea caves around Cyprus. Questionnaires as well as coastal surveys were used to locate all sea caves, with a special focus on the partially submerged ones. A total of 207 sea caves were recorded, mainly through coastal surveys (86%). Of the recorded caves, 89% are partially submerged, and the remaining 11% are fully submerged.

Keywords: *Sea caves, Eastern Mediterranean, Cyprus*
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1. Introduction

Sea caves constitute a typical feature of the Mediterranean rocky coastline. They host rich biodiversity, including a variety of sciaphilous communities (Gerovasileiou & Bianchi 2021). In addition, submerged or partially submerged sea caves are important resting, breeding and nursing grounds for the endangered Mediterranean monk seal *Moncahus monachus* (Karamanlidis *et al*. 2016), and are protected by the EU's Habitats Directive (Habitat Type 8330). According to the most recent census, approximately 3000 sea caves have been recorded in the Mediterranean Sea, with over 700 in the eastern basin (Giakoumi *et al*. 2013). However, at the time of Giakoumi *et al*. (2013) census, Cyprus caves remained unexplored despite the small population of monk seals inhabiting the region (Nicolaou *et al*. 2021). Herein, we present the results of a recent survey, which specifically focused on the detailed spatial assessment and description of sea caves around Cyprus.

2. Material and Methods

Data of the sea caves of Cyprus was collected through (a) questionnaires distributed to dive centres, scuba divers, free divers, and fishers, and (b) coastal surveys that were conducted along targeted areas of the coastline. During the coastal surveys researchers were scanning the coastline on board a research vessel. Whenever a potential cave was detected, researchers would approach and enter the cave to note the basic characteristics of the caves and take photographs. For each cave, information regarding the location (geographic coordinates), basic topographic features (e.g., length, width, depth, number of entrances), and submersion level (fully submerged or partially submerged) were recorded.

3. Results and Discussion

A total of 207 sea caves (Figure 1) were recorded, out of which 177 through coastal surveys, 19 through questionnaires, and 12 from data provided by the Department of Fisheries and Marine Research (DFMR) of Cyprus. Recorded caves are distributed in the following districts of Cyprus: Ammochostos District - 33 caves, Larnaca District – 26 caves, Lemesos District – 71 caves, and Paphos District - 76 caves. A considerable number of sea caves (81 caves) are located within the boundaries of Natura 2000 Protected Areas.

With regards to the 177 sea caves assessed through coastal surveys, the majority (163 caves) are partially submerged, whereas only 14 are fully submerged. Most of the caves recorded (60%) are quite small in size as their length does not surpass 10 m. Specifically, 50 caves have a length of 0-5 m, 56 caves a length of 6-10 m, 17 caves a length of 11-15 m, 24 caves a length of 20-25 m, and 15 sea caves a length of over 30 m. Among the 15 longest caves, 5 had a length of up to 50 m. Furthermore, 90% of the sea caves have the form of a "dead-end" cave, with one (75%) or multiple (15%) openings, while 8% have the form of a "running-through" tunnel. A large number of the sea caves identified during coastal surveys (83 caves) had an internal beach. Internal beaches were comprised of pebbles or sand (Figure 2), or solid rocky platforms, and their widths varied from 0.5 m to 8 m.

Figure 1. Distribution of all sea caves recorded around Cyprus, indicating the source of information and the marine Natura 2000 areas.

Figure 2. Example of a sandy (left) internal beach and a pebble (right) internal beach identified in the sea caves of Cyprus, all suitable for *M. monachus* resting.

Acknowledgements

This study was funded in the framework of the Project "Mapping and evaluation of *Posidonia* meadows and other important marine habitats under the European Habitats Directive (92/43/EEC), in the coastal waters of Cyprus", of the Department of Fisheries and Marine Research (DFMR) of the Republic of Cyprus (25% National Resources, 75% European Maritime and Fisheries Fund, EMFF, 2014-2020).

References

Gerovasileiou V., Bianchi C.N. (2021). Mediterranean marine caves: a synthesis of current knowledge. Oceanography and Marine Biology: An Annual Review 59, 1–88.

Giakoumi S., Sini M., Gerovasileiou V., Mazor T., Beher J., Possingham H.P., Abdulla A., Çinar M.E., Dendrinos P., Gucu A.C., Karamanlidis A.A., Rodic P., Panayotidis P., Taskin E., Jaklin A., Voultsiadou E., Webster C., Zenetos A., Katsanevakis S. (2013). Ecoregion-Based Conservation Planning in the Mediterranean: Dealing with Large-Scale Heterogeneity. PLoS ONE, 8(10), e76449.

Karamanlidis A.A., Dendrinos P., de Larrinoa P.F., Gücü A.C., Johnson W.M., Kiraç C.O., Pires R. (2016). The Mediterranean monk seal Monachus monachus: Status, biology, threats, and conservation priorities. Mammal Review 46(2), 92–105.

Nicolaou H., Dendrinos P., Marcou M., Michaelides S., Karamanlidis A.A. (2021). Re-establishment of the Mediterranean monk seal Monachus monachus in Cyprus: priorities for conservation. Oryx 55(4), 526–528.

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USE OF HBV LIGHT MODEL TO SIMULATE INTERMITTENT RIVER IN KARST AREAS: THE CASE STUDY OF DRAGONJA RIVER, SLOVENIA

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Abstract
As the hydrological processes in karst areas are difficult to assess, the investigators develop or adapt the hydrological models to improve the availability of good and sufficiently hydro-climatological long time series. Dragonja River (91 km^2, Slovenia), a typical Mediterranean intermittent river of karst geology, has chosen to investigate the effectiveness of the application of conventional hydrological models. The HBV-Light is a conceptual hydrological model extensively used in hydrological forecasting and water balance. The major model parameters with strong effectiveness in water flow simulation are related to the soil moisture, infiltration and stream routing. The calibration verified by the statistical indexes (NSE, PBIAS, RSR, d and md indexes) and the observed and simulated amounts of water flow are 29.1 Mm^3 and 26.6 Mm^3, respectively.
Keywords: *HBV-Light model, Karst Geology, Intermittent River.*
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1. Introduction

The impacts of climate change on catchment behavior have been extensively investigated the few last decades, as a result river flows are constantly reduced. The intermittent Mediterranean rivers are significantly affected by the new status, as it makes them important for investigating the effects on the flow regime (Gutierrez–Jurado *et al.* 2021). The hydrological models are composed of significant tools for specialized forecasting and analysis of hydrological events, if reliable and sufficiently long time series of climate and discharge observations for model calibration are available (Reynolds *et al.* 2017). Therefore, the aim of this study is to estimate the major hydrological processes and investigate the influence of flow intermittency on model calibration and application. The runoff-rainfall model HBV-Light is used to simulate a low flow hydrology system in Dragonja River Basin (Slovenia) in a karst area (Figure 1).

2. Material and methods

Study area

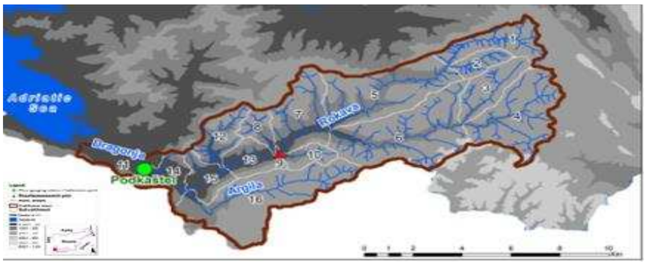

Figure 1: The Dragonja River Basin (Glavan *et al.* 2013).

The Dragonja River is located in SW Slovenia and NW Croatia (lat = 45.45293, long = 13.65905) and covers a catchment area of 91 km^2. The main land uses have been agricultural since the 1960s, combined with anti-erosion vegetation stabilization works, which caused natural reforestation of the land. Geologically, the Dragonja River is homogenous (Eocene flysch sediments predominate, marl and sandstones) and the bedrock is relatively soft and erodible (Sraj *et al.* 2016). The Dragonja River basin belongs to the sub-Mediterranean climate, with average annual Temperature of 12°C, and annual aerial Precipitation of 1075 mm.

Meteorological, climatological, and water data were produced by Slovenian Environmental Agency (http://meteo.arso.gov.si). Climatological data were collected from climatological station Potroz – Letisce (m.a.s.l. = 2 m) and flow data by Podkastel 1 (m.a.s.l. = 9.81 m). The mean annual precipitation is approximately 944 mm and the temperature is 14°C, the maximum flow is 50.61 m^3/s, for the period 01/01/1998 to 31/12/2019.

Modeling tools

The HBV Model, developed by the Swedish Meteorological and Hydrological Institute (SMHI), is used at many countries around the world. The HBV is a conceptual hydrological runoff model which produces a simple process (hydrological forecasting, monitoring of water balance). The entire catchment is divided into sub-basins in different elevation and vegetation zones. The model runs on daily rainfall, temperature and evaporation data with high resolution runoff data (Bergström 1992). The water balance formula is:

$$P-E-Q = d/dt\,(SP + SM + UZ + LZ + lakes)$$

Where P is precipitation, E is evaporation and Q is runoff. SP is the snowpack, SM is the soil moisture, UZ and LZ are the upper and lower ground zones, while the parameter "lakes" represent the level water routing of a sub-basin.

The runoff response routine gives the total flow value (ΔQ). The model has the ability to perceive the catchment as three reservoirs (soil, upper and lower), as their flow Q_0, Q_1 and Q_2. Thus, the model enables the handle to calibrate free parameters for better runoff (Bergström 1992).

3. Results and Discussion

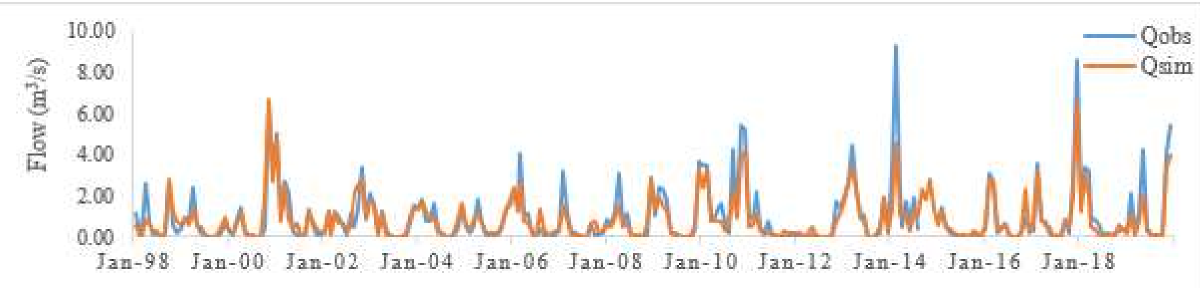

Figure 2: Evaluation of HBV-Light model processes at Dragonja River for period 01/08/1991 – 31/12/2019.

Figure 2 shows the observed (0.92 m^3/s) and simulated (0.84 m^3/s) amounts of water flow of Dragonja River for the period 1998 – 2019. The calibration process is carried out by changing manually the parameters and validating the results by examining the statistical indexes (NSE, PBIAS, RSR, d and md indexes). The HBV-Light model was calibrated by a number of parameters, which effectively control the simulation of surface water. The soil moisture routine parameters (FC, BETA and LP) show high sensitivity to NSE (0.94), RSR (0.25) and d (0.98) statistical indexes. The response routines (K0, K1 and K2) affect at PBIAS (8.93), d and md (0.91) statistical indexes, while the model coefficient is equal to 0.94 (> 0.8 - satisfactory). Further long-term hydro-meteorological monitoring studies are necessary to understand the conservation status and climate change impact especially for the Mediterranean intermittent rivers.

Acknowledgments

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References

Bergström S. (1992). The HBV model: Its structure and applications. Stockholm: Swedish Meteorological and Hydrological Institute RH No 4. Norrköping, 35 pp.

Glavan M., Pintar M., Volk M. (2013). Land use change in a 200-year period and its effect on blue and green water flow in two Slovenia Mediterranean catchments – lessons for the future. Hydrological Processes 27, 3964–3980.

Gutierrez – Jurado, K.Y., Partington, D., Shanafield, M. (2021). Taking theory to the field: streamflow generation mechanisms in an intermittent, Mediterranean catchment. Hydrology and Earth System Sciences 25, 4299–4317.

Reynolds J. E., Halldin S., Xu C.U., Seibert J., Kauffeldt A. (2017). Sub-daily runoff predictions using parameters calibrated on the basis of data with a daily temporal resolution. Journal of Hydrology 550, 399 – 411.

Šraj M., Bezak N., Rusjan S., & Mikoš M. (2016). Review of Hydrological Studies contributing to the advancement of hydrological sciences in Slovenia. Acta hydrotechnica 29/50.

CAROTENOID PROFILE OF A HYPERSALINE *Dunaliella* sp. STRAIN CULTIVATED IN DIFFERENT CONDITIONS

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Abstract
The hypersaline representatives of the genus *Dunaliella* are of great interest due to their carotenoid content and their ability to accumulate carotenoids when cultivated under certain conditions. In the present study, a carotenogenic strain of the species *Dunalliela salina* isolated from Greek saltworks were cultivated under different conditions (senescence and nitrogen deprivation) and the carotenoid profiles produced showed that the strain has a significant capacity for the production of β-carotene high in 9-cis-β-carotene when cultivated under nitrogen deprivation conditions.
Key words: *Dunaliella, Local Strains, Microalgae Cultivation, Carotenoids, β-Carotene, Nitrogen Deprivation*
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1. Introduction

The genus *Dunaliella* is common in aquatic environments and includes some species of great interest due to their ability to thrive in hypersaline waters. Strains belonging to this genus can accumulate great amounts of beta-carotene along with a variety of other carotenoids (Henríquez *et al*. 2016). This ability along with some exceptional physiological traits has led to its cultivation for a variety of industries such as human nutrition, aquaculture and cosmetics (Spiegler *et al*. 2012, Zhang *et al*. 2016). Herein, we present the investigation of the effect of different cultivation conditions on the carotenoid profile of a microalgal strains (AthU-Al Dun_30) isolated from Greek saltworks.

2. Materials and Methods

The studied strain is preserved it the AthU-Al (Athens University Algae) culture collection of the National and Kapodistrian University of Athens (NKUA). The strain, *Dunaliella salina* AthU-Al Dun_30, was isolated from the salt pans of Megalon Emvolon Thessaloniki and was cultivated in artificial salt water of 60‰ salinity. The cultures were preserved in a culture chamber ensuring steady conditions (temperature: 25 ºC; relative air humidity: ~35%; light intensity: 100 μmoles photons m^{-2} s^{-1}; light period: 12/12 h, light/darkness). For this set of experiments the cultures were scaled-up to a final volume of 5L in aerated Erlenmeyer flasks in quadruplets, using two different cultivation media. The cultivation media used were Walne's medium (CCAP 2002) and a modified version of this medium with the same composition except for the complete lack of a nitrogen source ($NaNO_3$). For the first condition (senescence) the cultures were harvested when they reached the stationary phase and demonstrated an orange color due to the accumulation of carotenoids. For the second condition (nitrogen deprivation), the cultures were harvested when they demonstrated an orange color due to the accumulation of carotenoids. The cultures were then centrifuged at 5000 rpm for 10 minutes and the wet biomass was harvested.

Identification and quantification of carotenoids was based on their chromatographic behavior on HPLC. Before HPLC analysis, the freeze dried biomass samples were extracted using acetone in a solvent to solid ratio of 10 mL/g dry biomass and were left overnight at 4ºC. The solutions were filtered before injection using syringe filters with pore size 0.45 μm and filter size 25 mm. HPLC analysis was performed with an HPLC Shimandzu HP 1100 Series (USA). Carotenoid compounds were analyzed with an YMC C30 (Germany) analytical column (5 m, 250 × 4.6 mm I.D.). Detection of carotenoids was accomplished using a diode array system at a wavelength of 458 nm. Carotenoids were identified by comparison to external standard (β-carotene standard) and quantified by use of a standard curve.

3. Results and Discussion

The carotenoid profiles of the strain Dun_30 are shown in Figure 1 and Figure 2 for senescence and nitrogen deprivation respectively. In both cases the carotenoids content is high. In the case of senescence the predominant carotenoid is the β-carotene with a concentration of 4.25 mg/ml and the presence of 9-cis-β-carotene is significant in total carotenoid content of the biomass. Additionally, very low amounts of 9-cis-astaxanthin and loroxanthin were detected in the amount of 0.08 mg/ml and 0.72 mg/ml, respectively. In the human organism, carotenoids are part of the antioxidant defense system. They interact synergistically with other antioxidants; mixtures of carotenoids are more effective than single compounds (Wilhelm & Helmut 2003). Therefore, the presence of other type of carotenoids apart from β-carotene in *Dunaliella salina* cultivated until senescence is important for its application in food, feed, cosmetic and food supplement sector. In the case of nitrogen deprivation, the predominant carotenoid is the β- carotene with a concentration of 6.5 mg/ml, while the presence of 9-cis-β-carotene is also significant (approximately 4mg/ml).

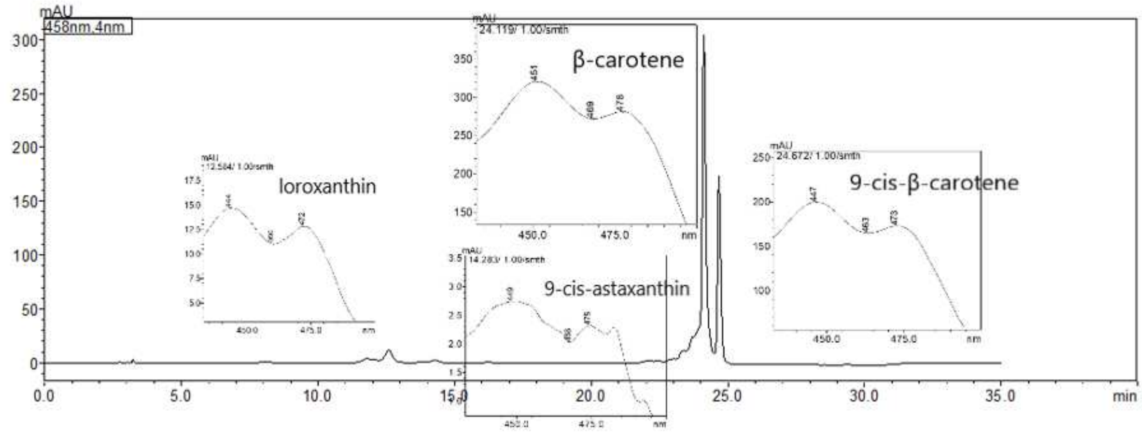

Figure 1. Carotenoids profile of AthU-Al Dun_30 cultivated under nitrogen senescence conditions.

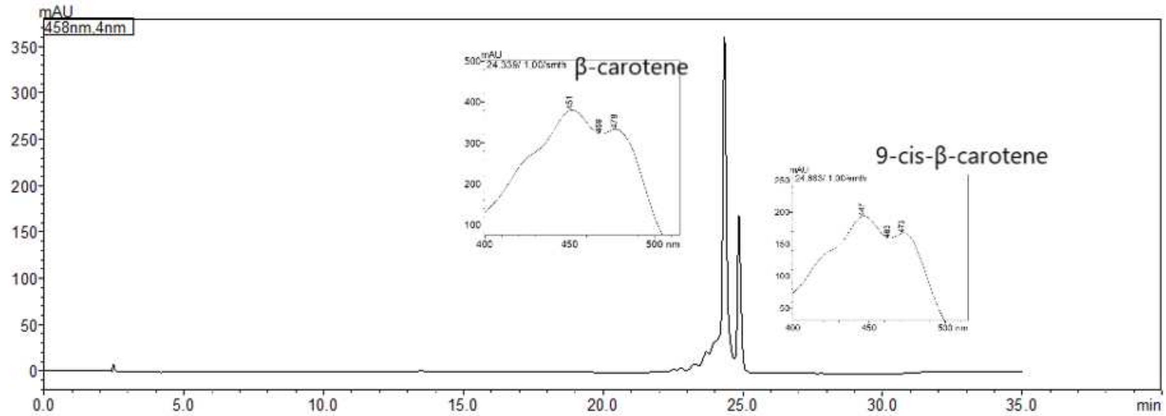

Figure 2. Carotenoids profile of AthU-Al Dun_30 cultivated in nitrogen deprivation conditions.

Acknowledgements

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References

CCAP (2002). Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory. https://www ccap.ac.uk/wp-content/uploads/MR_Walnes.pdf (accessed: 22-07-21).

Henríquez V., Escobar C., Galarza J., Gimpel J. (2016). Carotenoids in Microalgae. In: *Carotenoids in Nature* edited by Stange C. Springer, Subcell Biochem, 2016; 79: 219-37. doi: 10.1007/978-3-319-39126-7_8.

Spiegler E., Kim Y.K., Wassef L, Shete V., Quadro L. (2012). Maternal–fetal transfer and metabolism of vitamin A and its precursor β-carotene in the developing tissues.Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1821(1), 88–98. doi: 10.1016/j.bbalip.2011.05.003.

Wilhelm S., Helmut S. (2003). Antioxidant activity of carotenoids. Molecular Aspects of Medicine, 24: 6, 345-351, https://doi.org/10.1016/S0098-2997(03)00030-X.

Zhang Z.Q., Cao W.T., Liu J., Cao Y, Su Y.X., Chen Y.M. (2016). Greater serum carotenoid concentration associated with higher bone mineral density in Chinese adults. Osteoporosis International, 27:1593-1601.

FISHERIES TYPOLOGY OF *PONTASTACUS LEPTODACTYLUS* (DANUBE CRAYFISH (ESCHSCHOLTZ, 1823)) IN THE DAM LAKE OF POLYFYTOS (WESTERN MACEDONIA)

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Abstract
The paper focuses on the professional fishery in the Dam lake of Polyfytos in Western Macedonia in the late of 2010s. Surveys were conducted based on individual interviews conducted to professional fishers. Results revealed that the extended fishing on the Danube crayfish was started after 2012, their catches peaked in summer, and most of the quantities leaded to European fish markets. However, the most common problems encountered by the interviewed fishers were the intense fishery from the unlicensed fishers that illegally used crayfish traps with fishing vessels within the Dam lake.
Keywords: *Fishing tactics, Fisher's knowledge, Freshwater Fishery, Greek lakes*
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1. Introduction

During the mid-2010's there has been an increasing fisheries intensity on the Danube crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), the spread of which has been recorded as a fact for most freshwater systems in Northern Greece (Perdikaris *et al*. 2017). Fishers' knowledge, which represents a lifetime of accumulated ecological observations, is increasingly being used to fulfil data gaps as a source of complementary empirical data in marine and freshwater environments to preserve local knowledge of abundant and/or ecologically important species (for review see Hind 2015). The purpose of this work is to: (a) clarify the intensity of fishing for the Danube crayfish in the lake, (b) describe through a typology the professional fishing activity, and (c) capture the views of fishermen on its current situation and the problems they face in the exercise of fishing activity.

2. Material and Methods

On-the-spot interview survey was conducted during summer-autumn 2018 targeting on the licensed professional fishers operating in the dam lake. Contact details of the fishers were provided by the local fishery office, which is responsible for issuing fishing licenses and monitoring the fishing activity in the lake. The interviews were carried out privately (as one-to-one sessions, to prevent influences by others), in Greek by one person, ensuring that questions were presented identically to minimize sampling bias, and they took place around the fishers' mooring/landing sites and frequently on board of their own vessel.

Fishers were asked about issues related to: (a) fishing operation, (b) problems faced on operational and marketing issues, and (c) demographic features. It should be noted that questionnaires were designed to be compatible with those used in socio-economic surveys conducted in other Greek lakes (Trichonis and Volvi lakes: Roussi-Dimitriou *et al*. 1997, Moutopoulos *et al*. 2020, respectively) to facilitate comparisons.

3. Results and Discussion

A total of 12 professional fishers were participated to the survey, with a mean age of 44 years old, with half of the participants pertain the basic education and being married with up to three children. Fully dependent fishers (100% of their annual income) were 2/3 of the participants (66%) and all of them fished all year round, apart from the 40-days spring fishery banning, during when a 40% of them fished up to 30 days. Fishers started fishing the species between 2012-2015, whereas according to their statements, Danube crayfish was already existed in the Dam lake, but to lower quantities. The main fishing gear targeting this species was traps, whereas a significant percentage of fishers (60%) used the nets as the main fishing tool, targeting crayfish, Common carp (*Cyprinus caprio*), Wels catfish (*Silurus glanis*) and the Roach (*Rutilus rutilus*), and to a lesser extent the longlines targeting the Northern pike (*Esox lucius*), and European perch (*Perca fluviatilis*). Based on the fishers' statements the seasonal pattern of the Danube crayfish fishery catches per each fisher were the following: summer (4.5 t) > autumn (3.7 t) > winter (3.0 t) > spring (0.3 t). Other commercially important fish species caught were the Common carp and Wels catfish and to a lesser extend the Northern pike, and Roach.

The use of the information derived from fishers' knowledge sheds light on the role of a local freshwater fishery in a data-deficient system and leads to better evaluation of the management policies. Enhancing the knowledge of the fishery tactics of the professional fishing in Polyphytos Dam Lake, with emphasis on the high commercial value species of the Danube crayfish *P. leptodactylus*, is a key objective to approach the knowledge of the fishing exploitation of the species. An important parameter that also emerges from the present research is the fact that almost all the catches of professionals were sold at the wholesale trade (85%), whereas a small percentage (14%) was directed to the local fish markets and privately owned shops (1%). Fishers stated the positive impact that the local fisher's association have on the commercialization of the Danube crayfish, which is mostly (80% of the total annual catches) leaded to European fish markets (France), and the achievement of satisfactory economic price (around 4 euros). Even though the species was already present in the lake, systematic fishing and commercial interest on crayfish started only after 2012, when its quantities were increasing. This might be explained by the fact that all invasive species have a lag phase after release and establishment, which are determined by the invasive traits of the species (e.g., size, fecundity, high population density). For the study species an additional cause of its expansion might be the moderate levels of resistance to crayfish plague, probably due to slow evolutionary adaptation to the pathogen expressed by increased resistance of some host specimens or infection by less virulent strain of *A. astaci* (see Perdikaris & Georgiadis 2017).

Comparing the species composition of the professional fishery between a decade (2007-2018), there has been a shift of interest from the Prussian carp (*Carassius gibelio* (Bloch, 1782)) and carp, which contributed up to 80% of the fisheries catches in late 2000s and early 2010s (Papistas et al. 2007), to the Danube crayfish. This is due to the vast reduction of the commercial interest recorded for the Prussian carp mostly from other Balkan countries, which was a reality in early 2010s in the Northern Greek lakes (Moutopoulos *et al*. 2020), and the subsequently peak of interest for the Danube crayfish from European fish markets. Fishing exploitation in the study lake can balance the high unemployment rate occurred in the wider area (HELSTAT 2020) and is enhancing the importance of the lake fishery to the local economy. This is observed from the shift of the local fishery to crayfish fishing, having left to a lesser extent the focus on other traditional important species of the lake.

The actual impact of fisheries is difficult to be evaluated due to misreporting estimates and serious limitations in the official landing statistics. Considering the complete lack of knowledge regarding the catch of the recreational and illegal fisheries conducted in the lake especially in shallow waters when fish spawn, it can be assumed that the actual catches are much higher and thus the officially reported data could be very misleading (Moutopoulos *et al*. 2020). In freshwater systems, fishery landing data were reported based on a non-mandatory declaration of the professional fishers every two years, when they renew their professional license in the local fisheries office. Thus, fishers probably declared lower quantities in order to pay lower taxes. Nevertheless, due to the anonymity of the interviews conducted in the present study, fishers might provide more accurate catch estimates. Recreational fishery in Greek lakes is prohibited by vessels all year round, whereas the shore-based ones are allowed (except in the month of May). A serious problem also encountered by the interviewed fishers were the intense illegal fishery from the unlicensed fishers that illegally used crayfish traps with fishing vessel within the Dam lake. The crayfish fishery has currently collapsed in the lake due to overharvesting (legal and illegal) and accordingly, a 2-year harvesting ban has been established. This is a prominent example of the absence of scientifically-based management of such valuable source.

References

HELSTAT (2020). Labor Force Survey - Population, Education, Employment Status (1981 - 2020). www.statistics.gr

Hind E.J. (2015). A review of the past, the present, and the future of fishers' knowledge research: A challenge to established fisheries science. ICES Journal of Marine Science 72 (2), 341–358.

Moutopoulos D.K., Petriki O., Ramfos, A., Stoumboudi M.Th., Stergiou K.I., Bobori D.C. (2020). Portraying fisheries and ecological status of a Mediterranean lake. Acta Ichthyological et Piscatoria, 50, 71-83.

Papistas A., Kokkonis A., Papadopoulos E., Moutopoulos D.K. (2007). Fishery in dam lake of Polyfytos (NW Hellas; Kozani Prefecture). In: Proceedings of 12th Panhellenic Conference of Ichthyologists 13, 281–284. [In Greek.]

Perdikaris C., Georgiadis C. (2017). Co-occurrence of narrow-clawed crayfish (*Astacus leptodactylus* sensu lato) and noble crayfish (*Astacus astacus* L.) in the southwestern Balkans: The case of Lake Pamvotida (NW Greece). North-Western Journal of Zoology, 13(1).

Perdikaris C., Konstantinidis E., Georgiadis C., Kouba A. (2017). Freshwater crayfish distribution update and maps for Greece: Combining literature and citizen-science data. Knowledge and Management of Aquatic Ecosystems 418, 51.

Roussi-Dimitriou A., Dimitriou E., Kaspiris P. (1997). Fisherman's social character, fishing fleet structure and fishery production of lakes Lysimachia-Trichonida. In: Proceedings of 8th Panhellenic Conference of Ichthyologists, 25–28 September 1997, Thessaloniki, Greece. [In Greek.]

SEX RATIO AND ALLOMETRIC RELATIONSHIPS OF THE WATER FROG (*Pelophylax epeiroticus*) IN LAKE PAMVOTIDA (NORTHWEST GREECE)

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Abstract
A population of the water frog *Pelophylax epeiroticus* (Schneider, Sofianidou and Kyriakopoulou-Sklavounou, 1984) in the lake Pamvotida (Ioannina) was assessed for potential differences in sex ratio and allometric relationships. No significant difference in any of the morphometric measurements between sexes was indicated. Significantly lower abundance of females than expected was indicated. Significance of allometric relationships for the overall population and each sex was assessed. Significant difference in the allometric relationships among sexes was indicated between body length, femur length and between body length and snout eye distance.
Keywords: *Pelophylax epeiroticus*, Water frog, Allometric relationships, Sex Ratio, Lake Pamvotida
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1. Introduction

Pelophylax epeiroticus is an endemic water frog species of the Ionian zone, distributed mainly in the western part of Greece (Sofianidou & Schneider 1989). This frog species, according to Kyriakopoulou-Sklavounou *et al*. (1991), lives sympatrically and hybridizes to a small degree with *P. kurtmuelleri* (Gayda, 1940), which can be found from sea level up to 1000 m elevation (Valakos *et al*. 2008).

The aim of this study was to assess potential differences in the sex ratio, morphometric characteristics and allometric relationships in a population of water frog *Pelophylax* sp. in the lake Pamvotida (Ioannina).

2. Materials and Methods

The water frog *P. epeiroticus* was sampled from the lake Pamvotida on a monthly basis between March and May of 2012. Morphometric characteristics were recorded for all 85 individuals caught (31 females and 54 males) (Figure 1).

Data for statistical analysis were evaluated for normal distribution by employing the Shapiro-Wilk test for normality and homogeneity of variance by employing Levene's test. Student's T-Test was employed for comparisons since data were approximately normally distributed and homogenous. Chi-Square Goodness-of-Fit and Chi-Square Test for Association were employed for the comparison of categorical values. Statistical analysis was performed with Jamovi Software (2.0.0) (The Jamovi Project, 2021). Non-linear regression was performed with Minitab 20 software (Minitab, Pennsylvania, USA).

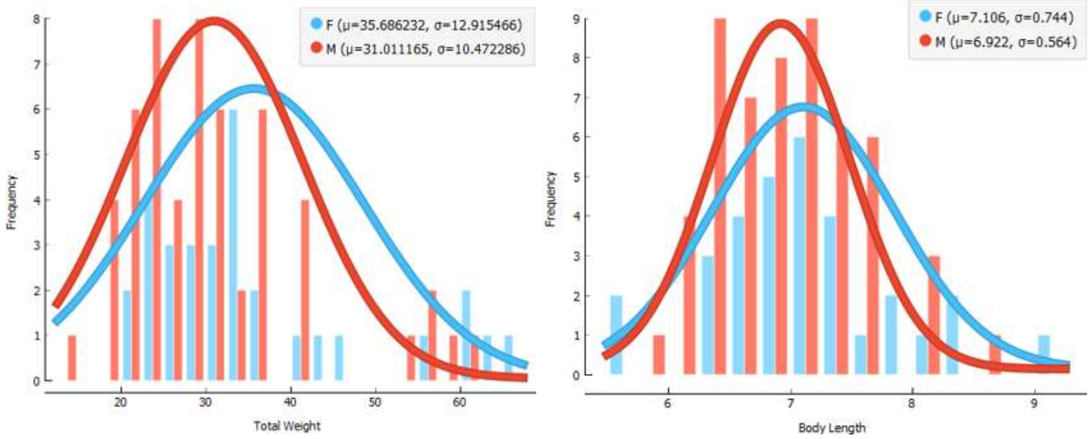

Figure 1. Total weight and Body length distribution for each sex.

3. Results

T-test indicated no significant difference in any of the morphometric measurements between sexes ($p>0.05$). Chi-Square Goodness-of-Fit Test (Table 1) indicated significant difference between observed and expected male and female abundance, with significantly lower abundance of females and higher in males than expected. Chi-Square Test for Association indicated no significant difference in the sex ratio between different months (p>0.05). Significant allometric relationship was indicated between body length, femur length, tibia length and snout eye distance. Significant difference in the allometric relationships among sexes was indicated between body length, femur length and between body length and snout eye distance (Table 2). No significant allometric relationship was indicated between total weight and body length as well as between maximum head length and maximum head width.

Table 1. Chi-Square Goodness-of-Fit Test for sex ratio.

Sex		Number of individuals	Proportion	χ^2 Goodness of Fit		
Female	Observed	31.0	0.36	χ^2	df	p
	Expected	42.5	0.50	6.22	1	<0.05
Male	Observed	54.0	0.64			
	Expected	42.5	0.50			

Table 2. Allometric equations between body length (BL), total weight (TW), femur length (FL), tibia length (TL), Maximum head length (MHL), maximum head width (MHW) and snout eye distance (SED) of Rana sp. population. N: number of individuals, R^2: coefficient of determination, t-test: statistical significance of the allometric relationship, allometry: allometric relationship between the two variables, slopes (b): statistical comparison between the slopes of the equations, intercepts (a): statistical comparison between the intercepts of the equations, significance level (ns: non-significant, *: p < 0.05, **: p < 0.01, ***P<0.001).

Morphometric Relationships						Equation Comparison	
Sampling Site	**Equation**	**N**	**R^2**	**t-test**	**Allometry**	**Slopes**	**Intercepts**
Total population	$TW = 0.0793345 \times BL^{3.08249}$	85	84.4%	ns	Positive		
Females	$TW = 0.147744 \times BL^{2.78335}$	31	85.7%	ns	Negative	ns	ns
Males	$TW = 0.0446303 \times BL^{3.36766}$	54	85.6%	ns	Positive		
Total population	$BL = 2.62897 \times FL^{0.723272}$	85	93.3%	***	Negative		
Females	$BL = 2.2427 \times FL^{0.839318}$	31	96.3%	ns	Negative	***	***
Males	$BL = 2.9084 \times FL^{0.647612}$	54	95.6%	***	Negative		
Total population	$BL = 2.90683 \times TL^{0.696432}$	85	92.8%	***	Negative		
Females	$BL = 3.13275 \times TL^{0.632722}$	31	94.7%	***	Negative	ns	ns
Males	$BL = 2.70909 \times TL^{0.758638}$	54	96.5%	***	Negative		
Total population	$BL = 6.14082 \times SED^{0.70285}$	85	87.8%	**	Negative		
Females	$BL = 5.81649 \times SED^{0.95948}$	31	92.9%	ns	Negative	***	ns
Males	$BL = 6.25962 \times SED^{0.592945}$	54	92.5%	***	Negative		
Total population	$MHL = 1.24333 \times MHW^{0.958627}$	85	94.8%	ns	Negative		
Females	$MHL = 1.41502 \times MHW^{0.829075}$	31	96.8%	ns	Negative	ns	ns
Total population	$MHL = 1.24333 \times MHW^{0.958627}$	85	94.8%	ns	Negative		

4. Discussion

Significantly lower abundance of females than expected could be potentially attributed to sampling bias. According to Shirose *et al*. (1993) differential mortality between sexes has been previously reported in frogs, where males exhibit more conspicuous behavior than females and can be easily detected by predators. Significant morphological variation reported across *Pelophylax* species, and particularly between mainland *P. epeiroticus*, *P. ridibundus* and *P. kurtmuelleri* species, does not always allow a reliable morphological discrimination of the different species in their overlapping regions (Sagonas *et al*. 2020).

References

Kyriakopoulou-Sklavounou P., Sjögren P., Jansson H. (1991). Discordant genetic and morphological differentiation between three *Rana ridibunda* populations from N Greece. Hereditas, 1991, 115.1, 51-61.

Sagonas K., Karameta E., Kotsakiozi P., & Poulakakis, N. (2020). Cross-species testing of nuclear markers in *Pelophylax* water frogs in Greece and examination of their power to detect genetic admixture. Amphibia Reptilia, 41(2) 253–259. https://doi.org/10.1163/15685381-20191229

Shirose L.J., Brooks R. J., Barta J.R., Desser S.S. (1993). Intersexual differences in growth, mortality and size at maturity in bullfrogs in central Ontario. Canadian Journal of Zoology 71, 2363–2369.

Sofianidou T.S., Schneider H. (1989). Distribution range of the epeirus frog *Rana epeirotica* (Amphibia, Anura) and the composition of the water frog populations in western Greece. Zool. Anz. 223, 13-25.

The Jamovi Project (2021). Jamovi (Version 1.6) [Computer Software]. Retrieved from https://www.jamovi.org

Valakos E.D., Pafilis P., Sotiropoulos K., Lymberakis P., Maragou P., Foufopoulos J. (2008). The Amphibians and Reptiles of Greece. Frankfurt am Main: Edition Chimaira. 463pp.

VISUALIZATION TYPES OF VARIOUS AQUATIC PHASES OF INTERMITTENT STREAMS

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Abstract
Flow data visualizations are useful visuals that give direct information that inspire, inform and enable people to address issues concerning water. The use of visualizations in order to describe a situation concerning runoff, flooding, or drought , needs water data and measurements shown in compelling and interactive ways when static images or written narrative can't effectively communicate the interconnectivity and complexity of water data issues or water management problems. As a result, a good quality of visualization is the key for communicating the issues concerning the aquatic phases in intermittent streams.
Key words: *Rivers, Visualization, Mapping, Watershed Management, Flow Data.*
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1. Introduction

Intermittent rivers are a very important category as it is estimated to make up 50% of the global river network (Datry 2014). They include many habitats, host a wide range of biodiversity (species of fauna and flora), include many different transitional ecosystems, and serve many anthropogenic activities. Visualizing the aquatic states of intermittent rivers provides an overview of the conditions prevailing along these rivers in relation to biotic, biochemical, and physicochemical factors that scientists need in the process of watershed management. Temporary streams interchange between aquatic states as a result of regular changes in catchment wetness, and in coordinated reaction to precipitation and snowmelt occasions. According to Gallart *et. al* (2016) there are six (6) aquatic states from the point of view of the aquatic organisms. These states are the hyperheic (flood conditions), eurheic (riffles dominant with pools present), oligorheic (scarce riffle areas connecting pools), arheic (isolated pools) and hyporheic or edaphic (dry bed conditions). The river regime is characterized by the temporal pattern of the relative frequencies of the diverse aquatic states, allowing the probability of occurrence of every aquatic state in every month to be determined (Gallart *et al*. 2016). Consistency and exactness of perceptions is guaranteed by a defined protocol followed by trained hydrologists.

Data visualization has an important role in every research concerning the intermittent flow and ephemeral streams (IRES) analysis and intermittent flow. The aim of the current study is to review the visualization types of IRES and suggest types of representations that can lead to a better understanding of patterns and trends, making the process of data analysis simpler and easier. Faster and clear understanding of data in the first glance and a creation of visually presentable data, in order to comprehend the information of each study.

2. Materials and Methods

The visualization of IRES is of critical significance in creating three-dimensional sedimentary architecture models of sheet deposits in distal fluvial systems (Sefton 2019). Depending on the visualization needs and data availability, most researchers use Line charts, Polygon charts, Scatter plots, Heat maps and Time-lapse videos (Figure 1). Simple heat maps visualize and measure the hydrological state along the river on each perception date by relegating the state watched at each site. The recurrence and consistency of perceptions were uncovered, and periods of lost data demonstrated. Visualization of aquatic states is a difficult task, because this phenomenon needs constant monitoring. Until now most observations are depicted through photos or frequency graphs. A new project has emerged called LIFE TRivers studies the hydrology and ecology of temporary rivers and aims at creating new tools to improve their management according to the objectives of the EU Water Framework. Last but not least , visual representation over time is very crucial in describing flood events in aquatic and hydrological states. Time-lapse videos are tools that can help in the visualization flow in rivers (Stamataki 2021).

In most of the studies that concern intermittent rivers, the data collection is done by field research or satellite and UAV imagery. After collecting these raw data, the process of visualizing them is done as shown by Figure 1. At this point, a decision must be made as to which visualization method should be used for the purposes of a research. Visualization tools such as GIS and programming tools will play a key role.

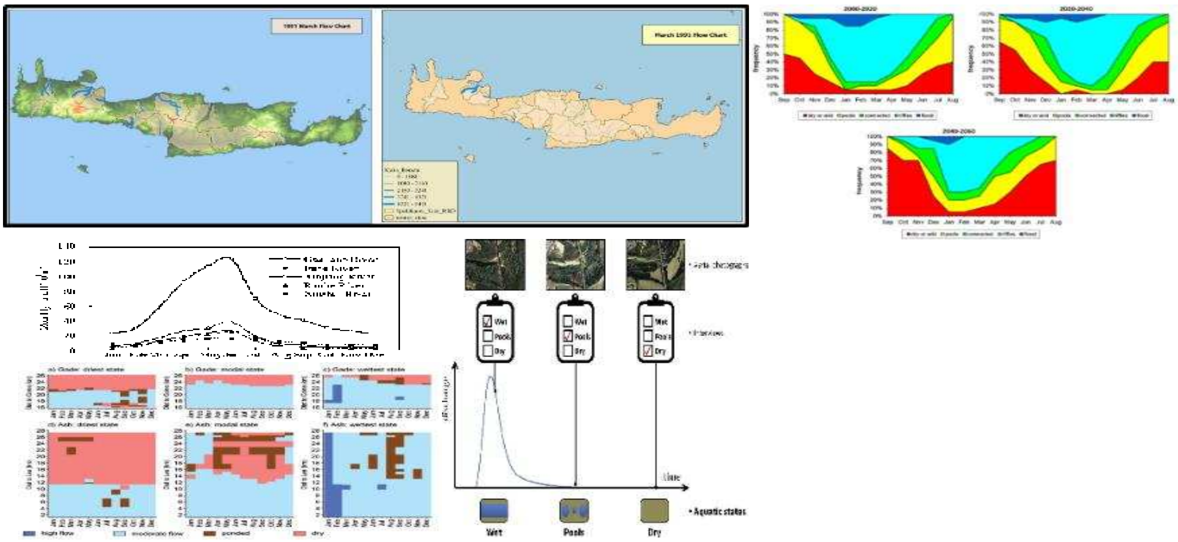

Figure 1. Visualization methods of intermittent flow streams

3. Results and Discussion

Seeing intermittent rivers as moving lotic, lentic, and terrestrial environment mosaics puts the onus on scientists to coordinate and combine concepts and strategies from lotic, lentic, and earthbound environments to oversee these environments viably (Bonada 2020). Data and strategies are required for hydrological and environmental ponders of IRES that capture their spatiotemporal inconstancy with a more detailed classification of aquatic state than can be inferred from gauging station information or wet/dry mapping alone. It is imperative to include the groundwater component within the basin model, as something else it is incomprehensible to estimate the response of the groundwater reflections for water systems to the change in river flow. In conclusion, being able to visualize and analyze aquatic states over a period of time is an essential step to understand hydrological patterns. Finding and providing the essential tools for visualization is very crucial for water managers (Querner 2016). Data existence is the core of each study. Having complete datasets, time series, good quality imagery and historical records is the best combination of tools to create quality imaging tools. Integrated Water Management in a period that climate change is happening is and should be a priority for researchers.

6. References

Bonada N, Cañedo-Argüelles M., Gallart F., Schiller D., Fortuño P., Latron J., Llorens P., Múrria C., Soria M., Vinyoles D., Cid N. (2020). Conservation and Management of Isolated Pools in Temporary Rivers. Water, 12–24.

Datry T., Larned T., Tockner K. (2014). Intermittent Rivers: A Challenge for Freshwater Ecology. BioScience 64, 229–235.

Gallart F., Llorens P., Latron J., Cid N., Rieradevall M., Prat N. (2016). Validating Alternative Methodologies to Estimate the Regime of Temporary Rivers When Flow Data Are Unavailable. Science of the Total Environment 565, 1001–1010.

Querner E.P., Froebrich J., Gallart F., Cazemier M.M., Tzoraki O. (2016). Simulating Stream flow Variability and Aquatic States in Temporary Streams Using a Coupled Groundwater-Surface Water Model. Hydrological Sciences Journal 61, 146–161.

Sefton C.E., Parry S., England J., Angell G. (2019). Visualising and Quantifying the Variability of Hydrological State in Intermittent Rivers. Fundamental & Applied Limnology/Archiv Für Hydrobiologie 193, 21-38.

Stamataki M.D., Tzoraki O., Sauquet E. (2021). Time-Lapse Graphical Representation Methods for Mapping of Intermittent Rivers and Ephemeral Streams (IRES). European Journal of Geography 12, 51-67.

PROTOCOLS FOR THE ENVIRONMENTAL MONITORING OF THE COASTAL AND TRANSITIONAL RIVER ECOSYSTEMS

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Abstract
Monitoring protocols are the guidelines which coastal and transitional river research can be based on, in order for the results to be compared and evaluated. But in most cases protocols differ according to the specific challenges of each scientific problem, and in that direction, remote sensing technology is used to acquire required imagery (UAV's, Satellite data). Recently, in the study of intermittent river health, protocols describing hydro-ecological conditions incorporate remote sensing technology. Five protocols are reviewed according to data acquisition they provide and the monitoring method, showing the advantages and disadvantages of each protocol.
Keywords: *Protocol, Monitoring, River Ecosystem, Coastal Ecosystem, Transitional Waters*
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1. Introduction

Transitional waters are bodies of surface water in the vicinity of river mouths that are partly saline in character because of their proximity to coastal waters, but which are substantially influenced by freshwater flows. Intermittent streams are defined by a periodic flow cessation and may experience a partial or complete loss of surface water. Transitions between flowing, pool, and dry phases result in greater temporal variation in environmental conditions of transitional waters (Stubbington *et al.* 2017). The conditions that transitional waters may create, can and should be monitored because the results can lead to crucial management decisions for the environment that they belong to. A scientist can observe conditions like salinity levels, depth measurement, water flow, subsoil measurement, water velocity, fauna and flora, and the natural habitat (Hentz *et al.* 2018). Nowadays, remote sensing technology has been the most efficient method to collect data in river systems. The aim of this work is to compare and evaluate monitoring protocols, which make use of remote sensing technology.

2. Materials and Methods

Monitoring is a process that can be done via a protocol system. A protocol is a predefined procedural method in the design and implementation of an experiment or research. Protocols are written whenever it is desirable to standardize a monitoring method to ensure successful replication of results by others in the same laboratory or by other laboratories. Stream assessment protocols often, but not always, include both biological and physical (geomorphological and habitat) variables. Creating a protocol for monitoring transitional waters is essential for comparing results and creating a database that can be comprehended by all scientists.

In most protocols the ways of observation and monitoring of a river and transitional ecosystem are done following the methods field research, Satellite imagery and UAV's (Figure 1). Depending on the research, one or more of these observation methods are selected. This review will present 5 random protocols created to serve surveys on different rivers around the world.

Figure 1. Monitoring types: a) Field research, b) Satellite, c) UAV's

3. Results

The Table 1 shows the comparison of five protocols, in which different monitoring methods have been used. The variables used in the research of each protocol are also analyzed (Casado *et al.* 2016, Hentz *et al.* 2018, Minnesota Pollution Control Agency 2014, Monteiro *et al.* 2021, Woodget *et al.* 2017). The study site of these protocols is either the whole river length or part of it (e.g. Transitional Ecosystems).

Table 1. Checklist of five different protocols depending on research variables and monitoring methods.

PROTOCOLS	Variables: Substrate	Salinity/ chemical analysis	Morphology	Water velocity	Aquatic state	Slope Angle	Vegetation	Bathymetry	MONITORING METHODS (FIELD RESEARCH/ DRONES/ SATELITE)
MSHA protocol (2017)	✓	✓	✓	✓	✓	✗	✓	✓	FIELD RESEARCH
Drones and Digital Photogrammetry (2017)	✗	✗	✓	✓	✗	✓	✓	✓	DRONES + SATELITE
Novel approach to enhance coastal habitat and biotope mapping with drone aerial imagery analysis (2021)	✓	✗	✓	✗	✗	✗	✓	✓	DRONES
Quantifying the Effect of Aerial Imagery Resolution in Automated Hydro-morphological River Characterization (2016)	✓	✗	✓	✓	✗	✗	✓	✓	DRONES + FIELD RESEARCH
Accuracy and Optimal Altitude for Physical Habitat Assessment (PHA) of Stream Environments Using Unmanned Aerial Vehicles (UAV)	✓	✗	✓	✗	✗	✗	✓	✓	DRONES + FIELD RESEARCH

4. Discussion

Stream monitoring is very important and the creation of a single protocol for the study of rivers is more necessary than ever (Koutalakis 2019). It is obvious that most of these studies use drones. In contrast, the aquatic state (existence of water, lakes, and dry riverbed) is important and has not been studied so much yet (especially via drone). Some variables are handled with the same monitoring tool, while others differ depending on the choice of the researcher. In addition, in most of the studied protocols, there was not much focus on research on transitional river ecosystems. There is a need in the development of new protocols which make use of remote sensing technology and may collect a higher variety of environmental data.

References

Casado M.R., Gonzalez R.B., Wright R., Bellamy P. (2016). Quantifying the Effect of Aerial Imagery Resolution in Automated Hydro-morphological River Characterization. Remote Sensing 8, 1-19.

Hentz A.M.K., Kinder P.J., Hubbart J.A., Elliott K. (2018). Accuracy and Optimal Altitude for Physical Habitat Assessment (PHA) of Stream Environments Using Unmanned Aerial Vehicles (UAV). Drones 2, 1–15.

Koutalakis P., Tzoraki O., Zaimes G. (2019). UAV's for Hydrologic Scopes: Application of a Low-Cost UAV to Estimate Surface Water Velocity by Using Three Different Image-Based Methods. Drones 3, 1–15.

Minnesota Pollution Control Agency (2014). MPCA Stream Habitat Assessment (MSHA) Protocol for Stream Monitoring Sites., 1-8

Monteiro J.G., Jiménez J.L., Gizzi F., Přikryl P., Lefcheck J.S., Santos R.S., Canning-Clode J. (2021). Novel Approach to Enhance Coastal Habitat and Biotope Mapping with Drone Aerial Imagery Analysis. Scientific Reports 11, 1–13.

Stubbington R., England J., Wood P.J., Sefton C.E.M. (2017). Temporary Streams in Temperate Zones: Recognizing, Monitoring and Restoring Transitional Aquatic-terrestrial Ecosystems. WIREs Water 4, 1–17.

Woodget A.S., Austrums R., Maddock I.P., Habit E. (2017), Drones and digital photogrammetry: from classifications to continuums for monitoring river habitat and hydro-morphology. WIREs Water 4, 1-20.

FATTY ACIDS QUANTIFICATION AND NUTRITIONAL QUALITY EVALUATION OF FRESHWATER FISH OF LAKE VOLVI

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Abstract: The aim of this work was to study fatty acids in muscle fillet of four fish species (common bream, macedonian shad, goldfish, common carp) sampled in Lake Volvi and to evaluate their nutritional quality. PUFA/SFA values ranged from 0.4 (macedonian shad) to 0.8 (common carp) and ω-6/ω-3 values from 0.3 (macedonian shad) to 2.8 (carp). The higher amount of Σ EPA+DHA (mg EPA+DHA/100 g raw fillet) was found in macedonian shad (215.8), followed by common bream (124.8), goldfish (100.2) then common carp (53.2). All fish species may contribute to prevent cardiovascular diseases but the macedonian shad and common bream were the most valuable sources of DHA.
Keywords: Fatty Acids, Nutritional Quality, Common Bream, Macedonian Shad, Goldfish, Common Carp
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1. Introduction

According to dietary guidelines fish should be consumed frequently because of their high content on ω-3 polyunsaturated (PUFA), eicosapentaneoic (EPA) and docosahexaenoic acid (DHA). In Greece, this dietary recommendation lacks detailed information regarding fatty acid (FA) profiles and quantity of commonly consumed fish, so health professionals do not know which fish species provide the highest content of beneficial fatty acids (FA) and therefore cannot provide the consumers with accurate recommendations of intake. This study aims to contribute to improving our knowledge of the nutritional quality of freshwater fish species of the Volvi Lake in Central Macedonia, Greece.

2. Materials and Methods

Adults of carp bream (*Abramis brama)*, macedonian shad (*Alosa macedonica),* goldfish (*Carrasius auratus*) and common carp (*Cyprinus carpio)* being caught during autumn (October 2020) from Lake Volvi were used to get a muscle sample in terms of a square trip behind the head. Lipids were extracted and converted to their corresponding fatty acid methyl esters (FAMEs) directly from freeze-dried samples under acidic conditions. The analysis of fatty acids (FA) was based on gas chromatography by using a Chromatec-Crystal 9000 (Russia) chromatograph coupled with a flame ionization detector. FA were first resolved on a 0.2 μm HP-88 column (100 m × 0.25 mm i.d., Agilent), then characterized according to their relative retention time and finally quantified. A 37-component standard mixture (Supelco) was utilized as reference; whist methyl behenate was used as Internal Standard. Three indexes were calculated for each fish species based on FA composition. PUFA/SFA refers to the ratio between polyunsaturated and saturated fatty acids, ω-6/ω-3 was calculated as Σ ω-6 PUFA/Σ ω-3 PUFA, and the index of hypocholesterolemic/hypercholesterolemic fatty acids (HH) was calculated as Zhang et al., 2020: HH = (18:1n-9 + 18:2n-6 + 18:3n-3 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3)/(14:0 + 16:0).

2. Results and Discussion

A total of 37 FAs were characterized and quantified. The highest amount of FAs was found in carp bream and Macedonian shad (2588.2 and 2156.9 mg/100 g fillet, Table 1). Saturated FAs (SFA) were the most abundant group in common carp, goldfish and macedonian shad but not for common bream (Table 1). PUFAs ranged from 18.8 (common bream) to 28.8% (common carp) of total FAs (Table 1). Among all FAs, the most abundant were: palmitic, oleic, lignoseric, linoleic, docosahexanoic (DHA), eicosatrienoic, palmitoleic, stearic, gondoic and myristic acids (Table 1). EPA was found in very low amounts ranging from 1.7 (goldfish) to 4.9 mg/100g fillet (macedonian shad). The highest amounts for DHA were found in Macedonian shad (211 mg/100 g raw fillet) followed by common bream (123.0), goldfish (98.0) and common carp (51.0, Table 1).

Excessive SFAs intake is not desirable as it is related to increased serum levels of both total and LDL-cholesterol levels (Calder 2015). Thus, for a healthy cardiovascular status ratio values ranging in levels higher than 0,4 are beneficial. PUFA/SFA values ranged from 0.4 (macedonian shad) to 0.8 (common carp) indicating that all fish species may contribute significantly to prevent cardiovascular diseases. Amounts of ω-3 PUFA were much higher than those of ω-6 PUFA. The lowest values of ω-6/ω-3 ratio, were calculated for macedonian shad and common bream (0.3 and 0.8 respectively, Table 2) indicating that consumption of those fish species contributes to a better balanced and healthy ω-6/ω-3 ratio. The hypocholesterolemic/hypercholesterolemic index (HH) is related with the effect of specific FAs on cholesterol metabolism, and higher values are considered more desirable for human health (Hosseini et al. 2014). High values of HH index were found for common bream, goldfish and common carp (1.5 to 2.1, Table 2) indicating that consumption of those fish species is favorable to regulate cholesterol metabolism. According to international recommendations, daily intake of EPA+DHA for healthy adults should reach at least 250 mg (or 1750 mg/week). This way, 115.8 g of fillet of macedonian shad provide the recommended minimal daily dose of 250 mg of DHA+EPA (Table 2) but higher amounts, i.e. 210.3 g of common bream, 320.1 g of goldfish and 700.5 g of common carp provide the same dose of 250 mg of DHA+EPA. Thus, considering the high ω-3 content (particularly DHA content) and low ω-6/ω-3 ratio, the macedonian shad (*Alosa macedonica*) an endemic species in Lake Volvi has the higher nutritional value in terms of ω-3 PUFA content and is highly recommended to promote health of consumers.

Table 1. Mean (±sd) concentration (mg FA /100g of raw fillet) and percentage (in parentheses) of saturated (Σ-SFA), monounsaturated (Σ-MUFA), polyunsaturated (Σ-PUFA) FAs to total FA (Σ-FA) and of the 10 most abundant FA and eicosapentanoic acid (EPA) in muscle tissue of freshwater fish from the Lake Volvi. Highest amounts among species for each FA are bold sized.

FATTY ACIDS (mg/100 g raw fillet)	Common bream	Macedonian shad	Goldfish	Common carp
Σ-SFA	950.2±634, (36.7%)	1177.1±558, (54.6%)	500.2±82, (44.8%)	688.2±459, (43.7%)
Σ-MUFA	1152.6±1347, (44.5%)	518.3±427, (24%)	254.8±50, (22.8%)	432.6±371 (27.5%)
Σ-PUFA	485.4±338, (18.8%)	461.5±127, (21.4%)	360.9±147, (32.3%)	454.3±212, (28.8%)
Σ-FA	2588.2±2321	2156.9±1085	1115.9±279	1575.2±1015
Palmitic, 16:0	474±389	**660±312**	278±21	437±318
Oleic, 18:1	**1015±1207**	167±129	173±23	247±213
Lignoseric, 24:0	**310±119**	255±139	117±59	80±50
Linoleic, 18:2 (ω-6)	187±189	90±65	169±71	**266±197**
Docosahexaenoic, 22:6 (ω-3)	123±39	**211±40**	98±52	51±30
Eicosatrienoic, 20:3 (ω-3)	94±33	**123±24**	61±26	57±41
Palmitoleic, 16:1 (ω-7)	33±34	**190±173**	20±19	118±110
Stearic, 18:0	66±33	**93±54**	58±7	88±40
Gondoic 20:1 (ω-9)	84±93	**127±100**	48±20	43±33
Myristic, 14:0	72±78	**93±90**	19±3	37±32
Eicosapentaenoic, 20:5 (ω-3)	1.9±0.1	**4.9±2.3**	1.7±0.8	1.8±0.7

Table 2. Nutritional quality indexes (PUFA/SFA, ω-6/ω-3, HH) and amounts of raw fish fillet needed to provide the minimum recommended daily amount of EPA + DHA (250 mg) for healthy adults according to international nutritional guidelines.

FA content (mg/100 g raw fillet)	Common bream	Macedonian shad	Goldfish	Common carp
Σ-PUFA (ω-6)	227.1±221.4	110.6±82.2	190.0±75.5	303.3±207.7
Σ-PUFA (ω-3)	258.3±118.3	350.8±65.1	170.9±76	151.0±100.1
Σ-DHA+EPA	124.8±38.5	215.8±40.4	100.2±51.9	53.2±30.7
PUFA/SFA	0.5±0.0	0.4±0.1	0.7±0.2	0.8±0.3
ω-6/ω-3	0.8±0.5	0.3±0.2	1.1±0.2	2.8±2.9
HH	2.1±0.9	0.7±0.1	1.5±0.4	1.5±0.4
Amount of fillet (g) to supply 250 mg DHA+EPA	210.3±65	119.2±23	320.1±211	700.5±577

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References

Calder P.C. (2015). Functional roles of fatty acids and their effects on human health. Journal of Parenteral and Enteral Nutrition, 39, 18S–32S.

Zhang X., Ning X., He X., Sun X., Yu X., Cheng Y. et al. (2020). Fatty acid composition analyses of commercially important fish species from the Pearl River Estuary, China. PLoS ONE 15 (1): e0228276.

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EVALUATION AND MODELLING OF SHRIMP MELANOSIS

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Abstract
Melanosis developed in shrimp is mainly attributed to polyphenoloxidase (PPO) activity and plays a significant role in consumer acceptance and market value. Shrimp surface turns black rapidly during distribution and storage at frozen and refrigerated conditions, as a result of inappropriate chilling and temperature fluctuations of the cold chain. The objective of the study was the development of a rapid evaluation method of shrimp melanosis and the mathematical modelling of the effect of storage temperature on the enzymatic browning in Pacific white shrimp (*Litopenaeus vannamei*) based on the correlation of PPO activity and shrimp appearance (color).
Keywords: *Shrimp*, *polyphenoloxidase (PPO)*, *enzymatic browning*, *image analysis, mathematical modelling*
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1. Introduction

Melanosis, or black spot, in shrimp is a harmless but objectionable discoloration (or darkening) of the shrimp surface. It has been recognized as a very common problem in crustaceans during post-harvest storage and consists of the natural formation of dark pigments mainly in the cephalothorax and joints regions, resulting from the biochemical reactions catalyzed by polyphenol oxidase (PPO) (Goncalves and de Oliveira 2016). Several studies have focused on preventing melanosis over the years through different techniques, mainly thermal processing or addition of PPO inhibitors, such as sulfiting agents and their derivatives. However, the increasing regulatory attention and consumer awareness of the risk associated with sulphur products, such as asthma and other symptoms of allergic responses in sulfite-sensitive people,has created a need for the effective monitoring and control of the enzymatic browning in shrimp (Lien *et al*. 2016, Kimbuathong 2020, Olatunde *et al*. 2021, Li *et al*. 2022). Since shrimp is a highly perishable seafood product, appropriate control of temperature during transportation and storage plays an important role in the quality maintenance of shrimp (Tsironi *et al*. 2009).

White shrimp (*Litopenaeus vannamei*, formerly *Penaeus vannamei*), also known as Pacific white shrimp or King prawn, is a variety of prawn of the eastern Pacific Ocean commonly caught or farmed for human consumption. Europe is the world's third largest market for Pacific white shrimp, after the United States and China. Europe's Pacific white shrimp imports in 2019 totaled approximately 370,000 tones, 7.5% less than in 2018. The biggest European importers are France, Spain, United Kingdom, Italy, Netherlands, Germany and Belgium, accounting for about 80% of the total European Pacific white shrimp imports (based on Eurostat 2020).

The objective of the study was the development of a method for rapid evaluation of shrimp melanosis and the mathematical modelling of the effect of storage temperature on the enzymatic browning in Pacific white shrimp (*Litopenaeus vannamei*) based on the correlation of PPO activity and shrimp appearance (color).

2. Material and Methods

Whole Pacific white shrimp (*Litopenaeus vannamei*) samples with an average weight of 18.0 ± 0.5 g and an average body length of 14.0 ± 0.5 cm were packed aerobically and stored at controlled isothermal conditions (0-15°C) in high-precision (± 0.2°C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature of the incubators was recorded using electronic, programmable miniature data loggers (Easylog EL-USEB-TC-LCD, Lascar) coupled with K-type thermocouples. Shrimp melanosis was monitored during storage by instrumental color measurement using the Eye1 Pro chromameter (X-Rite, Michigan, USA), and proper image analysis using the ImageJ image processing program (ImageJ 1.52h, Image processing and analysis in Java, National Institutes of Health, USA). PPO activity in shrimp cephalothorax was evaluated using a modification of the spectrophotometric method by Simpson *et al*. (1988). The enzyme extract was obtained by grinding of the shrimp shell followed by high-speed homogenization (Ultra Turax T-25 Basic, IKA WERKE), with 0.05 Msodium phosphate buffer (pH=6.5) solution containing 1.0 mol/L NaCl and 0.2 g/100 mL Triton X-100, stirring at 4°C and centrifugation (8000 g at 4 °C for 30 min). PPO activity was determined in the supernatant in triplicateusing L-dihydroxyphenylalanine (L-DOPA) as a substrate according to the method of Simpson et al. (1987). The enzyme activity unit (U) was defined as the increase of 0.001 units of the absorbance at 475nm per minute and per mL of the enzyme assay.

At least 5 specimens were evaluated at each sampling point. Melanosis was evaluated on the shrimp in the carapace of the cephalothorax, in the caudal zone and in the cuticle of the abdomen, mainly where the cuticle segments are joined and where the cuticle is connected to the pleopods cephalothorax at predetermined intervals during refrigerated storage.

Results from the analysis of PPO activity were correlated with the sensory acceptability of the products (appearance) and the color measurement. A score of 5 of sensory acceptability was taken as the average score for minimum acceptability. Values of the different measured indices were plotted vs. time for all temperatures studied and the apparent order of the reactions was determined based on the least square statistical fit. The temperature dependence of the deterioration rate constant, k, was modelled by the Arrhenius equation (1):

$$lnk = lnk_{ref} - \left(\frac{E_a}{R}\right)\left[\frac{1}{T} - \frac{1}{T_{ref}}\right] \quad (1)$$

where k_{ref} is the rate constant of the degradation of the respective quality index at a reference temperature, T_{ref} (4°C for refrigerated foods), T is the temperature in K, E_a is the activation energy of the studied reaction and R the universal gas constant. The activation energy (E_a) values were estimated from the slope of Arrhenius plots vs. ($1/T_{ref}$-1/T), by linear regression (Fu &Labuza, 1997).

3. Results and Discussion

At zero storage time, the shrimps had a very translucent and shiny body and shell appearance. Samples stored at higher temperatures (10-15 °C) developed severe blackening in the head and gill regions after less than 1 day of storage and consequently had a poor appearance and were unacceptable. The L-value showed a pronounced decrease during storage, even before blackening was visible, and was a good index for the progress of melanosis. The b-value in shrimp cephalothorax increased rapidly at the higher storage temperatures. L- and b-value changes in shrimp shell during storage was found to be adequately modelled by an apparent zero order reaction.

The images captured from the shrimp body initially and during refrigerated storage at isothermal conditions were analyzed and the results showed high correlations with PPO activity and sensory acceptability, indicating that color measurement and image analysis may be applied to rapidly recognize the melanosis and consequently the freshness level of shrimp.Based on the correlation of PPO activity with the sensory scoring and color of shrimp, the threshold values corresponding to sensory rejection due to enzymatic browning were determined. Time to reach these values was determined at different storage temperatures at the refrigerated range. The developed models may be valuable tools for the rapid determination of the quality level and remaining shelf life of shrimp at any point of the supply chain.

References

Fu B., Labuza T.P. (1997). Shelf life testing: procedures and prediction methods. In M. C. Erickson, & Y. C. Hung (Eds.), Quality in frozen food (pp. 377). New York: Chapman & Hall.

Goncalves A.A., de Oliveira A.R.M. (2016). Melanosis in crustaceans: A review. LWT-Food Science and Technology, 65, 791-799.

Kimbuathong N., Leelaphiwat P., Harnkarnsujarit N. (2020). Inhibition of melanosis and microbial growth in Pacific white shrimp (*Litopenaeus vannamei*) using high CO_2 modified atmosphere packaging. Food Chemistry 312, 312,126114.

Li Y., Lei Y., Tan Y., Zhang J., Hong H., Luo Y. (2022). Efficacy of freeze-chilled storage combined with tea polyphenol for controlling melanosis, quality deterioration, and spoilage bacterial growth of Pacific white shrimp (*Litopenaeus vannamei*). Food Chemistry 370, 130924.

Lien K.W., Hsieh D.P.H., Huang H.Y., Wu C.H., Ni S.P., Ling M.P. (2016). Food safety risk assessment for estimating dietary intake of sulfites in the Taiwanese population. Toxicology Reports3,544-551.

Olatunde O.O., Della Tan S.L., Shiekh K.A., Benjakul S., Nirmal N.P. (2021). Ethanolic guava leaf extracts with different chlorophyll removal processes: Anti-melanosis, antibacterial properties and the impact on qualities of Pacific white shrimp during refrigerated storage. Food Chemistry 341,128251.

Simpson B.K., Marshall M.R., Steven Otwell W. (1987). Phenol oxidase from shrimp (*Penaeus setiferus*): purification and some properties. Journal of Agricultural and Food Chemistry 35 (6), 918-921.

Simpson B.K., Marshall M.R., Steven Otwell W. (1988). Phenoloxidases from pink and white shrimp: Kinetic and other properties.Journal of Food Biochemistry 12, 205-217.

Tsironi T., Dermesonlouoglou E., Giannakourou M., Taoukis P. (2009). Shelf life modelling of frozen shrimp at variable temperature conditions. LWT- Food Science and Technology 42, 664-671.

AMINO ACID COMPOSITION OF *CERAMIUM RUBRUM* (RHODOPHYCEAE) FROM NORTH AEGEAN SEA, TURKEY

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Abstract
The amino acid profiles of red seaweed *Ceramium rubrum* was investigated in three locations in the North Agean Sea (Turkey). Total amino acid content was 44.49 g/100g in Yeniköy station, 44.08 g/100g in Ayazma station and 42.77 g/100g in Asos station. Both non-essential and essential amino acid content is higher in the Ayazma station than in other stations. In addition, all essential amino acids except tryptophan was detected in three stations. Our results showed that the highest essential amino acid ratios were found in Ayazma and Yeniköy stations. Seaweeds might be important sources of proteins with a high level of essential amino acids. However, the amino acid composition varies depending on the geographic locations.
Keywords: *Red seaweeds, Ceramium rubrum, Amino Acid, Functional Food, Nutrition*
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1. Introduction

Seaweeds are photosynthetic organisms that contain protein, essential fatty acids, carbohydrates, vitamins, and minerals in various amounts (Ak 2015, Pereira 2011). They are used in many areas such as food, feeds, fertilizers, biofuels and cosmetics industries (Abomohra *et al*. 2018, Aisha *et al.* 2019). Among the seaweed groups, red seaweeds (Rhodophyta) are an excellent candidate for functional foods consisting of highly valued nutritional compounds (Cotas *et al.* 2020). In addition to their nutritional value, they also have functional components exhibiting anti-oxidant, anti-microbial, anti-viral, anti-inflammatory, and anti-cancer properties (Cox *et al.,* 2010). Intertidal red macroalgae are subjected to a range of abiotic and biotic factors that cause biochemical variation between and individuals (Schmid & Stengel 2015). Therefore, the nutrient composition of seaweeds varies depending on the species and environmental conditions (i.e., light, temperature, and nutrients) as well as locations even over small geographic ranges (Dawczynski *et al.* 2007, Gaillard *et al.* 2018, Hafting *et al.* 2015). In order to effectively obtain and use the red algal amino acid composition, an understanding of their variability within locations is critical. In particular, there is a lack of knowledge on specific localized environmental effects on amino acid contents. Therefore, the aim of the study is to evaluate amino acid compositions of red seaweed *Ceramium rubrum* distributed along with the costs of the Noth Aegean Sea in Turkey.

2. Material and Methods

Ceramium rubrum C. Agardh samples were collected qualitatively by hand from intertidal at three locations from the Assos (39°29'16.16"N; 26°20'43.28"E), Yeniköy (39°55'25.18"N; 26° 9'26.41"E), and Ayazma (39°48'44.71"N; 26° 0'20.87"E) (Çanakkale/Turkey) were chosen as sampling areas where macroalgae species distributed intensively. The samples were identified according to Guiry & Guiry (2021). The thallus were cleaned from their epiphytes, dried at 30 °C, and milled into powder before extraction. The samples were extracted according to Çankırılıgil *et al.* (2020) and amino acid analyses were done according to Henderson *et al.* (2000).

3. Results

The amino acid composition of *Ceramium rubrum* collected from three different locations of the North Agean Sea is reported in Table 1. Total amino acid content was 44.49 g/100g in Yeniköy station, 44.08 g/100g in Ayazma station and 42.77 g/100g in Asos station. According to our results, the highest aspartic acid+asparagine, glutamic acid+glutamine, glycine, alanine, tyrosine levels were determined in the Ayazma station. In addition, the serine value changed between 0.17 to 0.29 mg/ g. While cystine was not found in Yeniköy and Asos, it was measured as 0.05 g/100g in Ayazma station. The highest isoleucine level was 0.16 g/100g in the Asos station. When the essential amino acid percentages between stations were examined, it was determined that the highest essential amino acid ratios were both in Ayazma and Yeniköy stations. Threonine levels ranged from 0.30 to 0.47 g/100g among the stations. The highest threonine, valine, methionine, phenylalanine, leucine and lysine levels were found in Ayazma station.

4. Discussion

Amino acid compositions of seaweed showed differences between locations. Gaillard *et al.* (2018) and Guihéneuf *et al.* (2018) reported similar results. Although, *Ceramium rubrum* shown alterations between stations in terms of amino acid compositions, all samples detected as rich in terms of essential amino acids. Considering the fact that, widely distribution of *Ceramium rubrum* and the rich amino acid compositions, this species can be used as feed ingredient and food supplement with proper processing techniques.

Table 1. Amino acid compositions of *Ceramium rubrum* collected from different stations

	Ayazma	Assos	Yeniköy
ASP+ASN	0.469	0.317	0.309
GLU+GLN	0.549	0.352	0.414
SER	0.231	0.166	0.288
HIS	-	-	-
GLY	0.369	0.204	0.158
THR	0.467	0.302	0.357
ALA	0.050	0.010	0.009
TYR	0.339	0.226	0.173
CYS	0.055	-	-
VAL	0.141	0.080	0.054
MET	0.140	0.085	0.088
TRP	-	-	-
PHE	0.238	0.090	0.023
ISO	0.117	0.158	0.061
LEU	0.334	0.207	0.223
LYS	0.398	0.306	0.387
TOTAL	3.898	2.503	2.544

*ASP: aspartic acid, ASN: asparagine, GLU: glutamic acid, GLN: glutamine, SER: serine, HIS: histidine, GLY: glycine, THR: threonine, ALA: alanine, TYR: tyrosine, CYS: cystine, VAL: valine, MET: methionine, TRP: tryptophan, PHE: phenylalanine, ISO: isoleucine, LEU: leucine, LYS: lysine.

References

Abomohra A.E.F., El-Naggar A.H., Baeshen A.A. (2018). Potential of macroalgae for biodiesel production: Screening and evaluation studies. Journal of Bioscience and Bioengineering, 125(2), 231-237.

Aisha A., Haider A. (2019). Seaweed: Nutritional and health benefits. The Pharma Innovation Journal, 8, 80-83.

Ak İ. (2015). Sucul ortamın ekonomik bitkileri; makro algler. Dünya Gıda Dergisi, Aralık, 88-97.

Cotas J., Leandro A., Pacheco D., Gonçalves A.M., Pereira L. (2020). A comprehensive review of the nutraceutical and therapeutic applications of red seaweeds (rhodophyta). Life, 10(3), 19.

Cox S., Abu-Ghannam N. Gupta S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible irish seaweeds. International Food Research Journal, 17, 205-220.

Çankırılıgil E.C., Berik N., Alp Erbay E. (2020). Optimization of hydrolyzation procedure for amino acid analysis in fish meat with hplc-dad by response surface methodology. Ege J. Fish. & Aquatic Sci., 37, 113-123.

Dawczynski C., Schubert R. Jahreis G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry, 103(3), 891-899.

Henderson J.W., Robert D.R., Brian A.B., Cliff W. (2000). Rapid, accurate, sensitive and reproducible hplc analysis of amino acids Application Note Publication No: 5980-1193.

Gaillard C., Bhatti H.S., Novoa-Garrido M., Lind V., Roleda M.Y., Weisbjerg M.R. (2018). Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. Animal Feed Science and Technology, 241, 210-222.

Guihéneuf F., Gietl A. Stengel D. (2018). Temporal and spatial variability of mycosporine-like amino acids and pigments in three edible red seaweeds from western ireland. Journal Applied Phycology, 30, 2573-2586.

Guiry M.D., Guiry G.M. (2021). Algaebase. Retrieved 13.2.2021, from https://www.algaebase.org

Hafting J.T., Craigie J.S., Stengel D., Loureiro R., Buschmann A.H., Yarish C., Critchley, A.T. (2015). Prospects and challenges for industrial production of seaweed bioactives. Journal of Phycology, 51, 821-837.

Pereira L. (2011). A review of the nutrient composition of selected edible seaweeds. In: V.H. Pomin (Ed.), *Seaweed: Ecology, nutrient composition and medicinal uses* (15-47). Coimbra: Nova Science Publishers.

Schmid M., Stengel D.B. (2015). Intra-thallus differentiation of fatty acid and pigment profiles in some temperate Fucales and Laminariales. Journal of Phycology, 51(1), 25-36.

MARINE BIVALVES MICROBIOLOGICAL MONITORING ORIGINATED FROM SEAFOOD MARKETS LOCATED IN THESSALONIKI (NORTH GREECE)

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Abstract:
Consumption of raw marine bivalves is a traditional trend throughout the Mediterranean countries. Despite their nutritional value and the high customer preference in consuming them unprocessed (thermal process) or minimally processed (salted, smoked, canned), raw consumption of bivalves is associated with a great human health risk on account of their filter-feeding nutritional behavior that may lead to the digestion of harmful microorganisms such as phytoplankton and bacteria. In this context, microbiological monitoring of marine bivalves originating from the two biggest local seafood markets in Thessaloniki was performed in the current study, in order to identify foodborne pathogens that pose public health threat. In particular, 330 *Ostrea edulis* and 540 *Callista chione* specimens were tested for the presence of *Escherichia coli*, *Salmonella spp*. and *Vibrio spp*. Neither pathogenic for human health *Vibrio sp.* nor *Salmonella spp*. were detected in marine bivalve molluscs intended for raw food consumption, while *E. coli* was only present at levels below the acceptable upper limit.
Key words: *Public Health*, *Raw food*, *Foodborne pathogens*, *Kapani*, *Nea Michaniona*
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1. Introduction

Seafood consumption provides a great variety of beneficial nutrients associated with good health, while specifically bivalve food consumption has been recommended towards the reduction of coronary heart disease and support of cognitive development and vision. Despite their high nutritional profile, raw bivalve consumption has been associated with foodborne disease outbreaks, due to their filter feeding activity and their exposure in aquatic pollutants and harmful microbial load. The genus *Vibrio* includes more than 12 pathogenic species that have been linked with foodborne diseases due to the consumption of lightly cooked or raw marine bivalves. The members of *Vibrio* spp. exhibiting the highest public health risk are *Vibrio cholerae*, *Vibrio vulnificus* and *Vibrio parahaemolyticus,* associated with pandemic of cholera outbreaks, gastrointestinal diseases and acute septicaemia in humans, respectively (Xu *et al.* 2017).

Therefore, poor handling in the fishing boat, abused temperatures in transportation, inappropriate manipulations and storage in the markets expose the consumer to microbial-spoilage risks. Additionally, microbial contamination is becoming more pervasive in fishing areas due to the local human activities (livestock farming, crop production), resulting in even more risks for human health (Oliveira *et al.* 2011). Regarding the bacteria contamination of the fishing areas due to human activities, *Salmonella* is the second most common cause of human gastroenteritis (Rubini *et al.* 2018). Apart from the mussels, which are lightly cooked, clams and oysters are intended for raw consumption. The main objective of this research is the bacterial foodborne disease monitoring of bivalves originating from seafood markets in Thessaloniki in an assessment of public health safety point of view.

2. Material and Methods

Samplings of *Callista chione* and *Ostrea edulis* that represent the species with the highest demand of raw consumption in Greece, took place during 2020 from two seafood markets in Thessaloniki, one located at the center of the city (Kapani) and the second in the South-east part of the city (Nea Michaniona). *O. edulis* samples were collected every two months, while *C. chione* samples were collected in a monthly basis excluding April, May and June in which fishing is prohibited for these species. Each sample consisted of 30 specimens collected.

Each specimen was dissected aseptically with the half kept for microbiological research while the rest was stored in deep freeze for molecular analysis. *E. coli* was enumerated according to the standard method ISO 16649-3/2005 and *Salmonella spp*. was isolated according to the ISO 6579-1:2017. Molecular detection of *Vibrio* spp. associated with foodborne diseases was performed applying a Multiplex-PCR as described in Xu et al., 2017.

3. Results

All examined samples were found negative for *Salmonella* spp., while 5 of them (17.5%) were positive for *E. coli* (3 *C.chione,* and 2 *O. edulis,* Table 1). The number of *E. coli* ranged between 0.2/100 g and 4.510/100 g of bivalve mollusc soft tissues. However, no sample showed *E. coli* counts higher than 230 MPN/100 g, which is the upper allowed threshold value by the EU legislation. Also, no positive sample was detected by multiplex-pcr for *Vibrio* spp. (Table 1).

Table 1: **Prevalence of positive bivalve molluscs for *Vibrio* spp, *Salmonella* spp and *E. coli* for each fish market studied between 2020-2021**

Seafood Market	*Ostrea edulis* (n=11)			*Callista chione* (n=18)		
	Escherichia coli	*Salmonella* spp.	*Vibrio* spp.	*Escherichia coli*	*Salmonella* spp.	*Vibrio* spp.
Kapani	16.7% 1/6)	0 (0/6)	0 (0/6)	0 (0/8)	0 (0/8)	0 (0/8)
Michaniona	20% (1/5)	0 (0/5)	0 (0/5)	30% (3/10)	0 (0/10)	0 (0/10)

4. Discussion

Bivalves as food components are characterized as easily digested food, with high nutritional profile, additive free and minimally processed fulfilling the modern demands of the customers for healthy trends (Oliveira *et al.* 2011). In this study no pathogenic strain was detected in non allowed limits, emphasizing the effective remediation procedure as well as the clarity of the aquatic ecosystems from which they originate. Keeping in mind the high importance of high productivity of Thermaikos Gulf in marine bivalves, these preliminary results provide valuable data for both trawlers and trade of bivalves. In conclusion, systematic monitoring for these three pathogens is proposed as a key strategy for ensuring the safety of marine bivalve market in Thessaloniki, Greece.

Acknowledgements

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project “Pathogens, associated with public health, assessment in marine bivalves distributed by the seafood markets in Thessaloniki” (MIS 5047858).”

References

Oliveira J., Cunha A., Castilho F., Romalde J.L., Pereira M.J. (2011). Microbial contamination and purification of bivalve shell fish: Crucial aspects in monitoring and future perspectives - A mini-review. Food Control 22, 805–816.

Rubini S., Galletti G., D’Incau M., Govoni G., Boschetti L., Berardelli C., Barbieri S., Merialdi G., Formaglio A., Guidi E., Bergamini M., Piva S., Serraino A., Giacometti F. (2018). Occurrence of *Salmonella enterica* subsp. enterica in bivalve molluscs and associations with *Escherichia coli* in molluscs and faecal coliforms in seawater. Food Control 84, 429–435.

Xu Y.G., Sun L.M., Wang Y.S., Chen P.P., Liu Z.M., Li Y.J., Tang L.J. (2017). Simultaneous detection of *Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seafood using dual priming oligonucleotide (DPO) system-based multiplex PCR assay. Food Control 71, 64–70.

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IDENTIFICATION OF THE PEARL OYSTER (*Pinctada imbricate radiata*), MISLABELING IN THE GREEK MARKET BY USING INTERNET-BASED TOOLS

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Abstract
Internet-based quantitative research was conducted between January 2021 and March 2021 to explore the market supply of the pearl oyster (*Pinctada imbricate radiata*), in Greece. Due to visual similarities between the pearl oyster, the native oyster Ostrea edulis and the pectenid *Aequipecten opercularis*, additional research took place to allocate the market supply of *O.edulis* and *A. opercularis* in Greece. Various keywords were used in the Greek language referring to the three species. Despite the presence of pearl oyster in seven regions, findings show that it is not offered in four of them, whereas *O. edulis* and *A. opercularis* are offered to all of them. Moreover, search results showed that various keywords referring to pearl oyster often led to specific websites about *O. edulis* or *A. opercularis*. Overall, it seems that there is a specimen mislabeling throughout Greece, thus, extraction of significant information about the market supply of pearl oyster is deficient. **Keywords:** pearl oyster, Greek market, mislabeling.
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1. Introduction

Pearl oyster (*Pinctada imbricate radiata*) is an exotic species with significant presence in the Greek seas and good potential for commercial exploitation (Moutopoulos et al., 2020). Up to now limited unregulated and unreported quantities are directed to the market with different names of the bivalve, due to lack of the relevant legal framework of the species. In the local markets referred with different names as "*stridokteno*", *tiganak*i", *margaritoforo stridi*", "*stridi",* "*xteni*" etc. The aim of the present effort is to identify the extent of the species presence in the supply chain and the potential mislabeling in the Greek seafood supply chain.

2. Material and Methods

This research started in January 2021 and finished in March 2021 intending to evaluate the presence of the pearl oyster in the markets of Greece in relation to the native oyster *Ostrea edulis* and the scallop (pectenid) *Aequipecten opercularis* (Figure 1).

Figure 1. External morphology of the bivalves searched through the keywords (from left to right) a) pearl oyster *Pinctada imbricate radiata*, b) native oyster *Ostrea edulis,* c) scallop (pectenid) *Aequipecten opercularis.*

Initially, there were used three popular search engines Google.com, Yahoo.com, Bing.com. Also a popular restaurant promotion platform was used, tripadvisor.com, to obtain indicative listings related to the market of *O. edulis* and *A. opercularis*.

3. Results

A total of 457 results about pearl oyster were found. 25 of them were related to the market of pearl oyster (Figure 2) and it is distributed in the market in three geographical areas in Greece. These areas include the Peloponnese, Central Greece and Thessaly (Table 1). A total of 88 results were found regarding *Ostrea edulis*. All of them were related to restaurants. The geographical distribution included all 9 regions of Greece: Thrace, Macedonia, Epirus, Central Greece, Thessaly, Peloponnese, Ionian Islands, Aegean islands, Crete (Figure 2, Table 1). A total of 208 results were found about *Aequipecten opercularis*. All of them were related to restaurants. The geographical distribution of these included all 9 regions of Greece: Thrace, Macedonia, Epirus, Central Greece, Thessaly, Peloponnese, Ionian Islands, Aegean islands, Crete (Figure 2, Table 1).

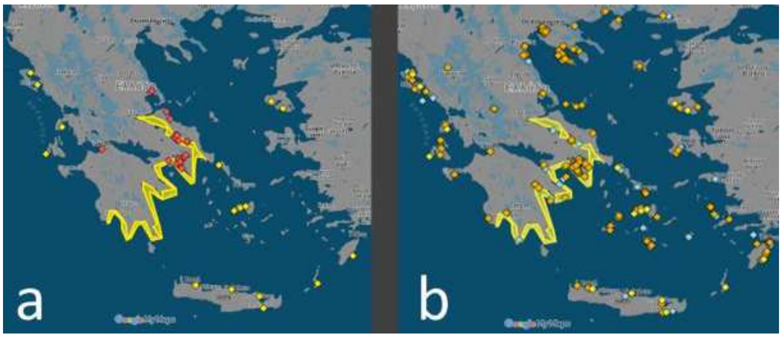

Figure 2. a) *Pinctada imbricate radiata* habitat (yellow) and market points (red), b) market points of *O. edulis* (blue) and *A. opercularis* (orange) in relation to *Pinctada imbricate radiata* habitat (yellow),

Table 1, Geographical distribution of *Pinctada imbricate radiata O. edulis* and *A. opercularis* market points habitat, *Pinctada imbricate radiata* habitat

Geographical region	Pearl oyster habitat	Pearl oyster selling points	*O. edulis* selling points	*A. opercularis* selling points
Thrace	No	No	Yes	Yes
Macedonia	No	No	Yes	Yes
Epirus	Yes	No	Yes	Yes
Central Greece	Yes	Yes	Yes	Yes
Thessaly	Yes	Yes	Yes	Yes
Peloponnese	Yes	Yes	Yes	Yes
Ionian islands	Yes	No	Yes	Yes
Aegean islands	Yes	No	Yes	Yes
Crete	Yes	No	Yes	Yes

4. Discussion

This research considered quantitative and geographical data related to the distribution of three species in the Greek market. The fact that pearl oyster is not an endemic species was also taken into account. They were first observed in Greek waters in the '60s (Theodorou et al., 2019). Furthermore, there is a certain visual similarity with the native species of *Ostrea edulis* and *Aequipecten opercularis* (Figure 1). In the areas of the eastern Peloponnese, in the Ionian Sea and in the Aegean Sea, despite the fact that they constitute habitat of the pearl oyster, no report of its availability to consumers was found. On the contrary, in areas that lives and grows, there were found dozens of references to the market of native oysters and scallops. Summarizing all the above, it seems that there is a specimen mislabeling throughout Greece, thus, extraction of significant information about the market supply of pearl oyster is deficient.

Acknowledgements

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References

Moutopoulos K. D., Ramfos A., Theodorou A. J., Katselis G. (2020). Biological aspects and population dynamic of Pearl Oyster in the Eastern Mediterranean. Regional Studies in Marine Science 45(5):101821, DOI:10.1016/j.rsma.2021.101821

Theodorou J.A., Perdikaris C., Spinos E. (2019). On the occurrence of rayed Pearl oyster *Pinctada imbricata radiata* (Leach, 1814) in Western Greece (Ionian Sea) and its biofouling potential. Biharean Biologist International Journal 13 (1): 4-7. http://biozoojournals.ro/bihbiol/index.html.

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PARTICIPATORY APPROACHES IN FLOOD RISK MANAGEMENT

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Abstract
Participatory approaches play a crucial role in understanding the complex nature of hazardous floods and their connections with human activities. Community-based approaches according to the methodology developed by the Red Cross, "Vulnerability and Capacity Assessment (VCA)" are used to evaluate Nature Based Solutions (NBS) in flood risk management of Kalloni basin (Lesvos). Groups of local stakeholders joined a series of five workshops and participated in the evaluation of nature-based solutions capable to mitigate flood risk. The participants highlighted NBS in terms of their expected effectiveness and changed their views on highly invasive technical projects.
Keywords: *Floods, Nature-based Solutions, Public participation, Stakeholders, Vulnerability and Capacity Assessment.*
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1. Introduction

A growing body of literature proposes that participatory processes help to resolve conflicts and promote collaboration. Using information gathered with public participation, a stronger foundation can be created for problem solving and uncertainty can be reduced, by leading environmental management processes to better and more sustainable solutions (Heitz *et al*. 2009). Furthermore, through participatory processes, the public can be given the opportunity to control the direction of change in environmental and social issues (Dell'Ovo *et al*. 2015). In this way decision-making processes can be more objective and transparent and emphasize the complexity of human and environmental interactions (Finger *et al*. 2019). Participatory methods that concern environmental risk management, are based on a variety of community research tools. These tools are selected and implemented to evaluate nature-based solutions in the river basin of Kalloni (Lesvos). Nature based solutions (NBS) are measures and actions that are inspired by nature, to provide flood risk mitigation and proactive adaptation options. The examined measures, such as the creation of small ponds, dry stone retaining structures, maintenance of land terraces at high altitudes, flood-based farming systems and the creation of rainwater harvesting in Kalloni, could enhance the resilience to flooding and eventually replace the need for difficult and costly technical projects.

2. Materials and methods

The tools of the methodology VCA (Vulnerability and Capacity Assessment) of the Red Cross, that is applied worldwide in the assessment of risk from natural and man-made disasters were modified to be used in the current study. The methodology used in the case of Kalloni, was selected with the ultimate goal of answering individual research questions related to flood risk management and the assessment of proposed solutions by the community itself. The participatory processes consisted of two steps: (i) individual interviews and one pilot workshop in the research site (ii) four small workshops, with each one of them focusing on a specific group of interest (schoolteachers, farmers, municipality employees/community leaders and freelancers in the scientific and technical sector, such as engineers, geographers, environmentalists and geologists). The groups of interest were invited to discuss the current situation in a focus group and to collaborate in designing problem and solution trees. In the final stage of each workshop, participants evaluated NBS and the participatory processes, using a questionnaire.

3. Results and Discussion

In total 51 participants were involved in this research, 67% were male and 33% were female. The vast majority of them (≈67%) aged more than 50 years old and 48% were municipality employees and decision makers, 21% were farmers, 19% were freelancers and 12% were schoolteachers. During the interviews, the participants expressed their anxiety about the phenomena and the lack of cooperation between residents and the municipality. They pointed to the extensive bureaucracy as a deterrent to the payment of compensation for damages that costed 500-10,000 euros. It is noteworthy that most participants were well informed about the causes of the flood, both due to the topography of the area and the causes related to human interventions in the natural landscape. In the stage of group discussion and problem-solution tree designing, it was clear that participants shared common beliefs regarding the causes that make the area vulnerable to flash floods. All agreed that topography and land use in the area in combination with management issues, play an important role in increasing risk. There were also common misunderstandings in the groups of municipality employees and farmers, as they believed that Natura 2000 areas are an obstacle to the adequate cleaning from debris. After a presentation of NBS by the team of researchers, participants were invited to complete a questionnaire and highlight flood protection measures. The order of priority to be given to each NBS, according to each group of interest, is illustrated in Figure 1.

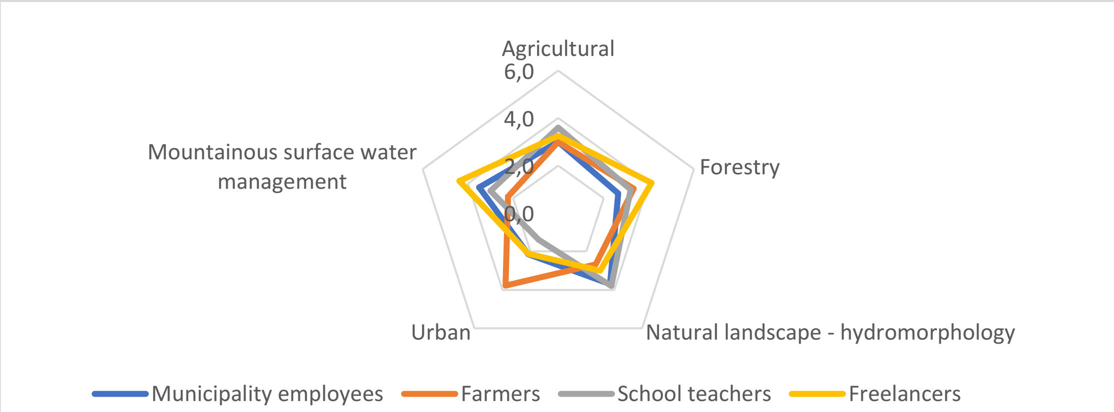

Figure 1. The order of priority to be given to each sector of NBS according to the groups of interest

In terms of flood control, throughout the actions (interviews and workshops), except for the last two workshops with teachers and freelancers, there was a strong preference for large-scale interventions. Proposals by the participants, such as diversion of the river and construction of a dam seem to be repeated as solutions to the problem. Following the presentation of the proposed NBS by the team of researchers, the views differed, as other solutions became known that are implemented without major interventions. The projects highlighted by the participatory workshops are expected to be evaluated using hydrological and hydraulic models, to investigate their effectiveness in the area. The participatory processes were evaluated by the participants using a questionnaire, with the aim of their subsequent improvement. Thus, 95% of the participants answered that they felt very comfortable expressing their opinion during the workshops. Also, 90.5% responded that the content of the action was quite understandable and 9.5% moderately understandable. So, based on these percentages it can be considered that the workshops were completed successfully, as the largest percentage understood the process and expressed their opinion freely.

4. Conclusions

During the implementation of VCA in Kalloni it was clear that the citizens were skeptical about the process in the beginning and tended to propose invasive solutions even if they did not clearly understand their function or appropriateness to the area. That quickly changed as they became familiar with the NBS proposed by the team. Public participation in flood risk management is at an early stage in Greece and especially in provincial areas and as a result, communities are not used to the involvement in decision making. The interaction between the public and scientists of different specialties, as well as the investigation of the social and environmental conditions that make an area vulnerable, is essential for the development and promotion of the effectiveness of participatory workshops related to protection against environmental hazards.

References

Dell'Ovo M., Torrieri F., Oppio A. (2015). How to Model Stakeholder Participation for Flood Management. Springer Nature 313, 67–75. DOI: 10.1007/978-3-319-90315-6_6.

Finger L., Stricevic F., Kapovic-Solomun Z. (2019). The Perception of Stakeholders to Implement Nature-Based Solution for Flood Protection in the Balkans and in Iceland. MDPI Proceedings 30 (1), p. 43. DOI:10.3390/proceedings2019030043.

Heitz C., Spaeter S., Auzet A.V., Glatron S. (2009). Local stakeholders' perception of muddy flood risk and implications for management approaches: A case study in Alsace (France). Land Use Policy 26 (2), 443–451. DOI: 10.1016/j.landusepol.2008.05.008.

APPLICATION OF THE DPSIR METHODOLOGY FOR SUSTAINABLE MANAGEMENT OF WATER RESOURCES IN THE MYGDONIA BASIN, GREECE

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Abstract
The Mygdonia basin is located in 10th Water Department of Central Macedonia with a surface area of 10.165 km^2 in which Mygdonia basin is occupying 2,060 km^2. Human intervention has altered the landscape of the area. In particular, there is a degradation of water resources, both qualitatively and quantitatively, mainly in the lakes Volvi and Koronia. The DPSIR (Driver-Pressure-State-Impact-Response) management model was developed in order to investigate the main parameters that affect this ecosystem with the ultimate goal of finding effective response measures. The proposed answers were based on the Water Framework Directive (WFD) 2000/60/EC, being a useful tool for land-use planning and water protection decision-making.
Keywords: *DPSIR, GIS, Mygdonia, Lake Koronia, Lake Volvi, Management*
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1. Introduction

In general, the Mygdonia basin is a single hydrological basin. However, for practical reasons it is divided into two sub-basins without a distinct boundary between them. One sub-basin is of the Lake Volvi and the other is of the Lake Koronia. The basin is characterized by intensive agricultural activities. Groundwater overexploitation via numerous boreholes has led in a decline of groundwater levels during the last decades. A large number of torrents, transient flow, are discharging in the two lakes throughout the basin. Although there is a large volume of water in the area, due to over-pumping of groundwater, there is a decline in the level of groundwater aquifers. The climate is a combination of Mediterranean and continental type with the Lake Volvi being its regulator (Ministry of Environment and Energy 2013).

Methodologies have been applied with the aim of taking effective measures to protect and manage water resources with an emphasis on basins. These methodologies reflect all the parameters that affect these ecosystems. In this study, the DPSIR management model was applied (Figure 1). During the application of the methodology, the interaction of the five indicators is studied, the initials of which also compose its name: Driving Force, Pressure, State, Impact and Response (Kristensen 2004; Karageorgis *et al*. 2005). The results are visualized using Geographic Information Systems (GIS).

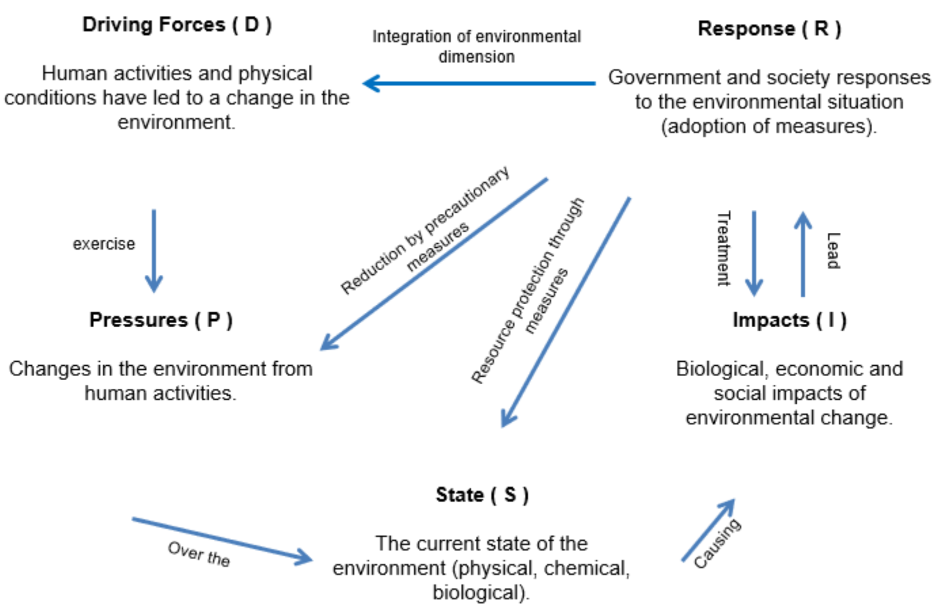

Figure 1. Schematic diagram of the DPSIR framework (modified by Kristensen 2004).

2. Materials and Methods

For the application of the methodology, the Driving forces were initially collected, including the main socio-economic conditions, which are parameters that strengthen or weaken the pressures in each environment. They include population trends, land uses, the development model of the area as well as natural and man-made processes that significantly shape the environment - such as natural runoff - or large-scale processes, the causes of which are located outside the study area.

Furthermore, the Pressures, originating from the driving forces, were collected. They are the main causes of burden on the system as they are demarcated around the perimeter of the lakes Volvi and Koronia and the hydrographic network. They include the water needs as well as the energy requirements of the system as a whole. An important assessment factor is the total pollutant load (kg/day), which is calculated on the basis of coefficients set by Directive 91/271/EEC. Bibliographic data were collected for State of the area, which result in both the "poor" groundwater status and the "good" chemical status, despite the sources of burden and the intense population growth / activity around the lakes. It is paradoxical that according to the European Commission in the field of polluted water, the Mygdonia basin is largely characterized as Nitrate Vulnerable Zone, meaning that the inflow of water is charged with high concentrations of nitrates. Nonetheless, 24 species of fish have been recorded, especially in Lake Volvi.

All the above result in the following Impacts. Although the population is declining in the basin region, the water balance of the region is in deficit. Surprisingly, the cultivated areas have increased, so the opening of more wells, combined with the reckless use of underground reserves, lead to falling water levels, deteriorating landscape, drought and a threat to the lake system. The lakes are affected by the use of agrochemicals, showing turbidity with a high pollution load even in the sediments, which are constantly increasing, due to the shallow nature of the lakes. The impact of these is the burden on the local flora and fauna. Specifically, Lake Koronia is the most degraded with only a scant presence of life.

3. Results and discussion

The DPSIR methodology is applied in order to note the pressures that the ecosystem receives in order to find the appropriate protection - management measures through the impact on the area and evaluating the "possibilities" of the system. The summary of the indicators, which were examined for the elaboration of the present study, in the context of the DPSIR methodology is presented below. Based on the results of DPSIR, a set of measures and appropriate policy responses are proposed (Figure 2).

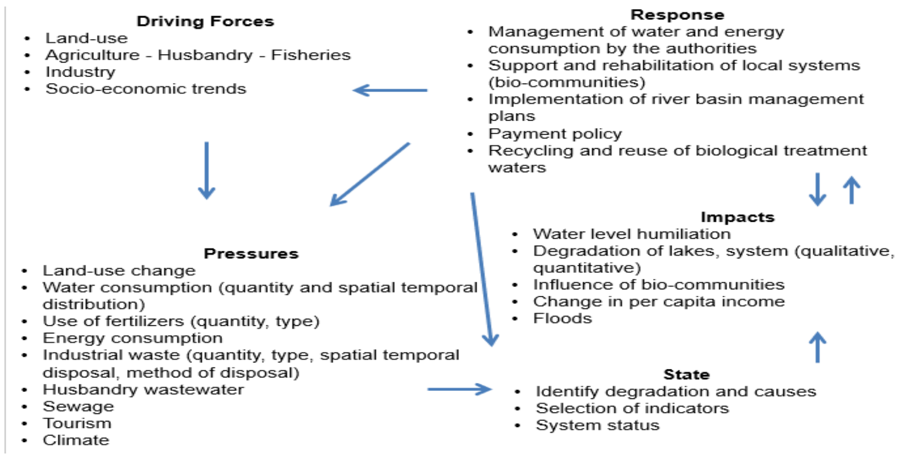

Figure 2. Proposed DPSIR method for the Mygdonia basin.

References

Karageorgis A.P., Skourtos M.S., Kapsimalis V., et al. (2005). An integrated approach to watershed management within the DPSIR framework: Axios river catchment and Thermaikos Gulf. Regional Environmental Change 5, 138–160.

Kristensen P. (2004). The DPSIR Framework. European Topic Centre on Water. European Environment Agency.

Ministry of Environment and Energy, Special Secretariat for Water, (2013). Strategic Environmental Impact Study, Water Department of Central Macedonia (GR10).

Hg IN SURFACE SEDIMENTS FROM THE HARBOR OF VOLOS, GREECE

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Abstract
The aim of this study is to evaluate Hg content and possible sediment contamination. The objectives of the study are to determine, total and bioavailable content and the distribution of Hg in Volos harbor, Thessaly region and to monitor sediment quality. For this purpose, 20 sediment samples were collected on July 2020. Preparation of the samples included drying at 35-40 °C and sieving, using 1mm sieve. For the determination of total and bioavailable Hg content, the samples were diluted following 3050B method as recommended by the United States Environmental Protection Agency (USEPA) and method «0.5N HCl» respectively. The analysis was made using Cold Vapor Atomic Absorption Spectroscopy (CVAAS). Results were compared with ERM-ERL Sediment Quality Guidelines values. Hg total and bioavailable content was found among ERL and ERM values indicating a range within effects would occasionally occur.
Keywords: *Mercury*, *Sediments*, *Volos harbor, Greece*
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1. Introduction

Surface sediment is the final recipient and therefore a source of Hg exposure to organisms. The distribution of Hg in sediment may be heavily influenced by various geochemical variables, in particular the content of the organic matter and the particle size (Xiao *et al*. 2017). Mercury is an unnecessary trace element and at high concentrations it is considered toxic among all its forms both inorganic (Hg^0, $HgCl_2$, Hg^{2+}, HgS) and organic compounds (CH_3Hg^+) and exposure of the organisms can cause adverse effects on their health (Driscoll *et al*. 2013). Transportation and mobility of Hg in the environment depends on the effects of various processes such as, oxidation, precipitation, dissolution, complexing and adsorption/desorption in aquatic ecosystems and these are crucial to the behavior of metal (Zhang 2006).

2. Material and methods

Sampling was carried out within the Commercial and Tourist harbor of Volos, Figure 1, in July 2020, by means of a Van Veen type sampler. Sediment samples were collected from the bottom surface from 18 points within the harbor and 2 outside as reference samples and the content of Hg was determined. The samples after drying, were sieved in a 1mm mesh sieve. For the determination of the total content, strong acid digestion (HNO_3-HCl), (USEPA, 1996) was performed, and the bioavailable fraction was obtained by extraction with «0.5N HCl». The analysis was made using Cold Vapor Atomic Absorption Spectrophotometry (CVAAS).

Figure 1. Sampling locations in Volos's harbor.

3. Results and Discussion

Figure 2 shows the content of the total and bioavailable fraction of Hg in the sediment from the bottom of the harbor of Volos at the sampling points.

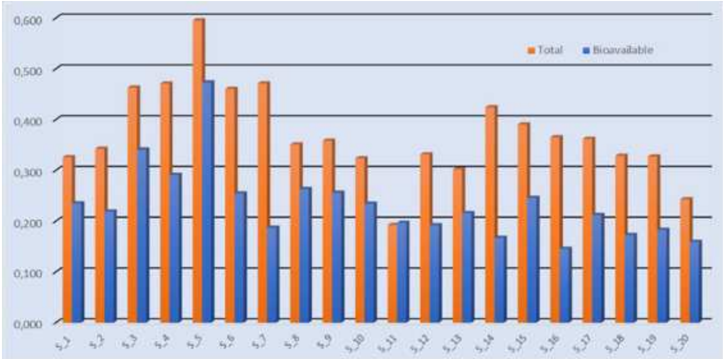

Figure 2. Total and bioavailable content of Hg at the sampling points.

Figure 3 shows the distribution of total (a) and bioavailable (b) Hg, respectively. It appears that, the increased content of Hg may result from a point source of pollution caused by the urban sewage system, and from the disposal and dispersion of waste due to increased sea traffic.

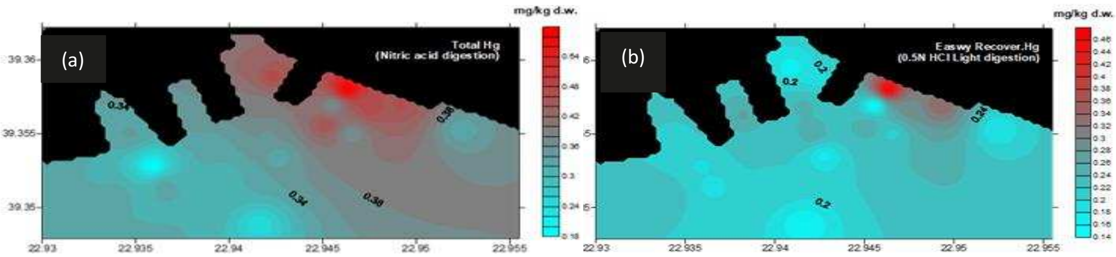

Figure 3. Spatial distribution of total (a) and bioavailable (b) Hg content in surficial sediments, Volos's harbor.

Table 1 shows the range and the mean for both total and bioavailable Hg content (ppm) and the international sediment quality guidelines values (Long & Morgan 1991).

Table 1. Total and bioavailable range and mean values of Hg.

Total			Bioavailable			SQGs	
Min	Max	Mean	Min	Max	Mean	ERL	ERM
0.193±0.01	0.586±0.03	0.38±0.02	0.145±0.7*10^{-2}	0.473±0.02	0.234±0.01	0.15	0.71
ERL: Effects range low, ERM: Effects range median							

Comparison showed that total and bioavailable Hg contents were among the corresponding SQGs values, indicating the prevailing sediment contamination and possible effects occurrence on aquatic benthic organisms.

References

Driscoll C.T., Mason R.P., Chan H.M., Jacob D.J., Pirrone N. (2013). Mercury as a global pollutant: sources, pathways, and effects. Environment Science Technology 47, 4967-4983.

Long E.R. & Morgan L.G. (1991). The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52, National Oceanic and Atmospheric Administration, Seattle, WA, pp. 175.

USEPA. (1996). Method 3050B: Acid digestion of sediments, sludges, soils. Revision 2. USEPA.

Xiao C., Jian H., Chen L., Liu C., Gao H., Zhang C., Liang S., Li Y. (2017). Toxic pollution in the Yellow Sea and Bohai Sea, China: distribution, controlling factors and potential risk. Marine Pollution Bulletin 119, 381-389.

Zhang H. (2006). Photochemical redox reactions of mercury. recent Developments In: Mercury Science. 37-79.

PRELIMINARY ACCESSEMENT OF INTERACTIONS BETWEEN DOLPHINS AND FISHERS IN ZAKYNTHOS ISLAND

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Abstract
This study examines the interactions between cetaceans and fishing boats in the area of Zakynthos Island, Ionian Sea, Greece. Relevant data were collected through interviews with local fishers between 2019 and 2020. The fishers who took part in these interviews identified two species, namely *Tursiops truncatus* and *Globicephala melas*, while it was noted that the main cause of interactions between cetaceans and fishing boats are due to food.
Keywords: Fisheries, Interaction, Cetaceans, *Tursiops truncatus*, *Globicephala melas*
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1. Introduction

Interaction between dolphins and coastal fisheries is an important issue of increasing concern in the Mediterranean Sea. Due to their experience and knowledge fishers should be seen as an invaluable source of information in the study of fisheries' complexity, and their voices should be taken into account in the design of relevant management strategies (e.g. Maynou *et al.* 2011). Although collisions between cetaceans and boats (tourist, cargo, fishing, to name a few) have been reported through the years in various parts of the world, such interactions are actually documented only in a very few areas (Laist *et al.*. 2001), whilst in the Mediterranean Sea and especially in Greece seems under-reported. In Greece and in the northern Aegean Sea, in specific, such interactions involve dolphins of the following species: common bottlenose dolphin (*Tursiops truncatus*), short - baked common dolphin (*Delphinus delphis*), Risso's dolphin (*Grampus griseus*) and stripped dolphin (*Stenella coeruleoalba*) (Pardalou & Tsikliras 2018). For one year work has been done on dolphin - fisher interactions along the coastline of Zakynthos, Ionian Sea, to identify the factors that affect these incidents by monitoring the actual damage on fishing gear, along with the characteristics and target species of the gear, including the potential compensation of fishers for damage suffered and income lost. This area provides a suitable habitat for the common bottlenose dolphin *Tursiops truncatus* and the *long-finned pilot whales* (*Globicephala melas*).

2. Materials and Methods

The dynamic of Zakynthos as far as the professional fishing vessels concerned is approximately 20, of which seven (7) were selected to allow the margin of repetition with the rest vessels. Fishers' empirical knowledge on human/dolphin interaction was incidentally collected during a summer research project focused on monitoring the damage caused by dolphins on the fishing gear. For this primary project 7 fishers were interviewed thoroughly and the damage to the fishing gear was recorded in detail during the summers of 2019 and 2020. The surveyed fleet included small-scale coastal fishery vessels that operated nets (gill nets, trammel nets, combined nets) as primary or secondary registered gear. Throughout the primary project, additional data on the type of fishing activities were gathered in parallel. The discussions were informal, although based on a questionnaire. All fishers were shown an identification catalogue of the dolphin species known (provided by ARION-Cetacean Rescue and Rehabilitation Research Center) and were requested to distinguish the ones interacting with their fishing activities.

3. Results

Among the species present in the waters of the Ionian Sea, all fishers were able to distinguish common bottlenose dolphins and long-finned pilot whales in the identification catalogue, as the species that primarily interacting with fisheries. Indeed, according to the literature, common bottlenose dolphins interact with coastal fisheries because of their coastal distribution (Frantzis 2007) and catholic diet (Blanco *et al.* 2001), whilst it is the first time that long-finned pilot whales have been recorded to interact with fisheries in the Ionian Sea. According to fishers' empirical knowledge dolphins tend to cause large holes or tears in the nets. Fishers operate with a range of gear types targeting the same species throughout the year but in multiple fishing grounds around the island of Zakynthos variation depending on season, weather, fish stock as well as dolphin occurrences. They also claim that 6 out of 7 fishing activities are unsuccessful due to the damage caused by dolphins (Figure 1). According to fishers, the factors which mainly affect these conflicts are the target species and seasonality. Particularly, due to the high mobility of tourist vessels in the summer there are approximately 20 herds of dolphin in the area. Furthermore, dolphins were described as 'picky choosers', mainly because they feed on commercially important species.

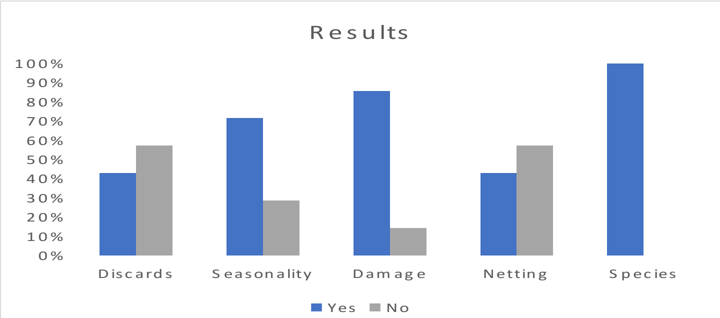

Figure 1: Main factors which have been taken into consideration throughout this preliminary research according to the empirical knowledge shared by the fishers of the Ionian Sea (spoiled catches: n=7, seasonality: n=7, total damage of fishing gear: n=7, netting: n=7, species: n=7)

4. Discussion

Dolphins were described as notably intelligent animals by the fishers, and also able to distinguish between different engine sounds and get used to pingers which are ineffective because dolphins became habituated to the signals produced. All questioned fishers agreed that since nothing can be done, they endure the destruction by constantly repairing their fishing gear. Moreover, they claim that there is no possibility to change their fishing spots although they respect the fact that dolphins need to feed. They illustrated that maybe their problems lie in their bad relationship with the government and not with dolphins (damage of fishing gear). Similarly, they mentioned that if there was an extra fund for the repair of their equipment, which costs enormous amounts of money each year, their relationship with marine mammals would be more peaceful. In fact, it was clearly demonstrated that all the questioned fishers were keen on finding an ecological friendly solution in respect of the environment. Although this study is based on a very small number of questionnaires, and as a preliminary assessment the research outputs may be biased, collection of further data (aka more fishers and regions) are ongoing process in an effort to better understand and evaluate the interactions of cetaceans and fisheries. The future findings will be of great importance toward the improved assessment of the requirements of both fishers and cetaceans, filling data gaps that can better inform what has to be done in order to reduce the possibility of an ecological loss.

Acknowledgements

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References

Blanco C., Salomón O., Raga J.A. (2001). Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. Journal of the Marine Biological Association of the United Kingdom, 81, 1053-58.

Frantzis A. (2007). Fisheries interactions with cetacean species in Hellas. In: "State of Hellenic fisheries", Papaconstantinou C., Zenetos A., Vassilopoulou V., Tserpes G. (eds). Hellenic Centre for Marine Research, Athens, Greece, p. 274-278.

Laist D.W., Knowlton A.R., Mead J.G., Collet A.S., Podesta M. (2001). Collisions between ships and whales. Marine Mammal Science 17, 35-75.

Maynou F., Sbrana M., Sartor P., Maravelias C., Kavadas S., Damalas D., Cartes J.E, Osio G. (2011). 'Estimating Trends of Population Decline in Long-Lived Marine Species in the Mediterranean Sea Based on Fishers' Perceptions. PLoS ONE 6, e21818.

Pardalou A., Tsikliras A.C. (2018). Anecdotal information on dolphin−fisheries interactions based on empirical knowledge of fishers in the northeastern Mediterranean Sea. Ethics in Science and Environmental Politics 18, 1-8.

FLOOD AND HAZARDOUS RISK OF LARNACA CITY UNDER THE CASE OF DIFFERENT SCENARIOS

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Abstract
Floods have always been frequent, but in many parts of the world flood frequency and intensity are increasing, not only because of the atmospheric precipitation but also because of other factors that may affect natural disasters. Cyprus island is one of the countries facing such issues with the historical floods that have been recorded to be quite frequent. There is a significant potential flood risk according to the flood risk maps made for each area following three different scenarios. These three scenarios involve low-probability floods with a 500-year recovery period, medium-probability floods with a 100-year recovery period, and high-probability floods with a 20-year recovery period. The study area that will concern us in the present work is the province of Larnaca in Cyprus, where the zones that are part of this province cover three areas, the area of Kamares-Larnaca, the area of Aradippou-Livadia and the area of Ormideia. This study estimates the buildings that will be damaged in the case of a flood, by flood risk maps, and the land registry for each hypothetical scenario. Analyzing the collected data, they are all gathered on maps and the damage curves are calculated with equations for each category separately. These categories relate to residential, commercial and industrial buildings, as well as transport, infrastructure/roads and agriculture. Finally, some preventive and regulatory ways to properly deal with the flood phenomenon are described.
Keywords: *Floods, Damage curve, European Policy, Larnaca City, Cyprus*
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1. Introduction

The main issue will concern us most in this study is the case of floods, this term refers to the condition of an area that usually is dry and over a period of time is covered by large amount of water. Furthermore, floods are based on the European Directive 2007/60/EC where the European Commission has recognized and established a new common framework for the calculation and monitoring of flood risks, with the main aim of reducing adverse impact on the environment, human health, cultural heritage and economic activities related to floods and directly to the community. The study area that will occupy us in the present work is Larnaca province. Larnaca is located in the southern part of Cyprus island and will have as its main objective the analysis and finding results presented in the flood risk maps of Cyprus at recovery time for 20, 100 and 500 years. Some of the data will be obtained from the Public Internet Service for flood risk (ERMIS-F), but also from google earth and other sources of remote sensing. At this study there is an effort to include: i) the estimation of buildings that will be affected by both land flood scenario according to flood defenses, ii) the creation of a prototype for the damage to the buildings due to floods, iii) the application of this prototype in the buildings of Larnaca and the damage curve that will be calculated and iv) suggestion of flood prevention and regulatory measures in this area.

2. Material and Methods

The island of Cyprus is located in the eastern part of the Mediterranean Sea, as a Member State of the European Union the European directive 2007/60/EC has been updated and incorporated into Cypriot Law with the Basic Law (Law 70 (I)/2010) that was submitted in 2010 and came into force in July of this year with the appropriate adjustments and in 2012 came into force the Amending Law (Law 153(I)/2012). According to the flood plans of ERMIS-F, the potentially high-risk zones in Cyprus are located in all the provinces of the island but we will focus and analyze more those which concern Larnaca province. The high-risk zones are divided into three areas, the first zone is located in Larnaca city called Kamares area. At the Kamares residential area there is an increase of risk due to the low terrain of the area and the small slope that exists in the riverbed. The type of flood that occurs this area is rapid flooding in combination with urban flooding. The river starts from the Aradipou settlement and passes through the northern area crossing the monument of Kamaron and the city north of Salt Lake and finally flows into the sea. The second zone covers the Aradippou and Livadia Municipality, which belong to the Larnaca Province. Through this area passes the Archangelos, Kammitsis, Parapotamos rivers ~10km long and the type of flood is rapid flood that usually prevails in this area. The Kammitsi river after its intersection with the Archangelos river flows and passing a route north to the area of Aradippou urban zone exist. Then the river continues to flow throw agricultural zone and enters the Livadia residential and from there flows until reaching the sea. The third and last area is the smallest area compared to the previous ones that mentioned above. This zone covers the Ormidia area where it is crossed by the river with 3.6km long and with the rapid flood being the type of flood that prevails in the area. This river consists of the main department ~4.5km long that starts south of Xylotympou high school, then passes through the Ormidia settlement and flows into the Dhekelia bay, while the secondary department starts from Vatainas dam, enters Ormidias settlement and joins the main department about 2.40km to the upper side of the estuary.

3. Results and Discussion

According to the flood risk maps (Water Development Department), the prevailing depth at all three study areas ranges from 0 to 7.4 meters. More specifically, the total number of buildings and infrastructure that may affected at the three study areas under the case of the three different scenarios (20,100 and 500 years) has been calculated. Furthermore, the total number of buildings for each category has been calculated separately. The categories that mentioned are single-story residence without basement (5A-1), two-story residence without basement (5A-2), three-story residence without basement (5A-3), single-story residence with basement (6B-1), two-story residence with basement (6B-2), the capital letters A and B correspond to buildings that don't have a basement and to those that do. Moreover, categories that are referred are the buildings with open pile foundation (7A), buildings with enclosed pile foundation (7B) and the category of "others" which mainly refers to churches, shops, schools, incomplete units etc. In addition, the damage curve for the categories that are mentioned above has been calculated and most likely, minimum and maximum damage curves are provided. One important finding is the role that presence or absence of a basement plays in developing damage curves. Finally, some possible remedies are suggested for flood prevention and control measures taking into account the hypothetical spatial planning scenario that have been created for Larnaca province.

References

European Commission (2014). Links between the Floods Directive (FD 2007/60/EC) and Water Framework Directive (WFD 2000/60/EC), Resource Document, Technical Report.

European Commission, 2000. Water Framework Directive (WFD 2000/60/EC), Technical Report.

Interreg Ελλάδα-Κύπρος. Flood Risk Maps for Cyprus. ERMIS-F. https://geoportal.ermis-f.eu/static_maps/cyprus.html

Spaulding M.L. et al., (2016). STORMTOOLS: Coastal Environmental Risk Index (CERI). Marine Science and Engineering, 31 August, pp. 1-27.

Water Development Department, 2011. Flood Risk Assessment. Republic of Cyprus. http://www.moa.gov.cy/moa/WDD/wfdf.nsf/All/5465B3AF75D2A79FC22582E80027E236/$file/1st_Ekthesi-rpokatartiki.pdf?OpenElement

Water Development Department. European Directive 2007/60/EC and the Cyprus Law on Floods http://www.moa.gov.cy/moa/WDD/wfdf.nsf/home_en/home_en?opendocument

Ευρωπαϊκή Επιτροπή, (2007). Οδηγία 2007/60/ΕΚ για την αξιολόγηση και τη διαχείριση των κινδύνων πλημμύρας. Επίσημη Εφημερίδα της Ευρωπαϊκής Ένωσης. https://eur-lex.europa.eu/legal-content/EL/TXT/HTML/?uri=CELEX:32007L0060&from=EL

Τμήμα Αναπτύξεως Υδάτων, (2016). *Σχέδιο Διαχείρισης Κινδύνων Πλημμύρας Λεκάνης Απορροής Ποταμού της Κύπρου για την εφαρμογή της Οδηγίας 2007/60/ΕΚ.* Κυπριακή Δημοκρατία. http://www.moa.gov.cy/moa/WDD/wfdf.nsf/All/EC4436C3C3241E17C22584A50028338A?OpenDocument

TOXICITY EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS TOWARDS MARINE MICROALGAE: A SYSTEMATIC REVIEW

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Abstract The present study is a systematic review of the scientific literature that has been published up to 31/10/2020 regarding the scientific research which has been conducted and concerns the evaluation of PAHs toxic effects towards marine primary producers such as microalgae. Based on the gathered information from the collected and classified articles, useful conclusions and observations are reported about research trends. At the same time, knowledge gaps and weaknesses in the current research are identified and highlighted. Furthermore, suggestions for future research on this topic are also discussed.
Keywords: *Petroleum Hydrocarbons, Ecotoxicological Assessment, Effects, Marine Microalgae, Inhibition*
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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are unsaturated organic substances that exhibit high molecular weights and low water solubility. Their occurrence in the ocean and marine ecosystems is attributed to both anthropogenic sources (pyrogenic and petrogenic PAH's) as much as to natural sources from oil regeneration processes. Petroleum hydrocarbons pollution is the most common type of marine pollution due to leakage from oil refineries or petrochemical factories, accidental spillage from tankers or/and through atmospheric fallout or waterway-transport from the land leading to a streak of negative effects for the marine environment. Marine microalgae are the primary producers of marine ecosystems, which have often been used for the evaluation of environmental effects and toxicity of a wide range of chemical pollutants due to their several different advantages, among which the small size, their rapid growth and reproduction, and finally their great sensitivity are included (Torres *et al*. 2008). For all the afore-mentioned reasons the scientific research focusing on the impacts of oil pollution towards these organisms that are the base of the marine food chain is a field that has been extensively researched worldwide in the scientific community. Therefore, the aim of the current systematic literature review is to summarize important information of the toxicity evaluation studies regarding PAHs towards marine microalgae species. According to the gained infrormation from the present review new areas for future studies can be highlighted and future research proposals in this scientific area are suggested.

2. Methods and Materials

The current systemic review was focused only on peer-reviewed journal articles that were published until 31 October 2020. The retrieve of the relevant published scientific articles was achieved by using Scopus Search (abstract and citation database). The selection of studies was made by using 14 different combinations of selected terms, as depicted in Table 1. It is worth mentioning that only documents which were available in English were included in this analysis. Based on the full article review the selected articles that met the criteria of the bibliographic research and that were included in the present systemic review were summarized using the following information: author (date), location of the study, PAH analyzed, target microalgae species, population (sample size), method used, and primary conclusions.

3. Results

Based on the data found and processed in the present systematic literature review, which provided the latest evidence that microalgae are an important test organism of PAHs and petroleum hydrocarbon pollution. Some highlights of selected studies are summarized in Table 2 in chronological order**.** According to the obtained data a wide range of effects has been observed and reported in the available scientific bibliography, such as growth and biomass inhibition (Li *et al*. 2020), amino acids synthesis (Li *et al*. 2019), fatty acids composition (Liu *et al*. 2019), gene mutations (Bopp & Lettieri 2007; Carrera-Martinez *et al*. 2011, 2010), etc. Moreover, based on the comparative studies that have been conducted it has been documented that the mainly toxic substances of PAHs towards selected marine biota are mostly the 2–5 rings containing molecules, whereas contradictory, PAHs with

>6 rings that have lower hydrophilic character and higher lipophilic capacity, are hardly dissolved in water and remain in water bodies for short times due to their volatility (Short, 2003; Li et al., 2019).

Table 1. Peer-reviewed journal articles with combination of terms/keywords related to PAHs

Combination of terms/keywords	Number of found documents
1. "Marine AND algae AND PAHs AND toxicity"	40
2. "Phytoplankton AND PAHs AND toxicity"	36
3. ''Phytoplankton AND PAHs AND effects''	61
4. "Marine AND algae AND PAHs AND ecotoxicity"	6
5. "Marine AND algae AND PAHs AND effects"	39
6. ''Marine AND algae AND PAHs"	83
7. "Marine AND algae AND PAHs AND bioassays"	11
8. "Marine AND species AND PAHs AND algae"	25
9. "Plankton AND PAHs AND toxicity"	17
10. ''Plankton AND PAHs AND bioassays''	3
11. "Plankton AND PAHs AND inhibition"	2
12. "Algae AND PAHs AND stress"	24
13. "Algae AND PAHs AND response"	71
14. ''Algae AND PAHs AND effects''	139
TOTAL	**557**

Table 2. Some selected references and their highlights found and reviewed in present systematic review.

Microalgae species	Primary conclusions	Reference
Nitzschia closterium	Growth and development effects that could be detected by the $\delta^{13}C$ (carbon isotope ratios) value.	Li N. *et al*. 2020
Nitzschia closterium, and *Heterosigma akashiwo*	Toxic effects were explored by using amino acids (on synthesis mechanism of amino acids in microalgae). Leucine $\delta^{13}C$ in *N. closterium* increased with time, whereas decreased in *H. akashiwo*. For Alanine, Threonine, and Methionine, $\delta^{13}C$ values showed unexpected trend.	Li N. *et al*. 2019
Ulva pertusa	Inhibition of algae biomass. Effect of oil spill stress on fatty acid stable carbon isotope composition.	Liu *et al*. 2019
Dunaliella tertiolecta	Cell gene mutations.	Carrera-Martinez *et al*. 2010
Thalassiosira pseudonana	Genes involved in the metabolism of studied microalgae are affected by PAHs.	Bopp & Lettieri 2007

Overall, the reviewed information provided a variety of numerous research approaches exploring the toxicity mechanism of primary producers under PAHs exposure and oil spill stress. Hence, it was shown that several successful research attemts have been made to monitor and evaluate the marine ecological risk of those persistent pollutants using marine algal species. Finally, marine phytoplankton is an effective and sensitive biomarker for the evaluation of PAHs' toxicity in seawater ecosystems.

References

Bopp S.K., Lettieri T. (2007). Gene regulation in the marine diatom *Thalassiosira pseudonana* upon exposure to polycyclic aromatic hydrocarbons (PAHs). Gene 396, 293-302.

Carrera-Martínez D., Mateos-Sanz A., López-Rodas V., Costas E. (2010). Microalgae response to petroleum spill: an experimental model analysing physiological and genetic response of *Dunaliella tertiolecta* (Chlorophyceae) to oil samples from the tanker prestige. Aquatic Toxicology 97, 151-159.

Carrera-Martinez D., Mateos-Sanz A., Lopez-Rodas V., Costas E. (2011). Adaptation of microalgae to a gradient of continuous petroleum contamination. Aquatic Toxicology 101, 342-350.

Li N., Liu Y., Liang Z., Liu Y., Lou Y. (2020). Effect of petroleum hydrocarbons on the carbon and nitrogen stable isotope composition of *Nitzschia closterium.* IOP Conf. Ser: Earth & Environmental Science 446, 032039.

Li N., Liu Y., Liu Y., Lou Y., Zhao, X. (2019). Effect of oil spill on compound-specific stable carbon isotope composition of amino acid in *Nitzschia closterium* and *Heterosigma akashiwo.* Science of the Total Environment 653, 1095-1104.

Liu Y., Liu Y., Li N., Lou Y., Zhao X. (2019). Effect of oil spill stress on fatty acid stable carbon isotope composition of *Ulva pertusa.* Science of the Total Environment 649, 1443- 1451.

Short J. (2003). Long-term effects of crude oil on developing fish: lessons from the Exxon Valdez oil spill. Energy Sources 25, 509-517.

Torres M.A., Barros M.P, Campos S.C., Pinto E., Rajamani S., Sayre R.T., Colepicolo P. (2008). Biochemical biomarkers in algae and marine pollution: a review. Ecotoxicology and Environmental Safety 71, 1-15.

HYDROLOGICAL SIMULATION OF THE STREAM SEDOUNTA OF PLOMARI LESVOS

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Abstract

Ephemeral streams are typical Mediterranean fluvial systems with high risk of flooding. The present work describes the hydrological simulation of the ephemeral stream Sedounta, which passes through the settlement of Plomari in Eastern Lesvos. The wider area of Plomari often faces hazardous floods and disasters and therefore needs further investigation. The software Hec-GeoHMS at ArcMap is used to calculate the hydrological characteristics of the area and HEC-HMS for the hydrological simulation of the six scenarios with and without the dam and for return period 50, 100 and 1000 years. According to the simulation results, the outflow of the basin is 39.5 m^3/s with return period 50 years and 90.5 m^3/s with return period 100 years. Also, there is a slight decrease in discharge in the dam scenarios by 0.51%. Therefore, according to the research, it is observed that Plomari village is extremely vulnerable to flash floods.

Keywords: *Hydrological Simulation, HEC-HMS, ArcMap, IDF curve, Floods*

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1. Introduction

Flooding is one of the major hazards in Mediterranean ephemeral streams (Camarasa-Belmonte 2016) and is controlled by a number of variables, one of which is rainfall intensity (Merz & Bloschl 2003). Floods cause significant damage, such as economic and environmental damage, and can potentially lead to loss of human life or health problems (Ohl & Tapsell 2000, WHO 2002, Hajat *et al*. 2003). The hydrological simulation determines the peak discharge of the sub-basins of a catchment area, thus helping to understand flood processes and suggest measures to mitigate flood risk. The objective of the current study is the hydrological analysis of the hydrological regime of the ephemeral stream of Sedounta, in Eastern Lesvos.

2. Materials and Methods

Hydrognomon software is used to calculate the Intensity-Duration-Frequency curve (IDF curve), which describes the relationship between rainfall intensity, rainfall duration, and return period. For the Sedounta stream in the wider area of Plomari, the IDF curve is calculated based on the 5-min precipitation from 2003 to 2020 of Agia Paraskevi meteorological station. Then, the Hec-GeoHMS is used to produce a series of spatial maps such fill sinks, flow direction, flow accumulation, stream definition, stream segmentation, catchment grid delineation, river length, river slope, basin slope. The hydrological software HEC-HMS is used to simulate the Sedounta stream. The SCS (Soil Conservation Service) unit routing model and the CN (Curve Number) are calculated using the land use and soil type maps. A total of 6 repetitive simulations were performed for the scenarios with or without the dam and for the return period of 50, 100 and 1000 years. For the scenarios with the dam, it is necessary to enter the Capacity-Height-Supply ratio of the Sedounta dam described in the technical plans of the Technical Works Service of the North Aegean Region for the study area.

3. Results and Discussion

According to the results of the digitization of the Sedounta catchment area, 7 sub-basins are delineated, as shown in Figure 1. The Figure also shows the location of the Sedounta dam in the basin. Figure 2 shows the IDF curve of Agia Paraskevi with return periods 50, 100 and 1000 years, which demonstrates that as duration rises, intensity gradually declines. Figure 3 shows the Peak Discharge of the outlet of the basin for the scenarios of the three return periods (50, 100 and 1000 years) and with and without the dam. For a return period of 50 the Peak Discharge is 39.5 m^3/s and for 100 and 1000 yrs are 48.7 m^3/s and 90.5 m^3/s correspondingly. There is a slight reduction in discharge, in the dam scenarios only for low return periods of precipitation. For instance, peak discharge of the stream's output of the model without the dam is 39.5 m^3/s, while the model with the dam is 39.3 m^3/s, with precipitation of a return period of 50 years.

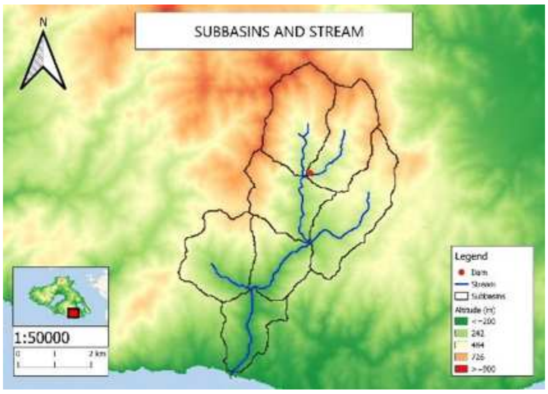

Figure 1: Sedounta's hydrographic network

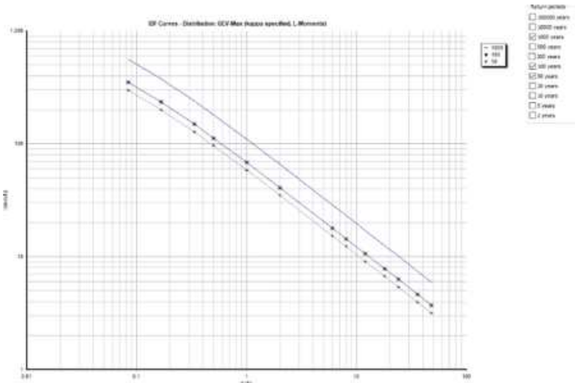

Figure 2: IDF Curve of Agia Paraskevi in which y= intensity(i) and x=duration(d)

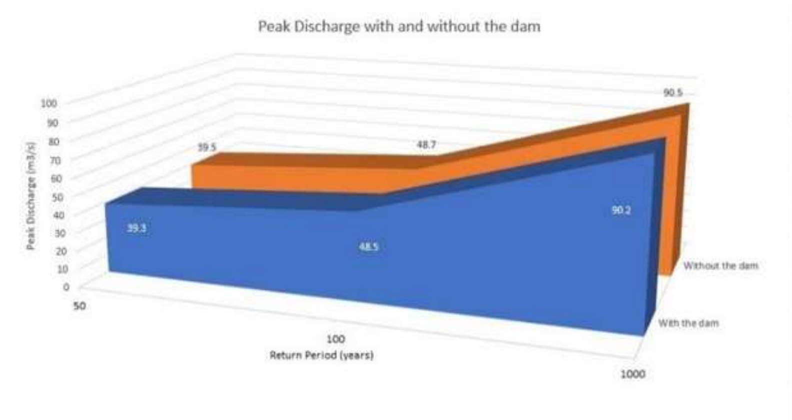

Figure 3: Peak discharge of the outlet of the basin with return periods 50, 100 and 1000 years, with and without the dam.

4. Conclusions

According to the results, the lower sub-basins show higher peak flows in comparison to the upstream ones. Also, while return period significantly affects discharge, dam scenarios show only a small reduction in total outflow. Plomari village is greatly vulnerable to floods and significant measures must be taken. In this direction, flood defense works can be considered that can help the region in this problem: the construction of numerous small capacity dams in the stream, the diversion of a tributary of the stream or the construction of another dam that controls water discharge.

References

Camarasa-Belmonte A.M. (2016). Flash floods in Mediterranean ephemeral streams in Valencia Region (Spain), Journal of Hydrology Part A 541, 99-115.

Hajat S., Ebi K. L., Kovats S., Menne B., Edwards S., Haines A. (2003). The human health consequences of flooding in Europe and the implications for public health: a review of the evidence. Applied Environmental Science and Public Health, 1(1), 13-21.

Merz R., Bloschl G. (2003). A process typology of regional floods. Water Resources Research 39 (12), 1340.

Ohl C.A., Tapsell S. (2000). Flooding and human health: dangers posed are not always obvious. British Medical Journal, 321, 1167–1168.

WHO (2002). World Health Organization of the United Nations, Floods: climate change and adaptation strategies for human health. https://apps.who.int/iris/handle/10665/347625

MEASURED AND ESTIMATED NITROGEN AND PHOSPHORUS LOADINGS OF FRESHWATER ARCTIC CHAR FISHFARMS

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Abstract
Fish farming, like nearly all forms of aquaculture and agriculture, generate significant amounts of waste, including nutrients, waste feed and fish feces. Monitoring and calculating N and P discharge from fish farms can be estimated using records of fish production and feed rate. Additionally the amount of N and P released by the fish farm can be estimated using feed conservation rates combined with chemical analyses of feed and fish and water. The aim of the present work is to compare estimated total nitrogen and phosphorus quantities with the previously mentioned methods, using data from Arctic char fish farms in Iceland. This analysis can be used to improve the ecological monitoring process and assess the validity of the aquaculture modeling.
Keywords: *Nutrient Loading, FTS land-based Aquaculture, Nutrient modelling*.
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1. Introduction

Fish farming, like nearly all forms of aquaculture and agriculture, generate significant amounts of waste, including nutrients, waste feed and feces, and by-products such as chemical residues; thus, the impact of aquaculture on the surrounding environment and ecosystem is a function of several parameters, such as nitrogen (N) molecules (NHX, NOx, DON), and phosphorus (P/POX) (Wallace 1993; Klaoudatos 2001; Karakassis *et al*. 2005; Coldebella *et al*. 2020). Changes in the surface and column of water ecosystems, as well as changes in the benthic ecosystems are exhibited due to aquaculture, reflecting changes in water quality and the ecosystem (Mavraganis *et al*. 2020). The FTS technology used in the Icelandic farms ranges from using only gravity flowing water to pumping water into tanks with oxygenation. Most of the Icelandic farms have limited or no treatment of the effluent water (Thorarensen *et al*. 2013). Monitoring and calculating N and P discharge from fish farms can be done using either records of fish production and feed used or feed conservation rates (FCR), such as FCR combined with chemical analyses of feed and fish (OSPAR 2000). The approach to quantifying N and P discharge from aquaculture to surface waters is described in Guideline 2 (OSPAR 2000). The aim of the present work is to compare estimated total nitrogen and phosphorus quantities with measured ones using data from Arctic char fish farms. This analysis can be used to improve the ecological monitoring process and assess the validity of the aquaculture modeling.

2. Material and Methods

Samples from land-based FTS Arctic char farms were collected by the Icelandic Environmental Agency and the School of Fish Biology and Aquaculture at the Holar University College for their productivity parameters, the total nitrogen and phosphorus loading annually in 2015. The modelled quantities of total nitrogen and phosphorus were calculated by using the OSPAR 2000 model.). The model for flow-through systems (FTS) is given as $L= 0.01*(ICi- PCf)$ in which L is the quantity of P or N in a water body (tonnes/year), I is the feed used (tonnes/year), Ci is the phosphorus or nitrogen content in the feed (%), P is the production (tonnes/year), and Cf is the P or N content in the resulting organisms (%). All the quantities transformed to tonnes per year and the data tested for normality using the Shapiro testing methodology in R. None of the groups were not normally distributed ($P<0.05$). Two tailed Mann Whitney testing analysis was applied to estimate the comparison of the groups means found to be not statistically significant ($U<0.05$).

3. Results and Discussion

It can be seen in Figure 1 the total nitrogen estimated model values to be higher than the measured ones, besides for the farm no5. The same can be seen in Figure 2 for three farms from total phosphorus quantities, while two farms showed to have higher measured quantities. In Table 1 the results from the comparison of the measured total nitrogen and phosphorus with the model estimated values can be seen to be statistically not significantly different.

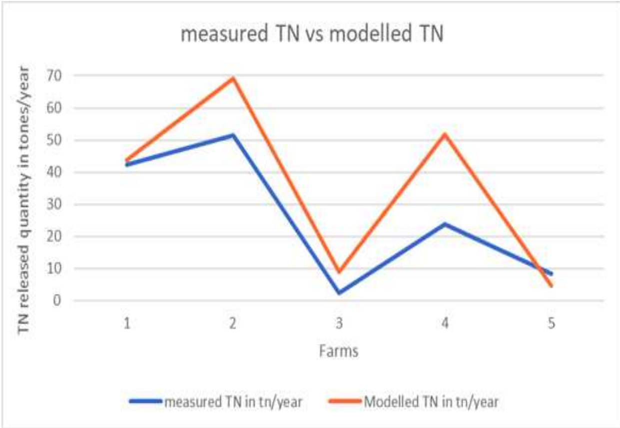

Figure 1. Measured total nitrogen and model estimated nitrogen quantities

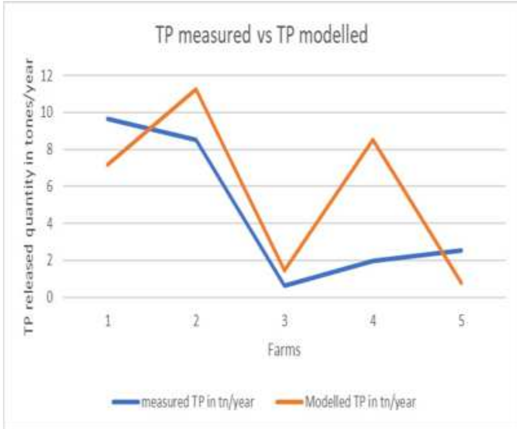

Figure 2. Measured total phosphorus and model estimated phosphorus quantities

Table 1. Comparison of the measured total nitrogen and total phosphorus quantities along with the model estimated ones using two tailed Mann Whitney U testing.

Farm Loading	U value	Z-score
Total Nitrogen	42,5	-0,53
Total Phosphorus	36,5	-0,98

Acknowledgements

Data collected through the Environmental Agency of Iceland and Holar University College. Sindri Mar Smarason assisted in translating some data from Icelandic to English.

References

Coldebella A., Godoy A.C., Gentelini A.L., Piana P.A., Coldebella P.F., Boscolo W.R., Feiden A. (2020). Nitrogen and phosphorus dynamics in Nile tilapia farming in excavated rearing ponds. *Research, Society and Development 9*(11), e1319119699-e1319119699.

Karakassis I., Pitta P., Krom M.D. (2005). Contribution of fish farming to the nutrient loading of the Mediterranean. Scientia Marina 69: 313–321.

Klaoudatos S.D. (2001). Environmental impact of aquaculture in Greece. Practical experiences. National Centre for Marine Research, Aghios Kosmas, 16604 Athens, Greece. 2001.

Mavraganis T., Choremi C., Kolygas M., Vidalis K., Nathanailides C. (2020). Environmental Issues of Aquaculture Development. Egypt Journal of Aquatic Biology of Fish 24(2), 441–450.

OSPAR Commission (2000). HARP-NUT Guideline 2: Quantification and Reporting of Nitrogen and Phosphorus Discharges/Losses from Aquaculture Plants. OSPAR Agreement.

Thorarensen H., Bergheim A., Dumas A., Jóhannsson R., Þórarinsdóttir R. (2013). *Tomorrow´s aquaculture systems, NORA Final Report*. Hólar, 2013-2 ISSN: 2298-3880.

Wallace J. (1993). Environmental considerations. pp.127-144. Salmon Aquaculture. 1993, Fishing News, Oxford, United Kingdom.

PUMPING COST MINIMIZATION WITH THE USE OF AN ITERATIVE ALGORITHM IN SIMANTRA SETTLEMENT, CHALKIDIKI, GREECE

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Abstract
Groundwater extraction is, in many cases, the predominant way of providing fresh water for serving the various water needs in a region. Any effort to minimize the energy consumed in this process has both environmental and financial benefits. In this perspective, the present paper studies the problem of minimizing the energy consumption during the abstraction of groundwater used for satisfying the water demands of the settlement of Simantra, located in Chalkidiki, Greece. The methodology followed herein consists of the linkage between a steady-state groundwater flow model developed to simulate the flow behavior in the aquifer under study and an iterative optimization algorithm used in order to find the optimal distribution of the well system flow rates. With regard to the optimization model, the energy losses due to friction that occurs within the pipes used for pumping and transporting water from the wells to a water tank were also taken into consideration. It turns out that the energy consumption is minimized when the stationary condition receives a common value for all managed wells.
Keywords: *Groundwater Management, Water-Supply Wells, Pumping Cost Minimization, Analytical Solution, MODFLOW, Nea Moudania Aquifer*
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1. Introduction

The problem of minimizing the energy consumption during groundwater abstraction is a frequent problem in the field of groundwater resources management. A common practice to deal with this problem is the linkage between simulation and optimization models. In this paper, an application of combining a simulation model with an optimization algorithm in the aquifer of Nea Moudania, Greece, is presented. The optimal solution is achieved when the stationarity condition is satisfied (Mallios & Tsiarapas 2021).

2. Materials and Methods

The problem considered in the present paper is to find the optimal distribution of the extracted flow rates of a system of six wells used for providing water in the settlement of Simantra, Chalkidiki, Greece. The optimal solution ensures the minimum energy consumption during both the pumping and transporting of water to a water tank that serves the water demands of the settlement. The general form of the objective function of the problem, which has to be minimized, is defined as the sum of the energy costs of all managed wells, i.e.:

$$Minimize E_{tot} = \frac{\rho \cdot g \cdot t}{1000 \cdot \eta_{pump}} \cdot \left[\sum_{i=1}^{n} \left(\frac{8f_i}{g\pi^2 D_i^5} \cdot Q_i^3 + Q_i \right) \cdot \left(\delta_i + \sum_{j=1}^{n} r_{ij} \cdot Q_j \right) + \sum_{i=1}^{n} \left(\frac{8f_i L_i}{g\pi^2 D_i^5} \cdot Q_i^3 \right) \right] \quad (1)$$

where ρ is the water density, g is the acceleration of gravity, t is the duration of pumping, n_{pump} is the efficiency coefficient of the pumps (which is considered constant and equal for all pumps), Q_i is the pumping rate of the ith well, f_i is the friction factor, δ_i is the initial lift at location i, L_i is the total length and D_i is the diameter of the pipes used for pumping and transporting water to the tank, and, finally, r_{ij} is the response of the hydraulic head at each location to the applied pumping rates and is called response coefficient (Ahlfeld & Laverty 2015). The response coefficients r_{ij} were calculated using a steady-state model developed for the study area by Siarkos & Latinopoulos (2016), while considering constant transmissivity conditions (Ahlfeld & Laverty 2015).
Mallios & Tsiarapas (2021) presented an analytical solution that considers the head losses occurring along the pipes used for pumping and transferring the water to a tank. This analytical solution leads to the following stationarity condition which is satisfied for the optimal pumping flow rates:

$$\sum_{j=1}^{n} \frac{8f_j}{g\pi^2 D_j^5} \cdot r_{ij} \cdot Q_j^3 + 3 \cdot \frac{8f_i}{g\pi^2 D_i^5} \cdot Q_i^2 \cdot (s_i + \delta_i + L_i) + 2 \cdot s_i + \delta_i = c \quad (2)$$

The settlement of Simantra belongs to the Nea Moudania basin, which is located in the southwestern part of Chalkidiki Peninsula, Greece (Figure 1). The settlement's total water needs are estimated to be equal to

274.41 m^3/day. This demand is satisfied from a system of six wells which pump water from the underlying Nea Moudania aquifer. As shown in Figure 1, the water is pumped from the aquifer and then transported to a water tank that serves the settlement's needs.

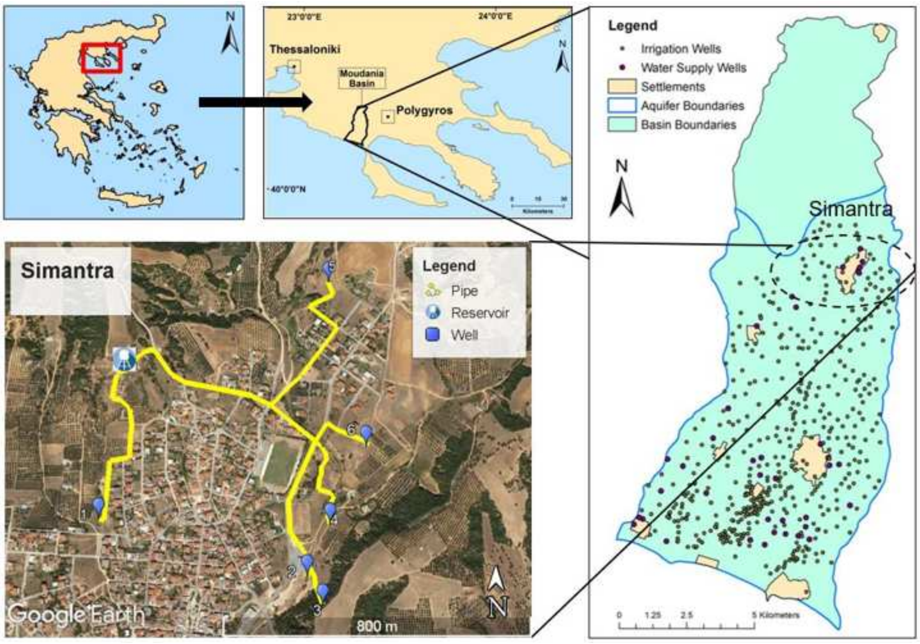

Figure 1. Study area location, along with the boundaries of the underlying aquifer, the wells (both irrigation and water supply wells) operating in the region, the water supply wells used for pumping optimization (managed water supply wells), the pipeline network and the water tank.

3. Results and Discussion

Table 1 presents the results of the optimization algorithm. More specifically, the table shows the flow rate of each one of the six managed wells, the initial lift at the location of each well, the length of the pipes that connect each well with the water tank, the drawdown at the location of each well, the head losses due to friction which occurs in the pipes used for transporting water to the tank, the value of the stationarity condition for each well, and the corresponding energy consumption. Based on the results, the calculated total energy consumption is 126.37 KWh per day, while the well pumping rates range from 28.54 (Well 3) to 61.69 m^3/day (Well 5).

Table 1: Optimal pumping flow rates distribution and the corresponding energy consumption

Well	Pumping, Q (m^3/day)	Initial lift, δ_i (m)	Pipe length, L_i (m)	Drawdown s_i (m)	Head losses, h_f (m)	Stationary condition P_i	Electricity consumption E (KWh/d)
1	59.57	112.98	569	1.74	3.73	127.661	27.47
2	35.30	117.01	1040	1.99	2.22	127.661	16.66
3	28.54	119.15	1114	1.93	1.55	127.661	13.62
4	40.24	115.81	919	2.05	2.58	127.661	18.87
5	61.69	104.60	1007	1.75	6.52	127.661	27.11
6	49.07	113.40	840	2.00	3.54	127.661	22.65
Totals	274.41						126.37

References

Ahlfeld D.P., Laverty M.M. (2015). Field scale minimization of energy use for groundwater pumping. Journal of Hydrology 525, 489-495.

Mallios Z., Tsiarapas A. (2021). An iterative algorithm for groundwater pumping energy cost minimization. Engineering Optimization, 1-15.

Siarkos I., Latinopoulos P. (2016). Modeling seawater intrusion in overexploited aquifers in the absence of sufficient data: application to the aquifer of Nea Moudania, northern Greece. Hydrogeology Journal 24, 2123-2141.

MOLECULAR PHYLOGENY OF LAND SNAIL SPECIES OF GREECE, FAMILIES HELICIDAE AND GEOMITRIDAE

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Abstract
In this research we achieved the construction of a phylogenetic tree with snail species of Helicidae and Geomitridae families from Greece. Findings support that Helicidae species form polyphyletic group, with *Theba pisana* (Helicidae) to be phylogenetically closely to Geomitridae species, possible due to species ecology and behavior.
Keywords: *Molecular Phylogeny, Snail species, Maximum Likelihood Method, Species Conservation, Habitat, Morphology, South-East Europe,*
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1. Introduction

Terrestrial gastropods have about 35.000 species and that makes them one of the most successful and diverse groups of terrestrial ecosystems (Barker 2001). Studies on snail species have been conducted, not only for geographic inference, but also to figure out how fitness related traits, such as morphology and behavior, correlates with the environment (Feher *et al*. 2013; Caro *et al*. 2019; Pfenninger *et al*. 2005). The aim of this study is the construction of a phylogenetic tree, including species of Greece, in order to describe the phylogenetic relations and use the data for future conservation analysis.

2. Materials and Methods

DNA from four different species were extracted using the phenol/chloroform protocol and Polymerase Chain Reaction for mitochondrial gene 16S was held, at annealing temperature of 50 °C.
Other species of Helicidae and Geomitridae families, that inhabit the region of Greece were searched at GBIF, and their 16S gene sequences were downloaded from NCBI database. Sequences were aligned using the program ClustalX. Maximum Likelihood tree (Figure 2) with 1,000 Bootstrap replications was constructed, using the General Time Reversable Model (GTR + I + G).

Figure 1: **Species and locations of the samples.**

3. Results

This analysis involved 30 nucleotide sequences. There were a total of 1056 positions in the final dataset. Helicidae species formed a polyphyletic group, with *T. pisana* to be nested along with Geomitridae species. Moreover, *Helix aspersa* and *Helix pelagonesica* are paraphyletic compared to other *Helix* species. *Helix schlaeflii* and *Helix vladika* formed a sister group with 90% posterior probability and they are most distantly related to *Helix aspersa* which is closely related to *Helix pelagonesica* with 91% bootstrap probability value. *Cochlicella Barbara* formed sister groups with *Cochlicella acuta* with maximal support of their node (100%).

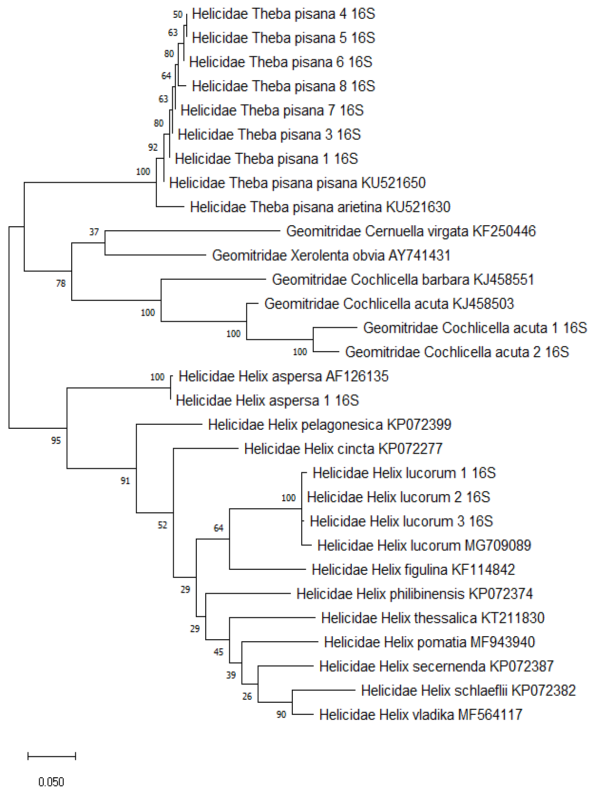

Figure 2: Maximum Likelihood tree, 1000 Bootstrap repetitions

4. Discussion

The probability that there are differences with respect to genetic mechanisms may increase the phylogenetic distance within a given family. This has been shown to other land snails' lineages (e.g. Neiber & Hausdorf 2015), where fitness related-traits, such as banding polymorphism or foraging strategies (Baker *et al*. 2012) may differ between species of the same Family. The adjustments they made over time to cope with the environmental pressure can be shown through phylogenetic relationships using mitochondrial genes and also by comparing other families' species together.

References

Baker G.H., Beckett S., Thammavongsa B. (2012). Are the European snails *Theba pisana* (Müller, 1774) (Helicidae), *Cernuella virgata* (da Costa, 1778) and *Cochlicella acuta* (Müller, 1774) (Hygromiidae) attracted by potential food odours? Crop Protection, 42: 88 -93.

Barker G.M. (2001). Gastropods on land: phylogeny, diversity and adaptive morphology. In: Barker, G.M. (Ed.), The biology of terrestrial molluscs. CAB International, New York, pp. 1–146.

Caro A., Neiber M.T., Juan Gómez-Moliner B., José Madeira M. (2019). Molecular phylogeny and biogeography of the land snail subfamily Leptaxinae (Gastropoda: Hygromiidae). Molecular Phylogenetics and Evolution, 106570.

Feher Z., Parmakelis A., Koutalianou M., Mourikis T., Eross Z.P., Krizsik V. (2013). A contribution to the phylogeny of Albanian *Agathylla* (Gastropoda, Clausiliidae): insights using morphological data and three mitochondrial markers. Journal of Molluscan Studies, 80(1), 24–34.

Neiber M.T., Hausdorf B. (2015). Molecular phylogeny reveals the polyphyly of the snail genus *Cepaea* (Gastropoda: Helicidae). Molecular Phylogenetics and Evolution 93:143-149.

Pfenninger M., Hrabáková M., Steinke D., Dèpraz A. (2005). Why do snails have hairs? A Bayesian inference of character evolution. BMC Evolutionary Biology, 5(1), 59.

OCCURRENCE OF ANTHROPOGENIC MICROPARTICLES IN *Boops boops* FROM LESVOS COASTAL AREA(NE AEGEAN)

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Abstract
Microplastics (MPs) in the marine environment have received increasing attention in recent years. In this study, the gastrointestinal tracts of 25 bogues (*Boops boops*) originating from the coastal area of Lesvos, were analysed to assess microplastic ingestion. Microplastics were detected in 80% of samples analysed. A total of 85 MPs were isolated, with fragments (61%) predominating over the fibers (39%). The size of the majority of ingested MPs was 400-1600 μm, while no correlation was found between the microplastic size and the fish body length ($p > 0.05$, ANOVA). The average of ingested microplastics was 4.25±0.59 MPs per fish. Statistical tests have shown that the ingestion of MPs does not depend on the sex of the fish ($p > 0.05$, Chi-squared test).
Keywords: *Microplastics, Boops boops, Gastrointestinal Tract, Lesvos*
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1. Introduction

Plastic litter accounts for 80% of marine debris from surface waters to deep-sea sediments, as a result of both land-based and sea-based human activities (GESAMP 2019). Microplastics (MPs) are produced either in a size of less than 5 mm or are derived from photolytic, mechanical and biological decomposition of larger plastic residues (Derraik 2002). The presence of MPs is widespread and ubiquitous in the marine environment causing harmful effects on marine life. Due to their small size, they are easily absorbed by organisms near the base of the ocean food chain, thus having the possibility of transporting these pollutants to human level (Gall & Thompson 2015). For benthopelagic habitats, the species *Boops boops* was identified as the most suitable organism for monitoring the ingestion of MPs throughout the Mediterranean basin (Bray *et al*. 2019).
The objective of the present study was to detect the presence and describe the characteristics of anthropogenic particles ingested by *Boops boops* specimens from the coastal area of Lesvos.

2. Material and Methods

Twenty five *Boops boops* specimens caught by local fishermen off Kalloni Bay on February 2019 were purchased from local marketplace in Mytilene and stored at -20 °C until further processing. In the lab, the morphological characteristics of each specimen were measured and then each fish was dissected to determine the sex and extract the gastrointestinal tract (GIT) for anthropogenic particle analysis. Each GIT, was weighted and then kept in the freezer. To degrade organic matter and enable detection of MPs, the GIT of each specimen was treated with 30% H_2O_2, 1:20 w/v and heated at 55-65°C on a hot plate, up to the point the tissue was completely degraded. After digestion, the remaining material was diluted with ultra clean water (Ultra-clear™ TP, TWF device, EVOQUA®) and filtered on fiberglass filters (Whatman® GF/C, pore size 1.2 μm,). MPs on the filter were examined visually using a Nikon SM800 stereomicroscope with a built-in Tucsen True Chrome HD camera, photographed, counted and categorized to different types (i.e., fiber, fragment etc) according to the guidelines of Hanke *et al*. (2013). Blank samples were kept at each step of the process, while precautions were taken to avoid contamination of the samples by airborne microparticles at all stages of the procedure. The recorded data were organized into Excel sheets, categorized to different types and then statistical analyses were implemented with Excel using the Data Analysis ToolPak.

3. Results and discussion

Length and weight of bogues as well as the weight of GIT are reported in Table 1 (according to sex; 8 males and 17 females). A total of 85 MPs were found in the GITs of bogues. It was found that 80% of the analyzed individuals (20 specimen) contained MPs in their GIT. Females showed the lowest ingestion of anthropogenic particles corresponding to a mean value of 3.69±0.66 items/individual, while the average number of microplastics ingested by the analysed specimen was 4.25±0.59 items/individual (mean ± SD; Table 1). Regarding the typology, fragments accounted for 61% (52 items), while fibers accounted for the rest 39% (33 items) (Table 1). The results obtained in the current study are similar with those reported for bogues from other areas of the Mediterranean Sea (e.g., Nadal *et al*. 2016).

Table 1. Biological parameters, occurrence and abundance of ingested microplastics (MPs) in *B. boops* from Lesvos coastal area

	N	Body Length (cm)	Body Weight (g)	GIT (g)	GITs with MPs	MPs & MPs/individual		
						Fibers	Fragments	total
Males	8	15,4-18,6 17,13±0,44	47,58-110,53 72,67±7,67	0,97-4,89 2,30±0,43	7	12 3,00±0,71	25 5,00±1,67	37 5,29±1,13
Females	17	15,4-20,0 17,85±0,33	28,02-149,6 93,09±7,97	2,54- 5,99 4,14±0,26	13	21 2,33±0,41	27 2,45±0,56	48 3,69±0,66
All	25	15,4-20 17,61±0,26	56,37-149,6 86,56±6,16	0.97- 5.99 3.60±0.11	20	33 2,54±0,35	52 3,25±0,69	85 4,25±0,59

Depending on the type of MPs found in their GITs, the 25 bogues specimen were divided into 4 categories. Males and females were classified in these categories differently (Figure 1), however statistical analysis showed that the ingestion of MPs does not depend on the sex of the fish ($p > 0.05$, Chi-squared test).

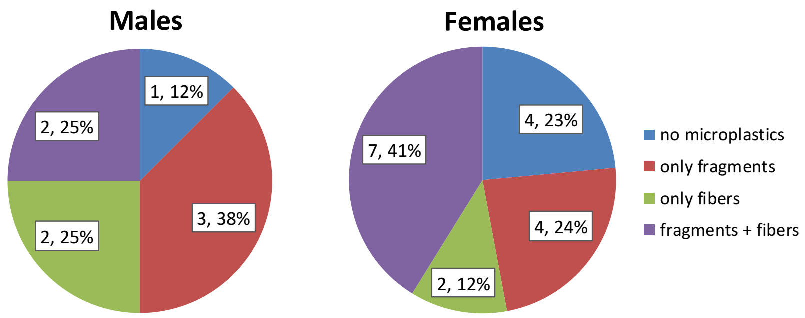

Figure 1. Percentage distribution of morphological types of microplastic particles (fragments and fibers) found in the GITs of male and female individuals examined.

The size (maximum length) of the fibers found in the GITs of the analyzed bogues ranges from 0.163 to 2.574 mm (average value: 0.10±1.06 mm), while the maximum length of the fragments varies between 0.024 and 0.228 mm (average value: 0.92±0.01 mm). The most common size class of ingested MPs was 400-1600 μm. No correlation was found between the microplastic size and the fish body length ($p > 0,05$, ANOVA).
In total 12 different colours were identified among the MPs. Fibers were classified in 9 colours and the most frequent one was green (28%), while fragments corresponded to 10 colours and the most common was brown (38%). Female bogues exhibited the largest colour variation of the ingested anthropogenic microparticles. Further investigation on bogues, as well as other commercial species, will contribute to identify the impact of MPs.

References

Bray L., Digka N., Tsangaris C., Camedda A., Gambaiani D., de Lucia G.A., Matiddi M., Miaud C., Palazzo L., Pérez-del-Olmo, A., Raga J.A., Silvestri C., Kaberi H. (2019). Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. Environmental Pollution 247, 1071-1077.
Derraik J.G.B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin 44, 842–852.
Gall S.C., Thompson R.C. (2015). The impact of debris on marine life. Marine Pollution Bulletin 92, 170-179.
GESAMP (2019). Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean. Kershaw P.J., Turra A., Galgani F. (eds), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 99, 130p.
Hanke G., Galgani F., Werner S., Oosterbaan L., Nilsson P., Fleet D., Kinsey S., Thompson R., Palatinus A., Van Franeker, J. A., Vlachogianni T., Scoullos M., Veiga J. M., Matiddi M., Alcaro L., Maes T., Korpinen S., Budziak A., Leslie H., Liebezeit G. (2013). Guidance on Monitoring of Marine Litter in European Seas. EUR 26113. Luxembourg (Luxembourg): Publications Office of the European Union; 2013. JRC83985.
Nadal M.A, Alomar C., Deudero S. (2016). High levels of microplastic ingestion by the semipelagic fish bogue *Boops boops* (L.) around the Balearic Islands. Environmental Pollution 214, 517-523.

OCCURRENCE OF ANTHROPOGENIC MICROPARTICLES IN *Boops boops* FROM LESVOS COASTAL AREA(NE AEGEAN)

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Abstract
Coastal protection from erosion and flooding is an important ecosystem service of marine benthic vegetation that needed to be further explored regarding the species morphology, especially in the Mediterranean Sea. We used the dissolution rate of gypsum cones (fd) measured as a percent dissolution per time to model the effect of *Ericaria barbatula's* forest in attenuating waves approaching the shore. The data showed that *E. barbatula* forest could attenuate waves, with species abundance estimated by the cover (%) being the best indicator. Such information is essential for developing indicators of the restoration success of fucalean for providing managers and policymakers with data on sustainable and cost-effective solutions for coastal defense.
Keywords: Dissolution rate of gypsum, Wave Attenuation, Coastal Protection, Ecosystem Service.
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1. Introduction

Canopy-forming seaweeds, generally large brown algae such as kelps and fucoids *(*e.g., species of the genera *Cystoseira, Ericaria,* and *Gongolaria)*, dominate shallow rocky coasts of the world's temperate and cold-water seas, providing valuable ecosystem services (Schiel & Foster 2006). They play a pivotal role in coastal areas offering biomass and nurseries (Steneck *et al*. 2002). Recently, there has been increasing interest in whether coastal habitats can provide another highly valued ecosystem service, i.e., coastal protection through wave attenuations. Climate change and coastal urbanization increase the risk of erosion and flooding along coastlines globally (Hinkel *et al*. 2014). Identifying the appropriate solutions for protection from contemporary and future hazards is one of the challenges facing coastal communities. A greater understanding is needed if fucalean forests in the Mediterranean Sea can provide coastal protection as documented by Kelps in other parts of the world (Morris *et al*. 2018). This study aimed to test the relationships between different parameters (cover %, height, and perimeter) of *Ericaria barbatula* forest with wave attenuation in a coastal area of the Kavala Gulf, Greece.

2. Materials and methods

The experiments were realized in the Kavala Gulf (Elaiochori beach), an area dominated down to 1-2 meters depth by dense fucalean forests. Pre-weighed gypsum cones were placed in a gradient of different abundances (% cover) *of E. barbatula* meadow to assess the effect of fucalean forest on hydrodynamics. These constructions (10 in number), were fixed on a base and left for 24 hours in the field at a depth of 60 to 110 cm, which corresponds to the average sea level where the daily hydrodynamics is intense. The dissolution rate of gypsum was measured as a percent dissolution per time, fd: fd (Dissolution rate of gypsum) = (Ma - Mb) / (Ma * t) * 100%, where Ma is the initial dry mass of the gypsum before the experiment, Wb is the dry mass at the end of the experiment, and t is the duration, in hours, of the experiment. This formula gives the percent dissolution of gypsum per h, and is a measure of the fractional amount of dissolved gypsum. Higher values of fd correspond to greater dissolution, resulting from an increase in water flux within the forest thalli (Reidenbach *et al*. 2006). Close to each gypsum cone *E. barbatula*'s cover (%) was estimated by using a metallic frame of 25 x 25 cm. For measuring thalli height and perimeter a thin non-elastic thread (fishing line) was laid along or around the branches of *E. barbatula* always at the same position and under maximum tension. Then the thread was placed on a tape measure and was counted the length between the two points held by thumb and finger of each hand.

3. Results

Figure 1 shows the negative linear regressions between *E. barbatula's* (a) cover (%), (b) thalli height, and (c) thalli perimeter and the dissolution rate of the gypsum (fd). Highest coefficient of determination (R^2 = 0.81) was estimated between fd and cover (%), that was described by the equation cover (%) = 3944.9 * Fd + 132. Therefore, cover (%) can be can be suggested as an indicator of the ecosystem service "wave attenuation". This result was also confirmed by using Spearman's non-parametric correlation coefficient analysis, where fd was better negatively correlated with cover (%) (ρ = -0.905), followed by perimeter (ρ = -0.697) and the height (ρ = -0.649).

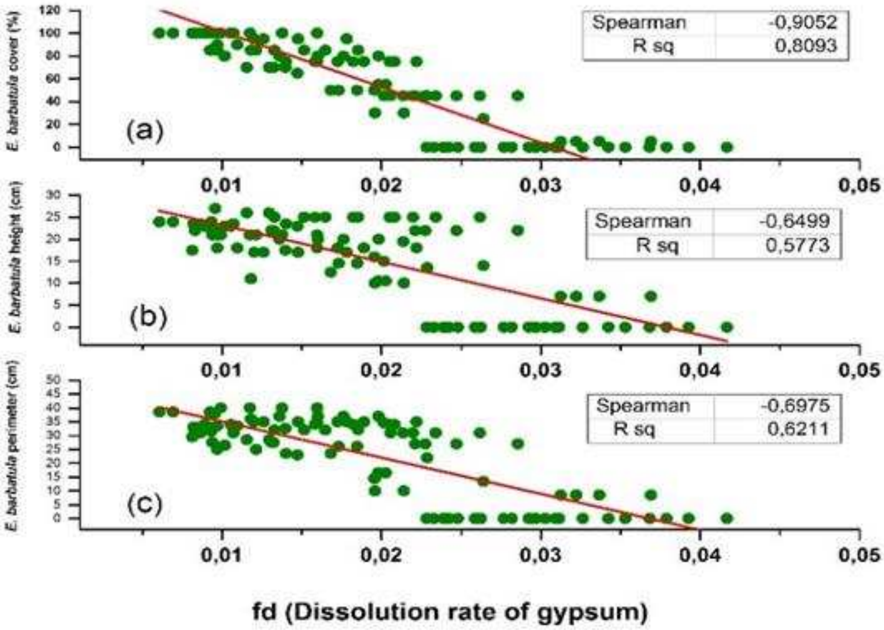

Figure 1. Linear regression of the fd (Dissolution rate of gypsum) with *Ericaria barbatula*'s (a) cover (%), (b) thalli height (cm), and (c) thalli perimeter (cm).

4. Discussion

Several studies have documented that coastal habitats, including benthic vegetation, have significant potential for reducing wave heights (Morris *et al*. 2020). The data of this study showed that *E. barbatula* forest could attenuate waves, with species abundance estimated by cover (%) being the best indicator. Paul *et al*. (2012) also showed that the attenuation waves in shallow water were dependent on a combination of shoot density and leaf length of submerged vegetation, which can be described by the leaf area index, a dimensionless indicator of quantity or abundance. Morris *et al*. (2020) found that kelp causing wave attenuation is not a universal truth but depends on kelp species morphologies and diversity, and thus there is a need to understand what species are able to provide coastal protection. We agree, and therefore we notice that this study took place in a period (middle of summer) where *E. barbatula* showed maximal abundance. Therefore, further study is needed to examine other geomorphic, ecological, and hydrodynamic factors that determine wave attenuation among different locations, seasons, and species. The results will be essential for developing indicators of restoration success providing managers and policymakers with data on sustainable and cost-effective solutions for coastal defense.

Acknowledgement

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References

Hinkel J., Lincke D., Vafeidis A.T., et al. (2014). Coastal flood damage and adaptation costs under 21st century sea-level rise. Proceedings of the National Academy of Sciences, USA 111: 3292–3297.

Morris R.L., Graham T.D.J., Kelvin J., Ghisalberti M., Swearer S.E. (2020). Kelp beds as coastal protection: Wave attenuation of Ecklonia radiata in a shallow coastal bay. Annals of Botany 125: 235–246.

Paul, M., Bouma T., Amos C. (2012). Wave attenuation by submerged vegetation: combining the effect of organism traits and tidal current. Marine Ecology Progress Series 444: 31–41.

Reidenbach M. A., Koseff J. R., Monismith S. G., Steinbuck J. V., Genin A. (2006). The effects of waves and morphology on mass transfer within branched reef corals. Limnology and Oceanography 51, 1134–1141.

Schiel D. R. & Foster M. S. (2006). The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. Annual Review of Ecology, Evolution, and Systematics 37: 343–372.

Steneck R.S., Graham B.J., Bourque D., Corbett J.M., Erlandson J.A., Estes M.J., Tegner M.J. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation 29: 436–459.

MONITORING MARINE LITTER IN PORTS OF THE NORTHERN AEGEAN SEA ACCORDING TO THE MARINE STRATEGY FRAMEWORK DIRECTIVE

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Abstract
Marine litter pollution has received increased attention over the last decades, constituting one of the main environmental issues of our times globally. The present study discusses data from the long-term project “Prosfero”, which aims at addressing litter in the marine environment and raising public awareness on the issue in Greece. In the first quarter of 2021, thirteen underwater clean ups were conducted on Thassos, Limnos, Lesvos and Agios Efstratios islands, where about 10 tons of litter were removed from about 80,000 m^2, while more than 5,000 items were recorded and removed from the marine environment. The surveyed sites could be categorized as relatively polluted, while most of the litter items belonged to plastics indicated the fishing, recreational and touristic activities as the main sources of litter assemblage. Although the present study evidences the current issue of plastic pollution, more research is needed due to the scarcity of the existing data.
Keywords: *Descriptor 10, Macroplastic Pollution, Marine Debris, Marine Habitat destruction,Single use plastics*
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1. Introduction

Marine litter is considered among the most significant problems in the Mediterranean Sea (Vlachogianni 2019), while major concerns have been raised on a global scale regarding plastic as the most abundant litter type (Landrigan *et al.* 2020). The seafloor is the ultimate sink of a vast variety of marine litter items, derived mainly from anthropogenic activities (Veiga *et al.* 2016). A variety of initiatives and instruments have been introduced in order to address marine litter pollution, such as the European Marine Strategy Framework Directive (MSFD; European Commission 2008; 2017) and the Mediterranean Action Plan (UNEP 2015). However, the current data on marine litter trends, distribution and effects are scarce and more research is recommended to further address marine litter (i.e. Galgani *et al.* 2021).

2. Materials and Methods

Thirteen ports located in the Northern Aegean Sea were cleaned in the context of “Prosfero” campaign aiming to achieve cleaner coasts, and to raise public awareness about marine litter pollution during May 2021. In particular, 6 underwater clean ups were conducted on Thassos, 1 on Limnos, 5 on Lesvos and 1 on Agios Efstratios islands (Figure 1).

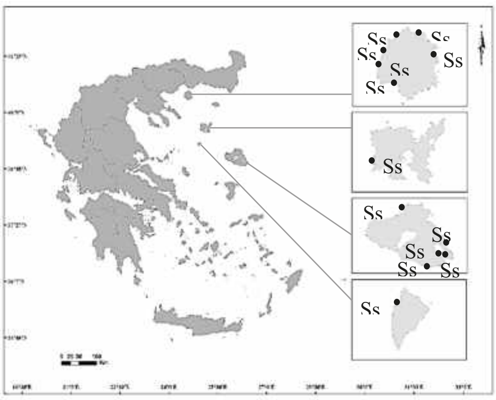

Figure 1. Map of the survey sites (Ss) on the islands of Thassos, Limnos, Lesvos and Agios Efstratios.

The clean ups were coordinated by 2 representatives of iSea, who were responsible for the coordination of the participants, the localization and the retrieval of marine litter and their recording to survey sheets. The methodology applied was aligned with the MSFD under Descriptor 10 following the recommendations of MSFD

Technical Subgroup on Marine Litter (2013). In addition, physical characteristics (i.e. habitat type, substrate, depth) of the survey site were also recorded and the litter density was calculated as litter items retrieved per m^{-2}.

3. Results and Discussion

In total, approximately 10 tons of marine litter were removed from a surface area of about 80,000 m^2, while more than 5,000 items were recorded and removed from the marine environment. The surveyed sites (Ss_1-Ss_13) could be categorized as relatively clean (Alkalay *et al*. 2007), as the mean value (± SD) of the collected litter was 0.12 ± 0.11 items m^{-2}. More specifically, most of the litter items on the survey sites belonged to plastics (64.42% ± 16.62), with the most abundant items being bottles, ropes and twines, fishing lines, bags, plastic pieces (>2.5 cm), and Single Use Plastic (SUP) cups and lids; to metals (18.11% ± 7.21) such as beverage and beer cans; to rubber (11.17% ± 15.67) such as vehicle tires; to glass (8.05% ± 8.13) such as glass bottles and pieces; while the types of fabric, paper and wood displayed low percentages ranged from 2.70% to 0.24% (Figure 2).

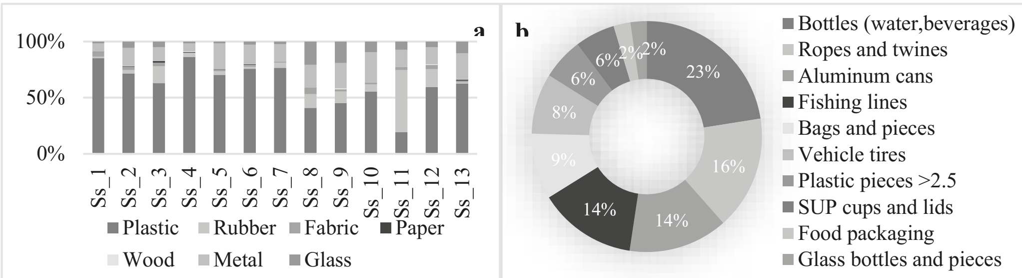

Figure 2. a. Collected litter per survey site (Ss) and type (%); b. Ten most common collected items (%).

The high percentages of ropes, twines, fishing lines, nets and aluminum cans observed could be attributed to the small-scale fisheries and recreational activities taking place in the area. Furthermore, the tourism activities could also contribute to marine litter accumulation. Thereby, the findings of the present study reflect the existing problem of marine litter and much more as for the plastic pollution and highlight the importance of further investigation. In addition, the present study could contribute to policy decision making to tackle marine litter.

Acknowledgements

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References

Alkalay R., Pasternak G., Zask A. (2007). Clean-coast index. A new approach for beach cleanliness assessment. Ocean & Coastal Management, 50, 352. Doi: 10.1016/j.ocecoaman.2006.10.002.

EC (2008). Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (MSFD).

EC (2017). Commission Directive (EU) 2017/845 of the European Parliament and of the Council as regards the indicative lists of elements to be taken into account for the preparation of marine strategies.

Galgani F., Brien A.So., Weis J., Ioakeimidis C., Schuyler Q., Makarenko I., Griffiths H., Bondareff J., Vethaak D., Deidun A., Sobral P., *et al*., Bebianno M.J. (2021). Are litter, plastic and microplastic quantities increasing in the ocean? Microplastics & Nanoplastics 1,2. Doi:10.1186/s43591-020-00002-8.

Landrigan P. J., Stegeman J. J., Fleming L. E., Allemand D., Anderson D. M., Backer L. C., *et al*., Rampal P. (2020). Human health and ocean pollution. Annual Global Health 86, 151. Doi: 10.5334/aogh.2831.

MSFD Technical Subgroup on Marine Litter (2013). Guidance on Monitoring of Marine Litter in European Seas: a guidance document within the Common Implementation Strategy for the MSFD. JRC, pp. 128.

UNEP-MAP (2015). Convention for the protection of the marine environment and the coastal region of the Mediterranean and its protocols- overview.

Veiga J.M., Fleet D., Kinsey S., Nilsson P., Vlachogianni T., Werner S., Galgani F., Thompson R.C., Dagevos J., Gago J., Sobral P., Cronin R. (2016). Identifying Sources of Marine Litter. MSFD GES TG Marine Litter Thematic Report, JRC Technical Report, EUR 28309. Doi:10.2788/018068.

Vlachogianni T. (2019). Marine Litter in Mediterranean coastal and marine protected areas – How bad is it. A snapshot assessment report on the amounts, composition and sources of marine litter found on beaches. Interreg Med ACT4LITTER & MIO-ECSDE.

PRELIMINARY RESULTS OF FISHING FOR LITTER IN GREECE 2020

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Abstract
The number of marine litter that ends up in the oceans, is increasing annually, causing problems to marine ecosystems, world economy and biodiversity (Gaglani *et al*. 2019). Marine litter can be found at the seashore or floating at the sea surface or on the seafloor, the latter accounts for 70% of the total litter entering the marine environment. Therefore, understanding more about the composition and distribution of benthic litter is vital. Yet, there are limited and costly options for its qualitative and quantitative research. Fishing for Litter (FFL) is a cost-effective scheme (Newman *et al*. 2015) that aims in the reduction of marine litter by the participation of the fishing industry. More specifically, it requires that professional fishers are collecting the litter that accumulates in their nets as part of their usual fishing activity. The aim of this preliminary study is to show the type of data FFL schemes can provide for benthic marine litter, as well as their composition, quantities, and spatial distribution.
Keywords: *Fisheries, Marine pollution, Plastic waste, Mediterranean*
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1. Introduction

Greece as an EU member state is required to achieve or maintain good environmental status of the marine environment according to the MSFD (2008/56/EC), and therefore, must evaluate and maintain data for the environmental status using the specific descriptors' criteria and the measures provided, such as for marine litter. Monitoring litter is of major importance, but in the case of benthic marine litter is both complex and costly. There are several methodologies, to study benthic marine litter; using divers, drones/ROVS or experimental fishing. In the Hellenic seas, all the aforementioned methodologies have been applied exploring the composition and amount of benthic marine litter in specific regions for a limited period of time (Ioakeimidis *et al*. 2015 and references therein). Fishing for Litter (FFL) has been also applied in Greece as a pilot Project in the Ionian Sea with 3 vessels participating for 13-month time window that collected 2.4 tons of litter (Ronchi *et al*. 2019). Among the strengths of FFL proved to be the valuable data that can be provided for the litter on the seafloor (Ronchi *et al*. 2019). In this paper we present, the preliminary results of Fishing for Litter Project that was implemented, during the fishing season of 2020 for bottom trawl fisheries (January – May, October – December), in the North Aegean Sea.

2. Material and Methods

In 2020, FFL took place in four ports of the North Aegean Sea, namely Nea Michaniona, Volos, Alexandroupoli, and Kavala in Greece. The fishers used a protocol developed in the context of the Project, to register the litter that was accumulated in their nets in every fishing trip. The protocol included 7 categories of litter (L1: Plastic, L2: Rubber, L3: Metal, L4: Glass, ceramics, and cement, L5: Fabric, L6: Processed wood, L7: Paper) and one category for exceptional items listed as Other Waste (i.e., beds, boats, etc.). For each category the participants filled the total weight, the total number of litter items in each category, and the number of specific litter items. To register the number of specific items, a list of 36 specific items were included within the main categories of litter. Furthermore, the protocol required from the fishers to register the duration of their hauls for the given fishing trip & the area of the fishing ground. Each form was accompanied with photos of the litter items.

3. Results

The data presented in this paper derived from the protocols filled by 17 fishing vessels. In total 658 forms were filled during January-May and October-December of 2020 within the Hellenic EEZ in depths from 50 – 350m. The vessels participating in this study used their usual net which is a typical otter bottom trawl with a 40mm mesh size in the cod end and head-rope with length ranging from 7 to 10 m according to the vessel's length. The vessels length ranged from 23-29m and from 6-8m width. The speed during the hauls ranged from 2.9 to 3.0 knots.

The fishing effort was consisted of 4,321 hauls of ~ 4 hours duration. The total amount of litter collected was 16,682.60 kg, from 23,037 litter items. The composition of litter was comprised from mostly three categories. *Plastic* exceeded the other categories with a 62.6% in weight and 82.8% in items, followed by L4 with 19.9% in weight and 2.6% in items and L3 with 10.5% in weight and 11.2% in items (Table 1). Out of all the fishing trips only in 61 litter was absent. The forms were separated in 9 areas of the North Aegean according to the fishing ground (Table 1). Interestingly, the semi-enclosed coastal areas had higher abundance in each category of litter with the Kavala Gulf – Strymonikos Gulf and Thermaikos Gulf exceeding the other marine areas (Table 1). The same is true for the abundance of litter items, with the gulfs having the most items, in Thermaikos Gulf in total 8,859 items were recorded, followed by North Evoikos Gulf with 3,261 items and Kavala gulf - Strymonikos gulf with 2,836 items. Even though, Thracean Sea had almost twice as much more forms had less litter items. In terms of the most abundant items found, plastic bags (23.01%) and plastic bottles (14.11%) were the most common items in all the regions and in total, followed by polypropylene ropes (10.34%), hard plastic (e.g., containers, buckets, barrels) (9.02%), aluminium tins (8.55%), octopus traps (5.03%), food packaging (3.17%), fishing nets (2.95%), ropes (2.93%), and lines (2.84%). Of those, more than 33% was related to fisheries' activities.

Table 1: The distinct regions of which the litter was collected and registered by FFL Project in 2020. N = the number of forms, L1:7= the categories of litter in weight (kg) and in brackets the number of litter items.

Regions	N	L1 Plastic	L 2 Rubber	L3 Metal	L4 Glass– Ceramic – Cement	L5 Fabric	L6 Wood	L7 Paper	Other Waste	Total Kg (No of items)
Central Aegean Sea	2	4(9)	0	0	0	0	0	0	0	4(9)
Mount Athos	1	0	0	5.5(3)	0	0	0	0	0	5.5(3)
North Evoikos Gulf	25	2167.5(2,998)	41(8)	269.8(241)	45(10)	37(3)	0	0	4(1)	2564.3(3,261)
Thermaikos Gulf	162	1066.7(6888)	102.3(100)	452.6(1328)	164(95)	174.6(276)	44.5(60)	4.9(84)	202.3(28)	2211.9(8,859)
Toronaios Gulf	3	18(57)	10(13)	5(11)	0	2(1)	0	0	0	35(82)
Trikeri Channel	17	673(899)	2.5(6)	78.35(195)	114(24)	37(8)	5	0	0	909.85(1,132)
Pelion - Sporades	18	713.5(1304)	36(7)	67.2(137)	85(10)	4(11)	0	0	0	905.7(1,469)
Kavala Gulf- Strymonikos Gulf	114	2056.38(2437)	163.7(15)	314.72(188)	906.4(151)	34.3(12)	31(11)	6(4)	6.6(18)	3519.1(2,836)
Thracean Sea	316	3749.22(4477)	79.3(34)	564.33(475)	2011.7(315)	39.5(25)	75.2(51)	8(9)	0	6527.25(5,386)
Total Weight in kg (No of items)	658	10448.3(19,069)	434.8(183)	1757.5(2,578)	3326.1(605)	328.4(336)	155.7(122)	18.9(97)	212.9(47)	16682.6(23,037)

4. Discussion

Plastic was the most dominant category of litter, Glass-Ceramic-Cement and Metal were the next most abundant categories aligning with the findings of other studies for benthic litter in Greece (Ioakeimidis *et al*. 2014 and references therein). Within plastic category bags (~30%) were the most abundant items being slightly lower than in the findings of Ioakeimidis *et al*. (2014) that bags almost accounted for 50% of the litter items in plastic category, and very close with 17% what was found in plastic bottles abundance, that in our case accounted for ~19%. Items related to fisheries were significantly higher (33%), than in other studies in Greece, yet more analysis must be conducted to examine the correlation between fishing intensity of an area and its litter composition.

Acknowledgements

We would like to acknowledge the A.C. Laskaridis Charitable Foundation for funding FFL Project and all the fishers who registered the forms.

References

Galgani L., Beiras R., Galgani F., Panti C., Borja A. (2019). Impacts of marine litter. Frontiers in Marine Science, 6, 208.

Ioakeimidis C., Papatheodorou G., Fermeli G., Streftaris N., Papathanassiou E. (2015). Use of ROV for assessing marine litter on the seafloor of Saronikos Gulf (Greece): a way to fill data gaps and deliver environmental education. Springer Plus 4(1): 1-9.

Ioakeimidis C., Zeri C., Kaberi H., Galatchi M., Antoniadis K., Streftaris N. et al. (2014). A comparative study of marine litter on the seafloor of coastal areas in the Eastern Mediterranean and Black Seas. Marine Pollution Bulletin 89(1-2): 296-304.

Newman S., Watkins E., Farmer A., Ten Brink P., Schweitzer J.P. (2015). The economics of marine litter. In: Marine Anthropogenic Litter (pp. 367-394), Springer, Cham.

Ronchi F., Galgani F., Binda F., Mandić M., Peterlin M., Tutman P., Fortibuoni T. (2019). Fishing for Litter in the Adriatic-Ionian macro-region (Mediterranean Sea): Strengths, weaknesses, opportunities and threats. Marine Policy 100: 226-237.

A PRELIMINARY ECOSYSTEM SERVICES ASSESSMENT OF GIOFYROS RIVER, CRETE

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Abstract
As today's society and life quality are directly linked with the Ecosystem Services (ES), their study and sustainable use are exceptionally essential. This research makes an initial effort to analyse the main ES provided by the Giofyros river in Heraklion, Crete. For the classification of the ES the Common International Classification of Ecosystem Services (CICES) was adopted. A survey is conducted about the river's ESs involved experts' opinions and an extended literature review was carried out. The main outcome indicates that Giofyros river may provide supplying ESs and to a lesser degree regulatory and cultural ESs. Development of recreational activities and agrotourism along the riparian zone should be considered.
Keywords: *Giofyros river, Ecosystem Services, provisioning, regulatory, cultural, CICES*
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1. Introduction

The term "Ecosystem Services" (ES) determines the benefits people obtain from ecosystems, either tangible or intangible (food, leisure, climate stability) (Daily 1997). As the importance of ES is exceptional on the wellbeing of people in urban areas (Bolund & Hunhammar 1999), the need for further study of this sector has been created as rivers are increasingly urban. The constant change and evolution of human life has led to the global loss of ES due to changes in land use (Costanza *et al*. 2014) and floodplains shrinkage. As ES management and monitoring is crucial for maintaining rivers viability, many leading practitioners have classified their ES (Costanza *et al.* 1997; De Groot *et al.* 2002; Mooney *et al.* 2005) mainly in three categories (provisioning, regulating, cultural services).

The study area was the Giofyros river of Heraklion, Crete, which drains an area with a total area of 186 km^2 at an average altitude of 355 m and 25 km long. Giofyros provides water for irrigation, offers food products, but also opportunities for research, education and recreation, contributes to the purification of surface water and recharges the aquifer. Its riparian vegetation regulates through complex hydro-geochemistry processes the soil biodiversity. In this preliminary work the ES of Giofyros river have been classified according to "The Common International Classification of Ecosystem Services" (CICES) (Haines-Yong & Potschin 2012).

The purpose of this work is the analysis of the ESs provided by the river and to highlight their environmental and social dimensions.

2. Material and Methods

A questionnaire was created which included questions on the ESs regarding the river Giofyros in Heraklion, Crete. The target audience was very specific as it consisted of scientists specialized in environmental and socioeconomic fields. In the survey, information was requested on the existence of riparian vegetation, the width of functional vegetation and its extension beyond the riverbank, the presence of emerging aquatic macrophytes and the fish biodiversity. Information was collected on flood risk monitoring, irrigation practices as well on wastewater treatment and disposal.

Then the topics of the aesthetics, education and leisure were analyzed in the context of the study of cultural ESs. Finally, for the supply ESs, the various uses of the fresh water were analyzed, the vegetables that are cultivated in the wider area, the land uses, but also information for the flora and fauna of the river basin.

More specifically, the survey was addressed to eight experts from Heraklion institutions, such as the Natural History Museum, the Directorate of Agricultural Economy and Livestock, and the Department of Environment and Spatial Planning of Crete Region.

The results of the questionnaire were then analyzed and completed with other data from an extended literature review.

3. Results and Discussion

From the preliminary analysis of the results, there was a clear indication that provisioning ESs were the main ones in the river's system. In more detail, in the provisioning ecosystem services it was found that the total irrigated area is 177 ha where the largest part concerns olive crops with a total of 90 ha (approx. 50%) followed by vineyards with a total area of 36 ha (approx. 20%) and the remaining 30% being hives. The total number of small industries/enterprises located near the river is 25.

As regards the regulating ecosystem services, it emerged that the absence of wastewater in the river, leads to a low risk regarding proliferation of macro-algae and/or phytoplankton at the mouth of the Giofyros. The riverbed consists of sand, stones, rocks, and in places cement. In the parts of the river where there are no constructions, there is rich vegetation along its entire length and on the banks. The dominant vegetation consists of common reed (*Phragmites australis*), plane trees (*Platanus orientalis*) and willows (*Salix babylonica*). In the valley there are myrtles (*Myrtus communis*), common reed (*Phragmites australis*), fennel pondweeds (*Potamogeton pectinatus*), and citrinuses (*Ranunculus acris*).

The riparian vegetation has a width of 1 to 5 meters and depends on the adjacent activities. Every year, usually during the autumn period, staff members of the Municipality of Heraklion clean the vegetation. The wetland of the area is rich in various bird species such as great cormorants (*Phalacrocorax carbo*), hooded crows (*Covrus cornix*), gulls of three or four species - including the Mediterranean gull *(Lanus melanocephalus*) and the black-headed gull (*Chroicocephalus ridibundus*) - herons (*Ardeidae*) and common kingfishers (*Alcedo atthis*). Also in the Pankritio Stadium located at the mouth of the river, birds such as peregrine falcons (*Falco peregrinus*) nest. Frogs, water turtles and sometimes ducks can be seen in the river, while reed warblers (*Acrocephalus scirpaceus*) have also been recorded as living in the reeds.

Part of the area of the Giofyros basin (Mount Juchta) is included in the Network of the Natura 2000 Protected Areas and in to Special Protection Area (SPA), which is an important bird area due to the presence of a large colony of vultures (*Gyps fulvus*) nesting on the western cliffs of the mountain. The area is also protected as an "Archaeological Site", as a "Landscape of Special Natural Beauty" and as a "Wildlife Refuge".

Finally, the cultural ESs include a variety of leisure activities (e.g., horse-riding, trekking, birdwatching), that the municipality has not exploited them fully. Agrotouristic activities could be an important growth sector which could be not only sustainable but profitable, too. Furthermore, the creation of a trail along the river, as a continuation of the coastal pedestrian path, would highlight the local scenery and would allow citizens-visitors to enjoy the route, thus enhancing the ecological awareness. The monitoring and mitigation of flood and drought phenomena in river Giofyros would protect human wellbeing and assets and sustain the rivers biodiversity.

Reference

Bolund P., Hunhammar S. (1999). Ecosystem services in urban areas. Ecological Economics, 29(2), 293-301.

Costanza R., D’Arge R., De Groot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., O’Neill R.V., Paruelo J., Raskin R.G., Sutton P., van den Belt M. (1997). The values of the world’s ecosystem services and natural capital. Nature 387, 253-260.

Costanza R., De Groot R., Sutton P., Van der Ploeg S., Anderson S.J., Kubiszewski I., Farber S., Turner R.K. (2014). Changes in the global value of ecosystem services, Global Environmental Change 26, 152-158.

Daily G.C. (1997). Introduction: what are ecosystem services. In: “Nature’s services: Societal dependence on natural ecosystems”, Daily G.C. (eds). Island Press, Washington DC, p.1-10.

De Groot R.S., Wilson M.A., Boumans R.M.J. (2002). A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological Economics 41, 393-408.

Haines-Yong R., Potschin M. (2012). CICES Version 4: Response to Consultation. Centre for Environmental Management, University of Nottingham.

Mooney H.A., Cropper A., Capistrano D., Carpenter S.R., Chopra K., Dasgupta P., Leemans R., May R.M., Pingali P., Hassan R., Samper C., Scholes R., Watson R.T., Zakri A.H., Shidong Z. (2005). Ecosystems and human well-being – Synthesis. Report of the Millennium Ecosystem Assessment, Island Press, Washington, DC, p. 1-100.

GOVERNANCE AND EVALUATION OF ECOSYSTEM SERVICES IN THE COASTAL AREA OF SOUTH – SOUTHEAST ATHENS SECTOR

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Abstract
This study, examines the bodies responsible for the administration and management of services of the ecosystem, in the South – Southeast coastal front of Athens. A questionnaire-based survey method was used to draw specific conclusions about the area under study. Our main goal was to investigate the effectiveness of management of coastal areas by the competed, as well as to investigate the knowledge of the inhabitants about the coastal area. Ecosystem services provided by the coastal environment in combination with anthropogenic activities often appear to be in conflict. The continuous increase of anthropogenic activities results in the environmental degradation of the quality of the coastal areas, which has a direct result of a continuous reduction of the biodiversity. The plethora of services offered by such ecosystems automatically lead them to competition in relation to terrestrial areas. Thus, the issue of 'location', the use of natural resources and also pressures on the natural environment often causes conflicts between people. In our research we also include a description of the coastal area under study, the administration and the legislation framework of Coastal Zone Integrated Management governing it, description of the region and the administrative bodies govern it, followed by a separate demarcation of each Municipality in our area.
Key words: *Ecosystem services, Management, Sustainability, Coastal Zone, S-SE Athens*
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1. Introduction

The term "nature service" is fully consistent with the term "ecosystem services" and is considered quite complex. It aims to identify and evaluate the benefits that nature 'offers' to humans (Costanza *et al*. 1997). The relationship that has been created between human societies and the natural environment could be characterized as "completely dependent". According to the MEA classification (Millennium Ecosystem Assessment: Provision Services, Cultural Services, Maintenance Services and Regulating Services) we distinguish the existing ecosystem services in the study area, which includes the area from the Municipality of Kallithea, to the Municipality of Voula – Vouliagmanei – Vari. Along the coastal area there are plenty of cultural services focusing on leisure, sports, landscape beauty and spiritual well-being. These last two parameters are considered quite important as the aesthetic beauty of the coast attracts people to 'invest' and take advantage of the area (Fisher & Turner 2008). In addition, a statistical analysis of the study area was performed, examining, both the administration field and the management field (Costanza *et al*. 2014). Finally, the issue of control is equally important, through which the functionality of the administrative and management sector is 'ensured', always in accordance with the legislation provided (Maes *et al*. 2013).

2. Material and Methods

Due to the pandemic of COVID-19 and the restrictions that prohibited close communications, a questionnaire was created through the platform 'Google Forms'. The questionnaire was sent to the administration bodies and services of the regions under study via email. Also, a QR code was created to ensure easier access for the participants directly to the website (https://tinyurl.com/1p0e3ihz). It should be mentioned that, after the request of some participants, the questions were conducted through a phone call, as they were not able to use any means to answer the questionnaire. The questionnaire was created in order to search the efficiency of management and the resident's knowledge of the ecosystem services in the coastal area. It consists of closed type questions and data analysis was performed using Excel. The criteria for the participants were that they had to be over 18 years old, residents or workers at the area under study and they had to answer all 33 questions. The questionnaire was divided in four parts: the first part consists of demographic questions of the participant, such as gender, age and the municipality they live. The second part, also evolves around the participants and their relation to the area studied. The third part concerns the view of the participants on the effectiveness of the management of the coastal area by the competent bodies. The last part is related to the knowledge of the participants about ecosystem services and various environmental issues.

3. Results and Discussion

Analyzing the collected data, it was observed that 62.3% of the participants is related to the coastal area. This suggests that our sample consists mostly of agencies and services operating in the coastal area. Also impressive is the fact that in the activities carried out in the coastal area, none of the participants mentioned the visit to the sights as an activity. Regarding the effective management, we conclude that while coastal cleanings are carried out quite often and the participants are already aware of it at a rate of 77% as shown in Figure 1, the majority (53%) doesn't take part in cleaning action as shown in Figure 2. Regarding the sports facilities, the participants are very happy with the maintenance of the sports facilities at a rate of 96.6%, in contrast to the cleanliness of the sports facilities, which, as shown in Figure 3 the 60% of the participants characterize it as average. In terms of participants knowledge and views on ecosystem services and various environmental issues, it was observed that 40% consider sustainability not a priority when the usage of the environment serves humans as shown in Figure 4. At this point it is worth to mention that when asked whether they would attend an information day related to environmental resources in the coastal area, 76.7% would like to participate. Proposal for the future is the research of the implementation of Integrated Coastal Zone Management, the protection of ecosystem services, the informing citizens about them and the most important for all this, is the supervisory control of services and responsible bodies for the administration sector.

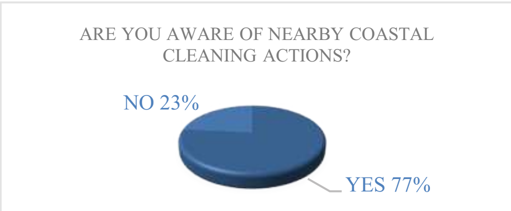

Figure 2. Awareness of nearby coastal cleaning actions

Figure 1. Participance in coastal cleaning actions

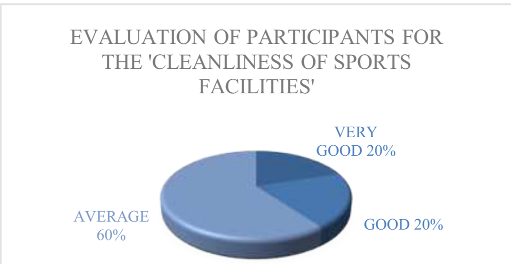

Figure 4. Evaluation of cleanliness of sport facilities

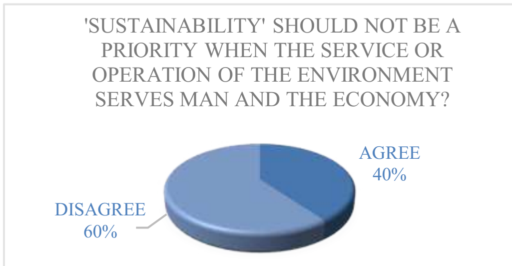

Figure 3. Sustainability as priority

References

Costanza R., d' Arge R., de Groot R. (1997). The value of the world's ecosystem services and natural capital. Nature 387, 253–260.

Costanza R., d' Arge R., de Groot R., Sutton P., van der Ploeg S., Anderson S.J., Kubiszewski I., Farber S. (2014). Changes in the global value of ecosystem services, 26, 152-158.

Fisher B., Turner R.K. (2008). Ecosystem services: Classification for valuation, Biological Conservation, 141, Issue 5, 1167-1169.

Maes J., Teller A., Erhard M., Liquete C, Braat L., Berry P., Egoh B., Puydarrieux P., Fiorina C., Santos F., Paracchini M.L., Keune H., Wittmer H., Hauck J., Fiala I., Verburg P.H., Condé S., Schägner J.P., San Miguel J., Estreguil C., Ostermann O., Barredo J.I., Pereira H.M., Stott A., Laporte V, Meiner A., Olah B., Royo Gelabert E., Spyropoulou R., Petersen J.E., Maguire C., Zal N., Achilleos E., Rubin A., Ledoux L., Brown C., Raes C., Jacobs S., Vandewalle M., Connor D., Bidoglio G. (2013). Mapping and Assessment of Ecosystems and their Services. An analytical framework for ecosystem assessments under action 5 of the EU biodiversity strategy to 2020, 14-23.

CAN MARINE SCIENCES STUDENTS SUPPORT THE OCEAN LITERACY FRAMEWORK? A PILOT STUDY FROM GREECE

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Abstract
The present pilot study aims at evaluating knowledge, attitudes, and behaviour concerning ocean issues in relation to university students in Greece. A 3-scale questionnaire was developed and administered to 106 students. Results revealed that Marine Sciences students were found to have better knowledge as well as pro-environmental attitudes and behaviour in relation to their counterparts from the Primary Education Department. However, the low to moderate knowledge scores observed in the participants of the survey reveal the need for integration of relevant concepts, and further education research on Ocean Literacy (OL) issues to ensure the sustainability of the ocean.
Keywords: *Ocean literacy, Tertiaty education, Students, Knowledge, Attitudes, Behaviour*
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1. Introduction

The United Nations (UN) recently declared a Decade of Ocean Science for Sustainable Development (2021-2030) to run along with the Agenda 2030 for Sustainable Development which includes 17 Sustainable Development Goals (SDG). For SGD 14 (the sustainability of the ocean and its resources) to be achieved, the need to increase societal awareness of Ocean Literacy (OL) is paramount (Eparkhina *et al.* 2021). The OL framework, consisting of 7 essential principles and 45 fundamental concepts, is now accepted worldwide for use in both formal (schools, universities) and non-formal (e.g. research centres) education settings: this is an invaluable support for citizens to obtain sound knowledge of ocean issues, enabling them to communicate about ocean issues and make informed and responsible decisions (Cava *et al.* 2005). To make progress in this direction, the present pilot study aims at evaluating knowledge, attitudes, and behaviour of university students (future marine scientists and/or educators) in Greece concerning ocean issues.

2. Material and Methods

A cross-departmental pilot study was conducted involving a group of university students from a Marine Sciences (n=37 of 1st year and n=34 of 3rd, 4th) and a Primary Education (n=35) Departments. Students from the Primary Education Department served mainly as a reference group, though they also attend several environmental classes. A 3-scale questionnaire concerning their knowledge, attitudes, and behaviour was developed and administered to the participants, taking into consideration previous research carried out within the framework of Ocean Literacy (NMEA 2010; NOAA 2013; Mogias *et al.* 2015, 2019; Fauville *et al.* 2018). Moreover, the normality (Kolmogorov-Smirnov and Shapiro-Wilk tests) and reliability (Cronbach α index) for all scales of the questionnaire were also checked.

3. Results

Marine Sciences students were found to be significantly more knowledgeable and possess more positive pro-environmental attitudes and behaviour, as expected, in relation to their counterparts from the Primary Education Department (Fig. 1). The majority of students of both Departments, however, seemed to be unaware of ocean issues such as the connectedness of the ocean basins and the origin of atmospheric oxygen. They also believe that fragile marine ecosystems will be lost if humans do not change their behaviour towards the ocean. Most of both groups collect their garbage when they spend time on the beach and they recycle plastic. Further in-depth analysis of the Marine Sciences students' sub-sample revealed a slight increase in all scales from year 1 to the end of their studentship (Fig. 2).

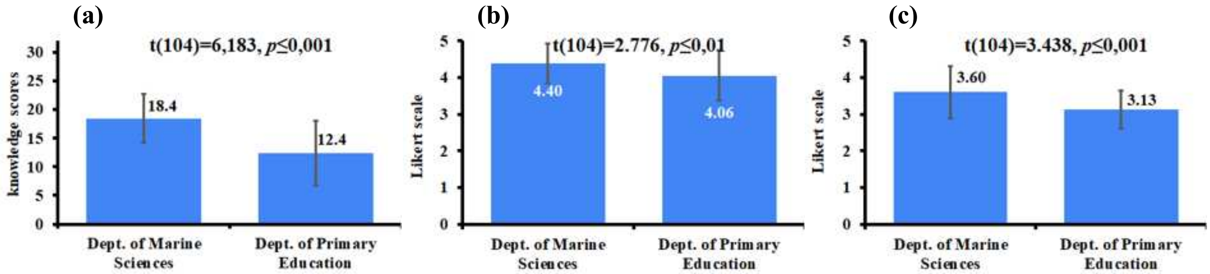

Figure 1. Mean knowledge (a), attitudes (b), and behaviour(c) values (a 5-point Likert scale was applied for (b) and (c)) of the total sample of participants

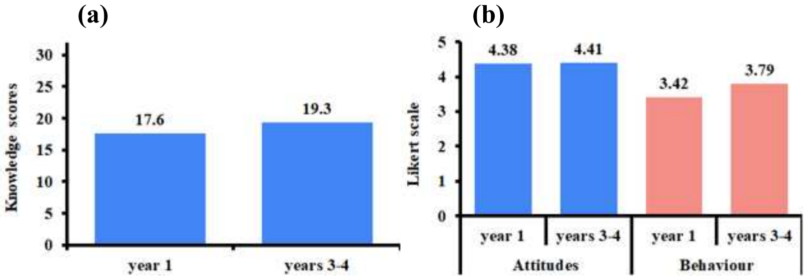

Figure 2. Mean knowledge (a), attitudes (b), and behaviour (c) values (a 5-point Likert scale was applied for b and c) of the Marine Sciences students on the basis of the year of enrollment

4. Discussion

The results of the pilot study showed an interesting pattern in the correct answers, regarding the most difficult and the easiest questions, which demonstrated that these are in line with other findings from the existing literature (e.g., Mogias *et al.,* 2015; 2019). However, results should be considered indicative as the study included a relatively small sample of students from both Departments. Nevertheless, the slight increase of Ocean Literacy in all scales of the Marine Sciences students from the lower to the upper academic years, taken along with the low knowledge scores from the Primary Education student group, point up the need to integrate relevant concepts (e.g., development of OL tools; Brennan *et al.,* 2019) and for further research to investigate in-depth OL issues in formal education.

References

Brennan C., Ashley M., Molloy O. (2019). A System Dynamics Approach to Increasing Ocean Literacy. Frontiers in Marine Science 6, 360.

Cava F., Schoedinger S., Strang C., Tuddenham, P. (2005). Science content and standards for ocean literacy: A report on ocean literacy. http://coexploration.org/oceanliteracy/ documents/OLit200405_Final_Report.pdf (Accessed 20 May 2020)

Eparkhina D., Pomaro A., Koulouri P., Banchi E., Canu D., Uyarra M., Burke N. (2021). Ocean Literacy in European Oceanographic Agencies: EuroGOOS recommendations for the UN Decade of Ocean Science for Sustainable Development 2021-2030. EuroGOOS Policy Brief. Brussels. Belgium.

Fauville G., Strang C., Cannady M.A., Chen Y-F. (2018). Development of the international ocean literacy survey: measuring knowledge across the world. Environmental Education Research 25, 238-263.

Mogias A., Boubonari T., Markos A. Kevrekidis T. (2015). Greek pre-service teachers' knowledge of ocean sciences issues and attitudes toward ocean stewardship. Journal of Environmental Education 46, 251–270.

Mogias A., Boubonari T., Realdon G., Previati M., Mokos M., Koulouri P., Cheimonopoulou M. (2019). Evaluating ocean literacy of elementary school students: Preliminary results of a cross-cultural study in the Mediterranean region. Frontiers in Marine Science 6, 396.

National Marine Educators Association (2010). Ocean Literacy Scope and Sequence for Grades K-12. Special Report #3 on The Ocean Literacy Campaign Featuring the Ocean Literacy Scope & Sequence for Grades K-12, USA.

National Oceanic and Atmospheric Administration (2013). Ocean Literacy: The Essential Principles and Fundamental Concepts of Ocean Sciences for Learners of All Ages. Version 2, USA.

LIFE CYCLE ASSESSMENT OF SMALL SNAIL FARMING SYSTEMS: THE GREEK CASE

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Abstract
Heliciculture recently has gained interest due to its potential for agricultural development and as an alternative for business opportunities. In the recent years, environmental sustainability has become a primary challenge in developed farming systems. In this paper we investigate the effect of snail farming systems to environmental sustainability by performing Life Cycle Assessment (LCA) on the carbon footprint. Our research analyzes the whole production chain from the farm to the plate and refers to the use case of small farming systems in the Greek region. Results shows that exists significant differentiation on the carbon foot printing evaluation between open and mixed field farms. For both types of farms, the largest contribution to the carbon footprint was the electricity, followed by the use of HDPE materials (mesh & watering tube pipelining). Finally, the least contribution to carbon footprint is due to the transport of raw materials.
Keywords: *Heliciculture, Life Cycle Assessment, Carbon Footprint, Environmental Sustainability.*
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1. Introduction

Ecologically and environmentally sustainable food production requires analysis and study of the supply chain, in a "farm to cradle" approach. Sustainability in this chain is a necessity, because profitability is a requirement in order to be compatible. For this reason, several supply chain scenarios must be developed fixing the total amount of the commodity (snail meat at hand) produced, but with various environmental performance factors. This is an important criterion in order to experimentally verify that the use/result of several environmentally friendly products (either incoming or outcoming) plays an important role in such sustainable environments. Based on the aforementioned presumptions, the goals of this work are to: a) measure the CO_2 impact of the snail farms and rearing supply chains and compare this with that of the present condition through Life Cycle Assessment (LCA). More specifically, we are interested in the effect of bio waste recycling (such as grape marc, carrots, red beets and other cultivated vegetables) in diminishing the carbon footprint of such farmed snail establishments and b) to measure the costs in primary snail production for reference scenarios, based on current cost levels. To achieve both criteria, we propose innovative strategies to be adopted by snail farmers to identify compound farming ventures. We illustrate an LCA research on a mixture of indoor and outdoor small snail farming systems in Greece as a case study. Because of the increasing environmental impact of livestock production, it is required to make fundamental changes on the demand of meat products and in the way alternative production systems can respond to this. Farmed snails might stand for an environmental alternative to usual macro-livestock (Forte *et al*. 2016). A comparison of the most common meat productions (cattle, pig and poultry) noted that edible snails show an essential reduction of impact in almost all categories [Zucaro *et al*. 2016]. In South Europe, snails continue to be collected in vast numbers for food. This fact leads natural populations to decrease, growing the importance of farming [Lazaridou – Dimitriadou *et al*. 1998]. After we impose the general characteristics of the study area and the snail farming systems, we perform an LCA study calculating the CO_2 emission of the supplementary feeding, and the effect of the raw material of the establishment construction. Tests performed in the OpenLCA software and can be used as a model for further inclusion of related processes.

2. Materials and Methods

Life Cycle Assessment

Our aim is to provide an integrated LCA on the environmental performance of snail rearing in Greece. We take under consideration that snail meat represents an environmentally friendly option in relation to conventional livestock. Our research focuses also on the possible environmental impacts of this farming over the product's life cycle from the first stage (purchase of raw materials) to the end-of-life treatment, recycling, and disposal. Table 1 depicts the material contribution to CO_2 emission for the outdoor and the mixed (indoor-outdoor) case. Note the categorization of raw material, electricity (energy), industrial structure and various auxiliary elements to support each state and phase of production along with the side buildings. For each of the inputs in Table 1 we calculated their durability which was then divided by their approximate lifetime to compute yearly values. Indicative values were gathered by the OpenLCA worldwide database system and slightly modified to represent the material used in most of the Greek surveyed establishments.

Table 1. Incoming and outgoing CO_2 contributions to carbon footprint

Flow	Category	Amount	Unit
Calcium carbonate (> 63 microns)	Materials production/Other ...	1.70660	kg
Carrot seed, conventional, at farm gat...	Plant Production/Vegetables	3.51000	kg
Chlorpyrifos	Emission to air/high populati...	3.33000	kg
drinking water	Materials production/Water	420.00000	kg
electricity mix	Energy carriers and technolo...	1260.00000	MJ
gasoline (regular)	Energy carriers and technolo...	14.78000	kg
Haying, with tedder - FR	Others/Agricultural Operations	10.00000	d
polyethylene high density granulate (P...	Materials production/Plastics	15.90000	kg
polypropylene granulate (PP)	Materials production/Plastics	1.02400	kg
Soil decompactation, with cultivator - ...	Others/Agricultural Operations	1.00000	d
Steel hot-dip galvanised coil	Materials production/Metals ...	10.36800	kg
Sunflower, seed, conventional, 9% moi...	Plant Production/Oil + protei...	1.17000	kg
Wooden pallet (EURO)	Production residues in life cy...	109.00000	kg
Zeolite	resource/in ground	83.33000	kg
transport in t*km	Transport services/Other tran...	25.00000	t*km

***(a)* *Outdoor Farming System* CF_{E1}=0.52728 Kg CO_2eq**

Flow	Category	Amount	Unit
aerated concrete block	Systems/Construction	18.18600	kg
Calcium carbonate (> 63 microns)	Materials production/Other mine...	503.57100	kg
Carrot seed, conventional, at farm gate - FR	Plant Production/Vegetables	0.45000	kg
Chlorpyrifos	Emission to air/high population ...	30.00000	kg
drinking water	Materials production/Water	44.74300	kg
electricity mix	Energy carriers and technologies...	6.42857E4	MJ
gasoline (regular)	Energy carriers and technologies...	0.59120	kg
polyethylene high density granulate (PE-HD)	Materials production/Plastics	8.55692	kg
polymethyl methacrylate (PMMA) beads	Materials production/Plastics	12.90300	kg
polypropylene granulate (PP)	Materials production/Plastics	4.76190	kg
Polystyrene, general purpose, GPPS, at plan...	plastics/polymers	20.87893	kg
polyvinylchloride resin (B-PVC)	Materials production/Plastics	61.53714	kg
Soil	Resource/in ground	357.14200	kg
Steel hot-dip galvanised coil	Materials production/Metals and...	33.66024	kg
Steel sections	Materials production/Metals and...	25.44183	kg
Transport, passenger car, diesel, fleet avera...	transport systems/road	1.00000	p*km
Wooden pallet (EURO)	Production residues in life cycle/...	62.14269	kg

***(b)* *Mixed [indoor-outdoor] Farming System* CF_{E2}=4.24146 Kg CO_2eq**

3. Results and Discussion

As Table 1 shows, there exists significant differentiation on the carbon foot printing evaluation between open and mixed field farms and this is due to the inclusion of the material/elements used in the calculation. The extensive CF amounted to 0.52728 Kg CO_2eq, and the intensive CF amounted to 4.24146 Kg CO_2eq, with the highest share, 79.6% for intensive farm and 98.3% intensive farm, from electricity consuming. The percentage of CF can be decreased by 18%, including possible CO_2 binding from snail shells. Note that for both types of farms, the largest contribution to the carbon footprint was due to the electricity consumption with slightly smaller percentages for open farming than mixed farming, followed by the use of HDPE materials (mesh & watering tube pipelining), the use of metal materials (sheet & fencing mesh) and the fuel consumption for agricultural machinery operation. Finally, the least contribution to carbon footprint is due to the transport of raw materials. Greenhouse gas emissions data are also presented and CF of other animal production (breeding cattle, pigs, poultry, etc.) are compared to the results of this study. In conclusion, snail farming is one of the most environmentally friendly sources of protein.

References

Forte A., Zucaro A., De Vico G., Fierro A. (2016). Carbon footprint of heliculture: A case study from an Italian experimental farm, Agricultural Systems 142, pp. 99-111.

Lazaridou-Dimitriadou M., Alpoyanni E., Baka M., Brouziotis Th., Kifonidis N., Mihaloudi E., Sioula D., Vellis G. (1998). Growth, mortality and fecundity in successive generations of *Helix aspersa* Muller cultured indoors and crowding effects on fast, medium and slow – growing snails of the same clutch. J. Moll. Stud. 64, 67-74.

Zucaro A., Forte A., de Vico G., Fierro A. (2016). Environmental loading of Italian semi-intensive snail farming system evaluated by means of life cycle assessment, Journal of Cleaner Production 125, 56 – 67.

SHORT-TERM SURVIVAL OF FAN MUSSEL (*Pinna nobilis* L.) DURING AN EXPERIMENTAL TRANSPLANTATION IN MALIAKOS GULF, GREECE: FIRST RESULTS

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Abstract
A pilot experiment regarding transplantation of 100 specimens of *Pinna nobilis* took place in Summer of 2019 in Maliakos Gulf, Greece. After 90 days, an assessment was carried out, estimating the survival rate. Transplantation, if well designed, could be a management solution for vulnerable species.
Keywords: *Pinnidae, Bivalves' Translocation, Environmental Management, Conservation*
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1. Introduction

The fan mussel *Pinna nobilis* L. is the largest endemic bivalve in the Mediterranean Sea, reaching up to 120 cm. Till 2016, natural populations had already declined significantly due to fishing and boat anchoring (Katsanevakis 2006). Because of the species' vulnerability, there is in place a well-established legislative framework protecting the fan mussel at national, EU and international scale (Presidential Decree 67/1981, Council Directive 92/43/EEC and Barcelona Convention, respectively).

Within the context of designing and performing preliminary actions first to document the transplantation's results and second to propose conservation and management scenarios and solutions, an attempt to transplant alive specimens was realized in Maliakos Gulf, Greece. Certain sites of the area have been renowned for thriving populations of the bivalve shellfish with known population structure and recruitment/growth parameters (Theodorou *et al*. 2017). The aim of this work was to present the results from the transplantation action. Thus, the option of transplantation of sedentary organisms might prove to be a solution to protect vulnerable species on condition that the transplantation-induced mortality remains at low levels.

2. Materials and Methods

Maliakos Gulf is located on the eastern side of mainland central Greece and part of the Aegean Sea (Figure 1; more characteristics of the area in Theodorou *et al.* 2017). The fan mussel is widely distributed in the coastal zone, having the anterior part of the shell partially buried in soft or hard substrata and is attached by threads of its byssus. On the 12th of June 2019, experienced, local divers removed 100 specimens, covering all visible mollusk sizes from a site near Stylida's port. As soon as mollusks were carefully removed from their natural habitat, paying attention not to lose the byssus threads, certain parameters including total length (tL), unburied length (uL), shell width (sW) in centimeters and total biomass (W) in grams (Figure 2), were measured on board. At the same time, fishing vessels were heading towards the transplantation site, about a mile away easternwards where molluscs were attentively placed, in a depth zone of 5 to 10m. Specimens were divided in two groups: one transplanted in soft bottom, the other in hard substrate. On the 5th of September 2019, the assessment of transplantation took place. Biometric parameters were measured along with the qualitative recording of survival or mortality.

Figure 1. Map of the study area

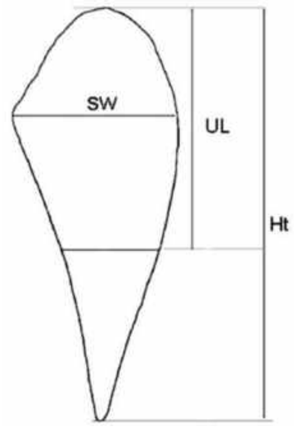

Figure 2. Biometry of *Pinna nobilis*: Total shell length (Ht), unburied length (uL) and shell width (SW)

3. Results and Discussion

Quantitative assessment showed 70% short-term survival rate and is in conformity with other pilot studies (Katsanevakis 2016). The rate of survival was different between the groups (group 1/soft substrate: 0.65, group 2/hard substrate: 0.775). Larger specimens survived less as shown in Table 1. The relationship between total Length (hT) and Biomass (W) was estimated and the graph is shown in Figure 3. Transplantation might prove to be a viable, cost-effective solution, incorporating local ecological knowledge and stakeholders' acceptance. More research is needed to design a suitable working protocol regarding the size and the density of transplanted specimens, and the appropriate time and place of transplantation.

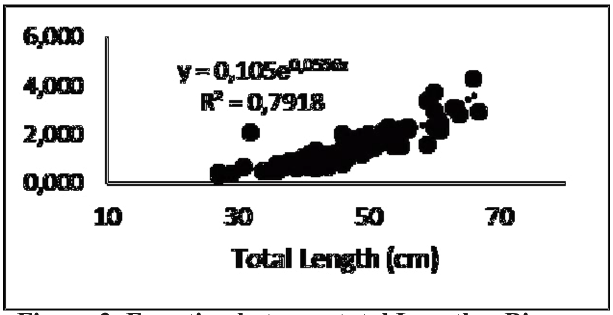

Figure 3. Equation between total Length – Biomass.

Table 1. Biometrics of transplanted / survived specimens. uL is unburied Length, SW is shell width.

parameter	transplanted (n=100)	survived (n=70)
	average/st.dev/var	average/st.dev/var
uL (cm)	27,30 / 5,51 / 16-42	24,90 / 5,47 / 16-43
SW (cm)	18,79 / 2,66 / 13-32	16,73 / 2,05 / 13-22

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References

Katsanevakis S. (2006). Population ecology of the endangered fan mussel *Pinna nobilis* in a marine lake. Endangered Species Research, 51-59.

Katsanevakis S. (2016). Transplantation as a conservation action to protect the Mediterranean fan mussel *Pinna nobilis*. Marine Ecological Progress Series 546, 113-122.

Theodorou J.A., James R., Tagalis D., Tzovenis I., Hellio C., Katselis G. (2017). Density and size structure of the endangered fan mussel *Pinna nobilis*, in the shallow water zone of Maliakos Gulf, Greece. Acta Adriatica 58(1), 63-76.

MARINE SPATIAL PLANNING: A GIS TOOLBOX TO ASSESS THE SIGNIFICANCE OF CONFLICTS OF ACTIVITIES IN COASTAL AREAS

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Abstract. In marine and coastal areas multiple activities are met which in several cases are overlapped with a high portion of them being competitive, leading to serious conflicts. Marine Spatial Planning (MSP) is widely recognized as an effective tool to implement strategic approaches in order to identify and reduce these conflicts, as well to plan future activities in the framework of sustainable development. A pre-requisite for this process is the delineation and evaluation of the significance of conflicts of activities at spatial scale. In this paper, assessment of the significance of such conflicts in the marine/coastal areas of the Cyclades, a group of islands in the Aegean Sea, Greece is performed. The methodology was based on the use of the AHP (Analytic Hierarchy Process) and was applied via a geo-processing tool developed and operated within a GIS environment. The assessment was based on selected criteria which reflected the degree of pressure that each conflict posed in the area and they were assigned appropriate weights. As a result, the zones of conflicts of activities were delineated and ranked according to their relative pressure. The outcome of the methodology can be visualized on thematic maps and is easily understandable by non-expert GIS users and decision-makers. The usefulness of such spatial tools in the implementation of MSP by stakeholders and local agencies is highlighted.
Keywords: *Geo-Processing Tools, Coastal Management, Multi-Criteria Analysis, Aegean Sea*
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1. Introduction

The continuous demand for marine space with diverse uses, i.e. maritime transport and fisheries, aquaculture areas, renewable energy, biodiversity and ecosystems' conservation, tourism etc., leads very often to the co-existence of competitive activities that lead to conflicts (Kyvelou & Ierapetritis, 2019). Two major forms of conflicts are stated; the "user-environment conflicts", which arise between the human uses and the environment, and the "user-user conflicts", when various activities are incompatible with each other, competing for space (Moore *et al.* 2017). A promising management approach to convert conflicting activities into solutions, managing simultaneously various marine activities is MSP (Pınarbaşı *et al.* 2017). Geographic Information Systems (GIS) which have been extensively used in decision-making and the management of marine and coastal areas, provide a suitable environment for analysis of spatial information and are considered valuable at various stages of the MSP process (Shucksmith *et al.* 2014). In literature, several methods are found for the assessment and management of conflicts when implementing MSP (Tuda *et al.* 2014; Ye *et al.* 2021). In this paper, the multi-criteria AHP method was applied to evaluate the significance of conflicts at spatial scale. AHP is widely known due to its simplicity and effectiveness to measure the consistency of results as well as its capability of applying weights of importance to the evaluation criteria (Saaty 2008). The methodology was developed in a GIS environment and became operational as a GIS toolbox. It was applied in the Cyclades area, Aegean Sea, Greece which was selected for the case study.

2. Material and Methods

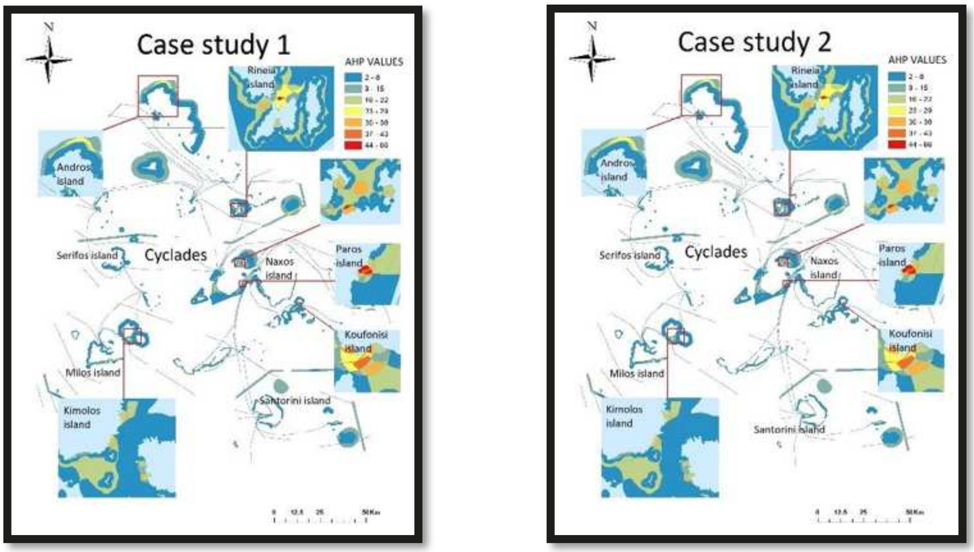
The Cyclades is a group of islands with common geographical and cultural characteristics, where a significant number of marine / coastal activities are met; therefore, the implementation of effective MSP is considered of high importance. The data sets related to the activities met in this area were acquired from numerous sources, among which various websites with open access data (i.e. geodata.gov.gr) as well as several public and private services. The ESRI, ArcGIS 10.8.1 software was used to design and develop a geo-database in a GIS environment, where all data sets were stored. In particular, the following 14 GIS layers representative of the activities taking place in the study area were stored in the geo-database: marine aquaculture, purse seining, coastal fishing, trawling, bathing waters, ports, passenger shipping routes, marinas, anchorages, submarine communication cables, wastewater disposal, underwater archaeological sites, Marine Protected Areas (Natura 2000 Network), and areas covered by the phanerogam *Posidonia oceanica*. In the next step, all activities were classified according to four criteria: (a) Mobility (fixed = 2, mobile = 1), (b) Spatial scale (large = 3, medium = 2, small = 1), (c) Time scale (long = 3, medium = 2, short = 1) and (c) Vertical scale (whole water column = 2, surface

/ sea floor = 1). In addition, a table called ‘Matrix of conflicts’ was created, where all conflicting activities were determined according to the existing European / Greek legislation and local / regional regulations.

The AHP was applied to calculate the level of conflict score via the developed GIS tool. The methodology involved the paired comparison of importance among criteria based on the 1 to 9 “Saaty scale” (value 1 is assigned to the judgment that the two criteria are equally important and 9 is assigned when the first criterion is extremely important compared to the second). The final weight of importance of each criterion was calculated using the paired comparison matrix. The conflict scores were calculated by multiplying the weights of importance of each criterion by the classification values of each activity assigned in the previous step and finally by adding the calculated values of the two activities that constitute a conflict; the higher conflict score, the greater pressure caused by the conflict.

3. Results and Discussion

The methodology was applied twice with different priorities set. In the first case (Figure 1a), the criteria were assigned equal priorities, while in the second one (Figure 1b) the criteria related to the duration of the presence of conflicts (mobility and time scale) were assigned higher importance. Both cases demonstrated the areas which were more pressed by the presence of conflicts. The comparison of the two cases indicated that the duration of the presence of activities did not have a significant effect on the ranking of the most affected areas by conflicts; small differences were observed. Further application of the methodology by assigning different weights to the criteria is planned.



(a) **(b)**

Figure 1: Result of the GIS tool by assigning (a) equal weights to the criteria and (b) higher weights to the criteria related to the duration of the presence of activities.

References

Kyvelou S.S., Ierapetritis D. (2019). Discussing and analyzing “maritime cohesion” in MSP, to achieve sustainability in the marine realm. Sustainability (Switzerland) 11, 1–29.

Moore S.A., Brown G., Kobryn H., Strickland-munro J. (2017). Identifying conflict potential in a coastal and marine environment using participatory mapping. Journal of Environmental Management 197, 706–718.

Pınarbaşı K., Galparsoro I., Borja Á., Stelzenmüller V., Ehler C.N., Gimpel A. (2017). Decision support tools in marine spatial planning: Present applications, gaps and future perspectives. Marine Policy 83, 83–91.

Saaty T.L. (2008). Decision making with the analytic hierarchy process. International Journal Services Sci. 1, 83.

Shucksmith R., Gray L., Kelly C., Tweddle J.F. (2014). Regional Marine Spatial Planning – The data collection and mapping process. Marine Policy 50, 1–9.

Tuda A.O., Stevens T.F., Rodwell L.D. (2014). Resolving coastal conflicts using marine spatial planning. Journal of Environmental Management 133, 59–68.

Ye G., Fei J., Wang Z., Jiang Q., Gaines S.D., Ming C.L. (2021). A novel marine spatial management tool for multiple conflicts recognition and optimization of marine functional zoning in the East China sea. Journal of Environmental Management 298.

OCCURRENCE OF ORGANOCHLORINATED PESTICIDES IN THE MARINE BIOTA OF THE MEDITERRANEAN SEA: A SYSTEMATIC REVIEW

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Abstract This survey consists a systematic review on the scientific literature that has been published up to 31/12/2019 regarding the scientific research which has been conducted and concerns the occurrence and detection of organochlorinated pesticides (OCPs) in the marine biota of the Mediterranean Sea. Based on the gathered information from the collected and classified articles, useful conclusions, and observations are reported about research trends. At the same time, knowledge gaps and weaknesses in the current research are identified and highlighted. Furthermore, suggestions for future research on this topic are also discussed.
Keywords: *Pesticide, Organochlorine Compound, Mediterranean, Micropollutants, Marine Organisms*
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1. Introduction

Pesticides, also known by the terms plant protection products or agrochemicals, are chemical compounds that are used and applied worldwidely for the protection of plants against pests, weeds and diseases which affect the quantity and quality of agricultural products. Especially the class of organochlorinated pesticides (OCPs) are synthetic organic compounds whose use has been banned by the relevant legislation in developed Mediterranean countries in the 1970s and 1980s due to their unique characteristics and physicochemical properties, such as lipophilicity, acute and chronic toxicity, environmental persistence, and their ability to bioaccumulate in biological tissues. However, their residual quantities into marine ecosystems are still monitored and detected nowadays (Milun *et al*. 2016, Necibi *et al*. 2020, Carro *et al.* 2021). Based on the published scientific literature, the occurrence and distribution of those persistent organic pollutants in several species of marine biota is a subject on which the interest of the global scientific community is focused undiminished since the effects of their extended use are evident even today. Hence, the aim of the present study is to carry out a systematic review concerning the scientific articles published until 31/12/2019, regarding the research of the presence of residual quantities of OCPs in the marine inhabitants of the Mediterranean Sea.

2. Methods and Materials

The current review was focused only on peer-reviewed journal articles that were published between 1st January 1970 to 31st December 2019. The retrieve of the relevant published scientific articles was achieved by using "Scopus Search" (abstract and citation database) and the selection of studies was made by using 7 different combinations of selected terms/keywords that were: (i)"Mediterranean" AND "organochlorine" AND "pesticides" (n=165 records), (ii) "Mediterranean" AND "organochlorine" AND "contamination" (n=100 records), (iii) "Mediterranean" AND "organochlorine" AND "pollution" (n=185 records), (iv) "Mediterranean" AND "organochlorine" AND "biomonitoring" (n=17 records), (v) "Mediterranean" AND "organochlorine" AND "monitoring" (n=160 records), (vi) "Mediterranean" AND "pesticide" AND "monitoring" (n=250 records), and (vii) "Mediterranean" AND "pesticide" AND "contamination" (n=189 records). Only documents which were available in English were included in this analysis, and the search provided a total of 1066 articles. After the elimination of duplicates, and triplicates (580 articles) 486 unique entries remained in the database. Afterwards, based on review of the abstract 254 of the 486 documents were excluded due to their lack of relevance. Finally, based on the full article review the selected articles that met the criteria of the bibliographic research and that were included in the present systemic review were summarized using the following information: author (date), location of the study, organochlorine pesticide(s) analyzed, type of analyzed marine substrate (water, sediment, biota), target species, population (sample size), method used, and primary conclusions.

3. Results and Discussion

Overall, among the 256 selected articles that met the criteria of the bibliographic research and thus were included in the present systemic review only the 170 records (as published yearly illustrated in Figure 1) provided relevant information for assessing the occurrence of OCPs in the biological tissues of marine biota, whereas the rest (86 records) contained published data regarding the presence of OCPs exclusively into the marine environment of the Mediterranean Sea (in water or/and sediments matrices). Based on reported information as summirized and depicted in Figure 2, residual levels of these pollutants have been detected and quantified into a wide variety of marine species belonging to different taxonomic classes, whereas most of them (74%) concerned fish.

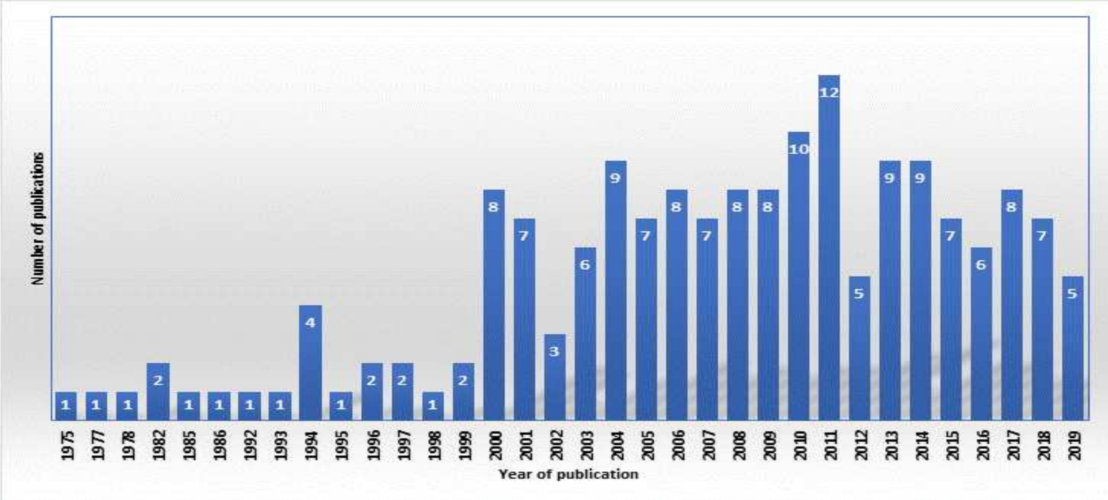

Figure 1. Number of relative published data *vs* year of publication

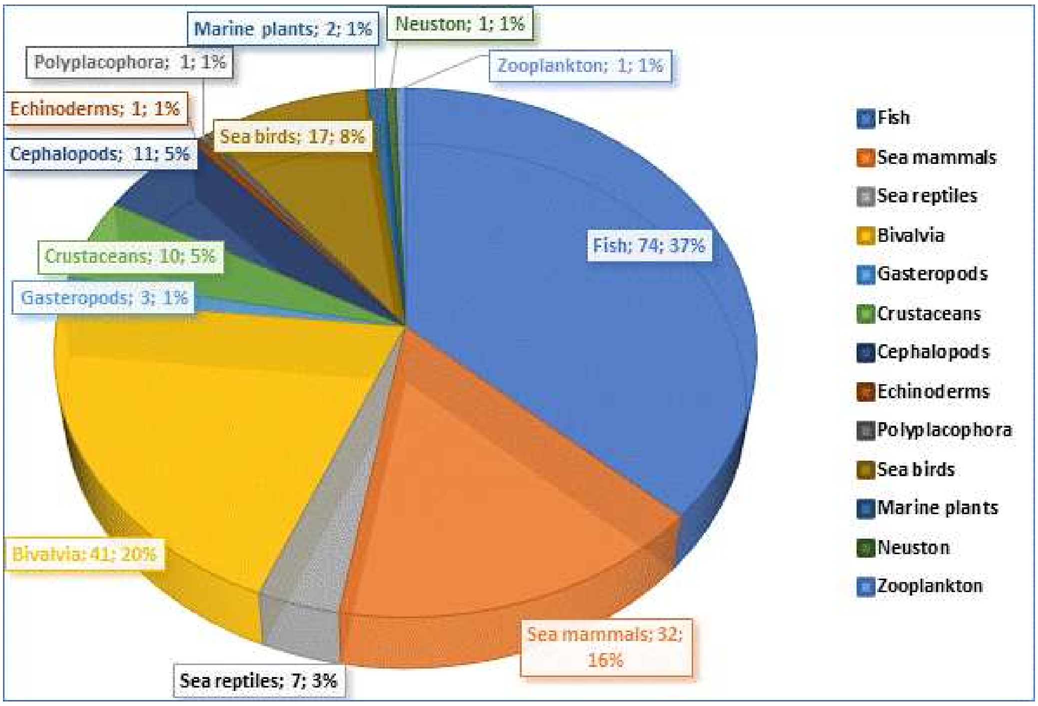

Figure 2. % relative frequency of OCPs detection in different taxonomic classes of marine environments

References

Carro N., Cobas J., García I. (2021). Organochlorine compounds and polycyclic aromatic hydrocarbons in mussels from Ria de Vigo (the Northern Spanish coast). Current levels and long-term trends (2010–2019). Relationship with human pressures. Regional Studies in Marine Science 44, Art. No.101742.

Milun V., Grgas D., Dragičević T.L (2016). Assessment of PCB and chlorinated pesticide accumulation in mussels at Kaštela Bay (Eastern Adriatic). Science of the Total Environment 562, 115-127.

Necibi M., Mzoughi, N. (2020). Distribution of organochlorine pesticides in sediment cores from the Bizerte Lagoon (Tunisia). International Journal of Environmental Analytical Chemistry 100, 1118-1132.

INVESTIGATION OF A MODEL DESIGN IN A LOW DAM FISH PASS HYDRAULIC CONSTRUCTION - THE CASE STUDY OF THE CONSTRUCTION NAMED «ROLLER PATH (RPT)»

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Abstract
The intention for increased exploitation of surface water resources, inevitably directs the strategic plans in the construction of special technical projects, mainly dams of various dimensioning. Today, one of the biggest challenges facing environmentalists is the ecological response to the disconnection of fish upstream and downstream of dams. The solution is offered through the rational design and construction of special hydraulic structures for fish passages like the "Roller PaTh (RPT)", a construction technique that approaches the concept of the environment, based on the principles of sustainable development. It is necessary to further investigate the operation of its construction, based on the hydrological characteristics that are formed, but also the behavioral profile of the fish that develops.
Key words: *Dams, Fish passes, Roller PaTh (RPT), Technical Plan, Sustainable Development*
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1. Introduction

One of the tensions in the exploitation of water resources is reflected in the construction of dams of various dimensions aimed at generating electricity, irrigation to meet the agricultural requirements of an area, regulating the diet of watercourses and ensuring soil stabilization of catchments, etc.

One of the most important consequences of the construction of dams in the natural environment and especially in the aquatic environment is the cessation of the continuity of the river and therefore the free movement of fish populations along it (Psilovikos 2014). It is noted that the construction of dams on rivers has led to fish populations declining in number or even extinction (Freyhof & Brooks 2011). The anadromous and catadromous fish species need unobstructed, fully accessible paths between the river and the sea (Fuller *et al.* 2015) to reach spawning grounds, often moving to fewer, smaller and isolated habitats. The European Union has also expressed its great concern, where through the European Water Framework Directive 2000/60/EC, it considers the efficient passage and undisturbed migration of fish as the key component for the restoration and sustainable river basin management.

Faced with this significant problem, the environmental managers had two solutions in front of them: either a) the partial or total removal of barriers (Bellmore *et al.* 2019), which is practically impossible, or b) the construction of special technical constructions of fish passages (Silva *et al.* 2018).

2. Materials and Methods

In the present paper, the design of a model hydraulic construction of low dam fish passages is attempted. This construction under the name "Roller PaTh (RPT)" refers to a technical design consisting of a series of tanks mounted on a sloping ramp (Figure 1). The tanks are placed in stages and are separated from each other by small dams that overflow with the help of water.

Each tank is distinguished: a) in the overflow orifice and the submerged orifice, through which the fish will descend and ascend respectively, b) in the concave bottom, which serves the concave flow of water, directing the fish upstream of the tank, c) the rotating roller/flow rate regulator, and d) the short-term fish rest area, located at the bottom of the tank and serving both descending and ascending fish. The "Roller PaTh (RPT)" construction is based on the holistic view of environmental perception, in which each parameter of the problem solution does not work one-dimensionally within the whole but is completely influenced and interdependent on the other parameters.

During the design of the hydraulic construction of the "Roller PaTh (RPT)", the following parameters were taken into account: a) the flow, the speed, the direction, the water depth and the hydrodynamic characteristics of the movement, b) the depreciation – destruction of energy, c) the rest areas, d) the water transport from individual areas, e) the traction speeds, f) the inlet vortices, g) the distance between individual tanks, and h) the species of fish to be passed.

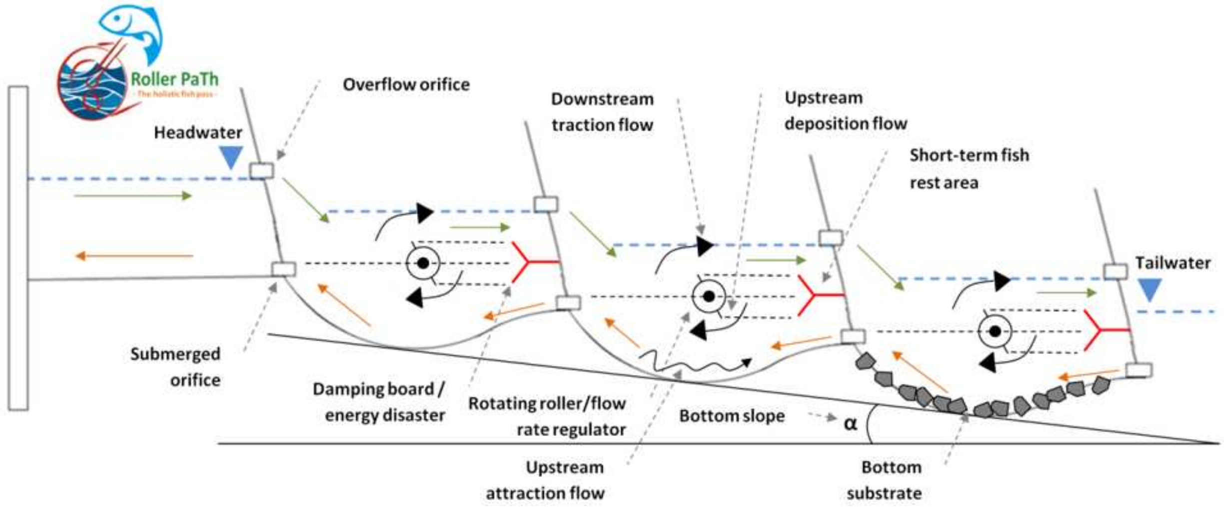

Figure 1: Side view of "Roller PaTh (RPT)" plumbing design.

3. Results

Hydraulic construction of "Roller PaTh (RPT)" fish passages is an early stage project. It is considered necessary to further develop and investigate both the design and the dimensioning of its basic and secondary parameters with the use of appropriate computer programs.

It is emphasized that the "Roller PaTh (RPT)" was designed as a rule to serve a large number of fish populations, to be attractive to them, to be autonomous, but also to serve other purposes, such as energy production and storage. The innovative "Roller PaTh (RPT)" function is based on: a) the concave bottom of each tank, b) the installation of special night lighting, and c) the rotating roller.

The rotation of the roller creates a rotating flow of water, which favors the fish in the upper water layer to turn to the overflow orifice and descend. In contrast to the lower aquifer, two streams are created, one upward (Zone A, around the rotating roller) and one downward stream (Zone B), which moves in the area of the hollow bottom. These two opposing flows help the fish to turn to the submerged stream and eventually ascend upstream, while preventing them from entering the upper water layer of the tank, resulting in disorientation.

4. Discussion

The evaluation of environmental interventions and at the same time the development of modern upgraded plumbing projects can overturn misconceptions about water exploitation and environmental protection, preventing further degradation. One such modern and sustainable construction is the "Roller PaTh (RPT)". The innovation of the "Roller PaTh (RPT)" lies in the combination of the following features that govern it: a) it is autonomous, b) its construction and installation is simplistic, c) it has cheap construction costs, d) it can generate electricity, hence profits, and e) may attract several fish populations.

The hydraulic construction of the "Roller PaTh (RPT)" is based on the principles of holistic vision, is a dynamic system based on the triptych: a) conservation and development of the environment, b) promotion of biodiversity, and c) pursuit of social welfare.

References

Bellmore J.R., Pess G.R., Duda J.J., O'Connor J.E., East A.E., Foley M.M., Wilcox A.C., Shafroth P.B., Morley S.A., Magirl C.S., Anderson C.W., Evans J.E., Torgersen C.E., Craig L.S. (2019). Conceptualizing ecological responses to dam removal: if you remove it, what's to come? BioScience 69, 26–39.

Freyhof J., Brooks E. (2011). European Red List of Freshwater Fishes. Publications Office of the European Union, Luxembourg.

Fuller M.R., Doyle M.W., Strayer D.L. (2015). Causes and consequences of habitat fragmentation in river networks. Ann. N. Y. Acad. Sci. 1355, 31–51.

Psilovikos A. (2014). Ecohydraulics. Tziolas Pub., Thessaloniki, pp. 536.

Silva A. T., Lucas M. C., Castro-Santos T., Katopodis C., Baumgartner L. J., Thiem J. D. et al. (2018). The future of fish passage science, engineering, and practice. Fish and Fisheries 19(2), 340–362.

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