3rd International Marine & Freshwater Sciences Symposium (MarFresh)

1-2 September 2026

İskenderun Technical University
İskenderun/Hatay, Türkiye

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Edited by

Prof. Dr. Tahir Özcan

September 1-2, 2026
İskenderun / Türkiye

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	Page No
SYMPOSIUM COMMITTEE	4
CONTENTS	6
FINAL DECLARATION	8
SCIENTIFIC PROGRAMME	9
PRESENTATIONS	13
Kevrekidis, K. (2026). Latest developments in research on the invasive blue crab (*Callinectes sapidus*) in the Mediterranean Sea. MarFresh Symposium., 3: 14.	14
Tarkan, A.S. (2026). Towards Predictive Invasion Ecology: From Species Distribution to Ecological Impacts. MarFresh Symposium., 3: 15.	15
Gammoudi, M. (2026). First Record of *Tubulanus nothus* (Bürger, 1892) (Nemertea: Palaeonemertea) from Tunisian Coastal Waters.	16
Berlajolli, V., Orqusha, N., Dreshaj, A., Behrami E., Płóciennik, M. (2026). *Prodiamesa olivacea* as an indicator of heavy metal contamination in upland streams (Drini i Bardhë River, Kosovo).	17
Krakowiak, M., Zawal, A. (2026). The impact of tectonic processes on the genetic diversity and population structure of Hydrachnidia in river systems of Iran.	18
Shumka, S. (2026). Threatened Fish of Albania, Conservation Management and Actions Needed.	19-26
Shumka, L. (2026). Current Ecological Pressures, Management Opportunities and the Connectivity Context of Aquatic and Terrestrial Landscape in Butrinti National Park and Unesco Site.	27-34
Hajirezaee, S., Khanjani, M.H. (2026). Pollutants and environmental stresses in aquaculture: Disruptions and the moderating role of medicinal plants from a metabolomics perspective.	35
Hajirezaee, S., Khanjani, M.H. (2026). Oocyte Ageing in fish from a Metabolomics Perspective.	36
Boumallassa, F.A., Moutaouakil, S., M'hamdi, F., Aroumy, F.E., Hachimi, T., Abouelaaz, F., Benlasri, M., Naimi, M., Ghamizi, M. (2026). Resource partitioning sustains a multi-endemic threatened barbels assemblage in a Mediterranean stream: evidence from the OLA KBA, Morocco.	37
Çek, Ş., Aydın, F. (2026). Serum-free Media for Fish Cells in Cultivated Seafood Production.	38-51
Başusta, N., Başusta, A. (2026). A New Record of Juvenile Shortfin Mako Shark (*Isurus oxyrinchus*) off the Coast of Side, Gulf of Antalya (Türkiye).	52-56
Başusta, N., Başusta, A. (2026). Records of newborn Marbled electric rays (*Torpedo marmorata*) off the coast of Alanya, Gulf of Antalya (Türkiye).	57-60
Deveci, Ö., Tiğrek, Ş. (2026). The Role of Ship Stability in Maritime Accidents: A Literature Review.	61-73
Başar, T., **Tiğrek, Ş.** (2026). A Review of Türkiye's Offshore Renewable Energy Potential.	74
Kutlu, B. (2026). Bibliometric Analysis of Microplastic Research in Freshwater Ecosystems: Global Trends, Scientific Collaborations, and Emerging Research Themes.	75-86
Özcan, G., **Dörtbudak, M.Y.** (2026). Research on Endemic Fish Species in the Orontes River: A Bibliometric Approach.	87-96
Shumka, L., **Kunt, F.,** Çiftçi, Ç., Argun, M. (2026). Application of the Integrated Beach Quality Index (BQI) for selected semi-urban, semi-rural, and urban types in Albania.	97-103
Jahan, S.N., Rahman, Md. A. (2026). Comparative Assessment of Nutritional and Organoleptic Properties of Sun-and Oven-Dried Small Prawns in Bangladesh.	104
Heß, M. (2026). Adaptation of clupeid visual systems in aquatic habitats.	105
Aydın, F., Çek, Ş. (2026). Zebrafish as a model organism in probiotic and postbiotic research for aquaculture.	106-132
Islam, A. (2026). Salted Hilsa Fishery Products: Supply Chain And Quality Issues in Bangladesh.	133
Galib, S.M., Kashmi, N.S., Parvez, Md.T. (2026). Dire conservation need for three co-occurring freshwater megafauna in a human-dominated river of Bangladesh.	134
Rasool, F., Azeem, M., And Fazeela, S.H. (2026). Growth Enhancement of Pangasius (*Pangasius hypophthalmus*) through Effective Treatment for NH3 Raised in Bio Flock System.	135
Muçollari, N., Shumka, S., Kullaj, A. (2026). Environmental Assessment of the Vjosa River Based on Heavy Metal and Microbiological Contamination in *Barbus prespensis* and *Squalius platyceps*.	136-142
Kozłowska, A., **Pietrzak, A.,** Zawal, A. (2026). Phylogeography and integrative taxonomy of water mites (Hydrachnidia) in Great Britain: implications for postglacial colonization processes.	143
Filiz, H., Gedikoğlu, S.Ö. (2026). Discovery of a new aggregation site of Sandbar Sharks (*Carcharhinus plumbeus*) in Alayurt Cove, Eastern Mediterranean.	144-148
Yavuz, Ş., **Yabanlı, M.** (2026). Food Safety Evaluation of Edible Bivalves from the Marmara Sea: Potentially Toxic Elements, Dietary Exposure, and Human Health Risk Assessment.	149-158

Tosya, M., Acar, S. (2026). Development of a Hemocyanin-Based Wound Care Gel Prototype from Blue Crab (*Callinectes sapidus*).	159
Özcan, G., **Dörtbudak, M.Y.** (2026). Exploring Fish Otoliths in Orontes River Research: A Bibliometric Approach.	160-169
Elglid, A., Enajjar, S., Saidi, B., Bradai, M.N., Chatti, N. (2026). Genetic erosion and demographic signatures in Mediterranean white sharks (*Carcharodon carcharias*): insights from Tunisian waters.	170
Özgen, Ö., **Acar, E.** (2026). Crustacean Fauna of Iskenderun Bay: A Review of Nearly a Century of Studies.	171-188
Yeşmil, B., **Acar, S.,** Ateş, A.S. (2026). Crustacean Fauna of *Zostera marina* Meadows off the Dardanos Coast, Çanakkale Strait: A Preliminary Study.	189
Filiz, H., Kunduz, A., Taşkavak, E. (2026). Growth, Reproduction, and Population Structure of Silver Scabbardfish (*Lepidopus caudatus*, Euphrasen, 1788) in Gökova Bay (Southern Aegean Sea, Türkiye)**.**	190-198
Aroumy, F.E., M'hamdi, F., Boumallassa, F.A., Hachimi, T., Elbouhali, K., S'khifa, A., Haddou, N., Ghamizi, M. (2026). Groundwater Biodiversity in the Taza Region (Morocco): Hydrobiidae Distribution and Conservation Priorities.	199
M'hamdi, F., Boumallassa, F.A., Elbouhali, K., Aroumy, F.E., Hachimi, T., S'khifa, A., Haddou, N., Teixeira, A., Ghamizi, M. (2026). Conservation of the Bouhlou Key Biodiversity Area, A hope for the Critically Endangered Freshwater Mussel *Pseudunio marocanus* (Pallary, 1918) (Mollusca: Margaritiferidae), Morocco.	200
Iftekhar, F., Zawal, A. (2026). Geographical and Environmental Distribution of Water Mites (Acari: Hydrachnidia) in Pakistan: An Integrative Morphological and DNA Barcoding Study.	201
Asif, M., Zawal, A. (2026). Tectonic History and Water Mite Diversity in Pakistan: A Biogeographical Hypothesis.	202
Elbouhali, K., Boumallassa, F.A., M'hamdi, F., Aroumy, F.E., Hachimi, T., Benlasri, M., Sousa, R., Ghamizi, M. (2026). Competitive Effects of the Invasive Nile Tilapia *Oreochromis niloticus* (Actinoptyrigii: Cichlidae) on Growth and Survival of the Endemic Moroccan Barbel *Carasobarbus fritschii* (Actinoptyrigii: Cyprinidae).	203
Belogurova, R.E., Alyomova, A.S. (2026). Phylogenetic Relationships of Pumpkinseed Sunfish (*Lepomis gibbosus*) from Water Bodies of the Northern Black Sea Region and across Its Native Range.	204
Ahnich, A., **Boumallassa, F.A.,** Khalki, H., Naimi, M. (2026). Seasonal Feeding Ecology of Endemic Barbels in a North African River: The Zat Basin Case.	205
Kapranov, S.V., Gureeva, E.V., Kapranova, L.L., Toichkin, A.M., Dikareva, J.D., Ryabushko V. I. (2026). Rare Earth Element Bioaccumulation in Biota of the northern Black Sea (Crimea).	206-212
Özdoğan, H.B.E. (2026). Morphometric Characterization of Cocoons in the Southern Medicinal Leech (*Hirudo verbana* Carena, 1820).	213
Hossain, M.I., Samad, M.A., Afrin, S., Istiaque, S. (2026). Assessment of Pond Productivity through Carp with *Macrobrachium rosenbergii* Culture at Rajshahi, Bangladesh.	214
Özcan, T., Dağlı, E., Çiçek, B.A. (2026). Macrofauna assemblages along the new Jetty in Iskenderun Bay, Mediterranean Sea, Türkiye.	215-218
Azaiez, K., **Killi, N.** (2026). Jellyfish along the Tunisian Coasts: Occurrence Patterns and Stinging Cases.	219-224
Momen, K.A. (2026). A Vision for Sustainable Aquaculture Development in Eastern Libya: Opportunities, Challenges, and Pathways to a Resilient Blue Economy.	225-235

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Final Declaration

3rd International Marine & Freshwater Sciences Symposium (MarFresh 2026), which took place at the Iskenderun Technical University (ISTE), İskenderun, Türkiye, 1–2 September 2026, was organised by the Iskenderun Technical University (ISTE) with the support of Harran University, AssosiaTech Publishing and Academic Union Association (Akademik Birlik Derneği).

The scientists participating in the symposium- MarFresh 2026, held both online and in person, included 75 experts from 5 research centres and institutions and 31 universities across thirteen different countries (Albania, Bangladesh, Germany, Iran, Libya, Morocco, Pakistan, Poland, Kosovo, Russia, Tunisia, Grece and Türkiye). Out of the 47 presentations given at the symposium, 20 were from Türkiye and 27 were from 12 different countries.

In final closing speech of the symposium, a fully agreed idea for continuation of this symposium as to organized once in two years was reached. Also, most importantly, in the next meetings (first to be planned in 2028) establishing some working groups on special and interesting issues, and organizing open discussions in round table fashion to reach joint resolutions along with initial steps towards the implementation of solutions were suggested by participants.

Day 1 - Tuesday, 1 September 2026			Day 1 - Tuesday, 1 September 2026	
Conference Hall- TURKUAZ in person			**Conference Hall- TURKUAZ Online**	
9:00-09:30	**Registration of Participants**		9:00-09:30	**Registration of Participants**
Conference Hall- TURKUAZ in person			**Conference Hall-1 Online**	
09:30-09:35	**Opening Ceremony**		09:30-09:35	**Opening Ceremony**
09:35-09:40	**Prof. Dr. Tahir Özcan**		09:35-09:40	**Prof. Dr. Tahir Özcan**
	Symposium Chairman			Symposium Chairman
09:40-10:00	**Prof. Dr. Mehmet Duruel**		09:40-10:00	**Prof. Dr. Mehmet Duruel**
	Rektor of the Iskenderun Technical University			Rektor of the Iskenderun Technical University
10:00-10:30	***Key-note speech***		10:00-10:25	***Key-note speech***
	Dr. Kosmas Kevrekidis			**Dr. Kosmas Kevrekidis**
	Latest developments in research on the invasive blue crab (*Callinectes sapidus*) in the Mediterranean Sea			Latest developments in research on the invasive blue crab (*Callinectes sapidus*) in the Mediterranean Sea
10:30-11:00	Coffee Break		10:30-11:00	Coffee Break
Conference Hall- TURKUAZ in person			**Conference Hall-1 Online**	
Chairman	**Prof. Dr. Şehriban Çek**		**Chairman**	**Assoc. Prof. Dr. Nurçin Killi**
11:00-11:15	**Syeda Nusrat Jahan,** Md. Arifur Rahman		11:00-11:15	**Sergey V. Kapranov,** E.V. Gureeva, L.L. Kapranova, A.M. Toichkin, J.D. Dikareva & V.I. Ryabushko
	Comparative Assessment of Nutritional and Organoleptic Properties of Sun- and Oven-Dried Small Prawns in Bangladesh			Bioaccumulation of rare earth elements in various phyla of marine organisms from the Crimean coastal area (northern Black Sea)
11:15-11:30	**Faycal Ait Boumallassa**, Abdelilah Ahnich, Soumia Moutaouakil, Fatima M'hamdi, Fatima ezzahra Aroumy, Tarik Hachimi , Fatimazahra Abouelaaz , Mokhtar Benlasri , Mohamed Naïmi and Mohamed Ghamizi		11:15-11:30	**Hasan Batuhan Emre Özdoğan**
	Resource partitioning sustains a multi-endemic threatened barbels assemblage in a Mediterranean stream: evidence from the OLA KBA, Morocco			Morphometric Characterization of Cocoons in the Southern Medicinal Leech (*Hirudo verbana* Carena, 1820)
11:30-11:45	**F. M'HAMDI,** F. AIT BOUMALLASSA, K. ELBOUHALI, F. E. AROUMY, T. HACHIMI, A. S'KHIFA, N. HADDOU, A. TEIXEIRA and M. GHAMIZI		11:30-11:45	Gülnaz Özcan, **Muhammed Yaşar Dörtbudak**
	Conservation of the Bouhlou Key Biodiversity Area (KBA): A hope for the critically endangered freshwater mussel Pseudunio marocanus (Pallary, 1918) (Mollusca: Margaritiferidae), Morocco			Exploring Fish Otoliths in Orontes River Research: A Bibliometric Approach
11:45-12:00	T. Başar & **Şahnaz Tiğrek**		11:45-12:00	**Fayyaz Rasool**, Muhammad Azeem, Shakeela Parveen and Fazeela
	A Review of Türkiye's Offshore Renewable Energy Potential			Growth Enhancement of Pangasius (Pangasius hypophthalmus) through Effective Treatment for NH3 Raised in Bio Flock System
12:00-12:15	**Shams M. Galib**, Nadia S. Kashmi, Md. Taskin Parvez		12:00-12:15	**R. Belogurova**, A. Alyomova
	Challenges with the conservation of freshwater megafauna in the Ganges River of Bangladesh			Phylogenetic Relationships of Pumpkinseed Sunfish (*Lepomis gibbosus*) from Water Bodies of the Northern Black Sea Region and across Its Native Range
12:15-14:00	Lunch break		12:15-14:00	Lunch break

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Conference Hall- TURKUAZ in person	
Chairman	**Prof. Dr. Şahnaz Tiğrek**
14:00-14:15	**Şehriban Çek,** Fatmagün Aydın Serum-free Media for Fish Cells in Cultivated Seafood Production
14:15-14:30	**Fatmagün Aydın,** Şehriban Çek Zebrafish as a Model Organism in Probiotic and Postbiotic Research for Aquaculture
14:30-14:45	**Özlem Deveci,** Şahnaz Tiğrek The Role of Ship Stability in Maritime Accidents: A Literature Review
14:45-15:00	**F. A. Flora,** A. Islam Salted Hilsa Fishery Products: Supply Chain and Quality Issues in Bangladesh
15:00-15:15	**Tahir ÖZCAN,** Ertan DAĞLI, Burak Ali ÇİÇEK Macrofauna assemblages along the new Jetty in Iskenderun Bay, Mediterranean Sea, Türkiye
15:15-15:30	Khouloud Azaiez, **Nurçin Killi** Jellyfish along the Tunisian Coast: Occurrence Patterns and Stinging Cases
15:30-16:00	Coffee Break

Conference Hall-1 Online	
Chairman	**Prof. Dr. Nuri Başusta**
14:00-14:15	**M. Heß** Adaptation of clupeid visual systems in aquatic habitats
14:15-14:30	Aleksandra Kozłowska, **Agnieszka Pietrzak**, Andrzej Zawal Phylogeography and integrative taxonomy of water mites (Hydrachnidia) in Great Britain: implications for postglacial colonization processes)
14:30-14:45	**Yuliya Andryushina** First discovery of Plagiorchis elegans cercariae from snails Lymnaea stagnalis in Biyuk-Karasu River (Southern Russia)
14:45-15:00	Ozge Ozgen, **Elif Acar** Crustacean Fauna of Iskenderun Bay: A Review of Nearly a Century of Studies
15:00-15:15	**Karema AlMabrouk Momen** A Vision for Sustainable Aquaculture Development in Eastern Libya: Opportunities, Challenges, and Pathways to a Resilient Blue Economy
15:15-15:30	Gülnaz ÖZCAN, **Muhammed Yaşar DÖRTBUDAK** Research on Endemic Fish Species in the Orontes River: A Bibliometric Approach
15:30-16:00	Coffee Break

Day 2 - Wednesday, 2 September 2026

Conference Hall- TURKUAZ in person	
Chairmen	**Prof. Dr. A. Suat Ateş**
09:00-09:30	
09:30-10:00	*Key-note speech* **Prof. Dr. Ali Serhan Tarkan** Towards Predictive Invasion Ecology: From Species Distributions to Ecological Impacts
10:00-10:15	
10:15-10:30	
10:30-11:00	Coffee Break

Day 2 - Wednesday, 2 September 2026

Conference Hall-1 Online	
Chairmen	**Prof. Dr. A. Suat Ateş**
09:30-10:00	*Key-note speech* **Prof. Dr. Ali Serhan Tarkan** Towards Predictive Invasion Ecology: From Species Distributions to Ecological Impacts
10:00-10:15	Begüm Yeşmil, **Seçil Acar,** A. Suat Ateş Crustacean Fauna of *Zostera marina* Meadows off the Dardanos Coast, Çanakkale Strait: A Preliminary Study
10:15-10:30	**Miray Tosya,** Seçil Acar Development of a Hemocyanin-Based
10:30-11:00	Coffee Break

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Conference Hall- TURKUAZ in person	
Chairmen	**Dr. Shams M. Galib**
11:00-11:15	**Halit Filiz,** Altan Kunduz, Ertan Taşkavak Growth, Reproduction, and Population Structure of Silver Scabbardfish (*Lepidopus caudatus*, Euphrasen, 1788) in Gökova Bay (Southern Aegean Sea, Türkiye)
11:15-11:30	**Halit Filiz,** Sait Özgür Gedikoğlu, Ertan Taşkavak Discovery of a New Aggregation Site of Sandbar Sharks (*Carcharhinus plumbeus*) in Alayurt Cove, Eastern Mediterranean
11:30-11:45	Khadija ELBOUHALI, **Fayçal AIT BOUMALLASSA,** Fatima M'HAMDI, Fatima Ezzahra AROUMY, Tarik HACHIMI, Mokhtar BENLASRI, Ronaldo SOUSA and Mohamed GHAMIZI Competitive Effects of the Invasive Nile Tilapia *Oreochromis niloticus* (Actinoptyrigii: Cichlidae) on Growth and Survival of the Endemic Moroccan Barbel *Carasobarbus fritschii* (Actinoptyrigii: Cyprinidae)
11:45-12:00	AROUMY. F., M'HAMDI. **F., AIT BOUMALLASSA.** F., HACHIMI. T., ELBOUHALI. K., S'KHIFA. A., HADDOU. N., and GHAMIZI. M., Groundwater Biodiversity in the Taza Region (Morocco): Hydrobiidae Distribution and Conservation Priorities
12:00-12:15	Şükran YAVUZ, **Murat YABANLI** Food Safety Evaluation of Edible Bivalves from the Marmara Sea: Potentially Toxic Elements, Dietary Exposure, and Human Health Risk Assessment
12:15-14:00	Lunch break
Conference Hall- TURKUAZ in person	
Chairman	
14:00-14:15	
14:15-14:30	
14:30-14:45	
14:45-15:00	
15:00-15:15	
15:15-15:30	
15:30-16:00	Coffee Break
Conference Hall- TURKUAZ in person	
16:00-16:15	**Conclusion Meeting**

Conference Hall-1 Online	
Chairmen	**Prof. Dr. Ali Serhan Tarkan**
11:00-11:15	**Spase Shumka** Threatened Fish of Albania, Conservation Management and Actions Needed
11:15-11:30	**Laura Shumka** Current ecological pressures, management opportunities and the connectivity context of aquatic and terrestrial landscape in Butrinti National Park and UNESCO site
11:30-11:45	**Maja Krakowiak,** Andrzej Zawal The impact of tectonic processes on the genetic diversity and population structure of Hydrachnidia in river systems of Iran
11:45-12:00	**Nertila Muçollari,** Spase Shumka and Adela Kullaj Environmental Assessment of the Vjosa River Based on Heavy Metal and Microbiological Contamination in *Barbus prespensis* and *Squalius platyceps*
12:00-12:15	**Mehrez Gammoudi** First Record of *Tubulanus nothus* (Bürger, 1892) (Nemertea: Palaeonemertea) from Tunisian Coastal Waters
12:15-14:00	Lunch break
Conference Hall-1 Online	
Chairman	**Prof. Dr. Fayçal AIT BOUMALLASSA**
14:00-14:15	**Fareeha Iftekhar,** Andrzej Zawal Geographical and Environmental Distribution of Water Mites (Acari: Hydrachnidia) in Pakistan: An Integrative Morphological and DNA Barcoding Study
14:15-14:30	**Maryam Asif,** Andrzej Zawal Tectonic History and Water Mite Diversity in Pakistan: A Biogeographical Hypothesis
14:30-14:45	**Nuri Başusta,** Asiye Başusta A new record of a juvenile shortfin mako shark (*Isurus oxyrinchus*) off the coast of Side, Gulf of Antalya (Türkiye)
14:45-15:00	**Nuri Başusta,** Asiye Başusta Records of newborn Marbled electric rays (*Torpedo marmorata*) off the coast of Alanya, Gulf of Antalya (Türkiye)
15:00-15:15	**Abdelilah Ahnich**, Fayçal Aitboumalassa, Hanane Khalki and Mohamed Naimi Seasonal Feeding Ecology of Endemic Barbels in a North African River: The Zat Basin Case
15:15-15:30	**Banu KUTLU** Bibliometric Analysis of Microplastic Research in Freshwater Ecosystems: Global Trends, Scientific Collaborations, and Emerging Research Themes
15:30-16:00	Coffee Break
Conference Hall-1 Online	
16:00-16:15	**Conclusion Meeting**

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

y 1 - Tuesday, 1 September 2026 / Poster Presentati	
Conference Hall- TURKUAZ in person	
PPT-1	**Violeta Berlajolli**, N. Orqusha, A. Dreshaj, E. Behrami & M. Płóciennik
	Prodiamesa olivacea as an indicator of heavy metal contamination in upland streams (Drini i Bardhë River, Kosovo)
PPT-2	**Saeed Hajirezaee**, Mohammad Hossein Khanjani
	Pollutants and environmental stresses in aquaculture: Disruptions and the moderating role of medicinal plants from a metabolomics
PPT-3	**Saeed Hajirezaee**, Mohammad Hossein Khanjani
	Oocyte Ageing in fish from a Metabolomics Perspective
PPT-4	**Aziza Elglid,** Samira Enajjar, Bechir Saidi, Mohamed Nejmeddine Bradai and Noureddine Chatti
	Genetic erosion and demographic signatures in Mediterranean white sharks (*Carcharodon carcharias*): insights from Tunisian waters
PPT-5	**Md. Istiaque Hossain,** M.A. Samad, S. Afrin and S. Istiaque
	Assessment of Pond Productivity through Carp with *Macrobrachium rosenbergii* Culture at Rajshahi, Bangladesh
PPT-6	Laura Shumka, **Fatma Kunt,** Çiğdem Çiftçi, Musa Argun
	Application of the Integrated Beach Quality Index (BQI) for selected semi-urban, semi-rural, and urban types in Albania

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PRESENTATIONS

MarFresh 2026- International Marine & Freshwater Sciences Symposium
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Latest developments in research on the invasive blue crab (*Callinectes sapidus*) in the Mediterranean Sea

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Abstract

The Atlantic blue crab (*Callinectes sapidus*) is widely considered one of the most prominent invasive species across all taxa in the Mediterranean Sea. This prominence stems from its ecological plasticity, high fecundity, and opportunistic omnivorous nature, which allows it to prey on all available benthic resources while experiencing minimal natural predation. Studies on the trophic ecology of the blue crab across the Mediterranean, including stable isotope analyses, highlight a systemic crisis: The species occupies a high trophic level and its isotopic niche overlaps with that of native predators. This overlap leads to intense resource competition and supports the invasive success of *C. sapidus* in the Mediterranean Sea. Thus, the blue crab acts as an apex predator in Mediterranean lagoons and estuaries; it has a competitive dominance that poses a significant threat to biodiversity (e.g., depletion of native fauna, habitat alteration), as well as having negative impacts on socioeconomics (e.g., fisheries and aquaculture production decline). These findings underscore the urgent need for targeted management interventions to mitigate blue crab impacts within the basin, especially in areas with significant ecological reserves, commercial fisheries and aquaculture. The most effective strategy would be to intensify harvesting efforts during the peak abundance of ovigerous females, targeting the preferred areas and depths where spawning occurs, using the most suitable fishing gear (double-chamber trap). The above aims to reduce reproductive output and recruitment success; thus, minimizing the population size of the blue crab. However, the implementation of this management policy requires a comprehensive knowledge of the complex life cycle of the species in every invaded habitat. Another management strategy is the economic valorization of the blue crab. The species is a commercially important resource; its populations in many Mediterranean countries are currently subject to intense fishery exploitation, turning an ecological challenge into an opportunity. Furthermore, blue crab by-products can be utilized across various sectors, creating new industrial opportunities. The invasive blue crab *C. sapidus* is emerging as a new keystone species in Mediterranean coastal areas. While its proliferation leads to severe ecological and socioeconomic impacts, it simultaneously offers new opportunities for commercial fishery exploitation and resource valorization.

Keywords: blue crab, *Callinectes sapidus,* invasion, Mediterranean Sea, apex predator, impacts, biodiversity, socioeconomics, *targeted management interventions,* economic valorization

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Towards Predictive Invasion Ecology: From Species Distribution to Ecological Impacts

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Abstract

Biological invasions are increasingly interacting with environmental change, yet predicting their ecological impacts remains challenging because impact depends not only on where an invader occurs, but also on trophic interactions, environmental conditions and the performance of ecological interactions. This presentation outlines an integrative framework for moving from species-distribution predictions towards spatially and temporally explicit projections of potential ecological impact. The framework combines four complementary dimensions of predator–prey interactions: trophic reliance derived from stable-isotope data, isotopic niche width, predator–prey spatial overlap from ecological niche models, and temperature-dependent feeding performance derived from functional-response experiments and thermal-performance-curve modelling. Eurasian perch (*Perca fluviatilis*) was used as a global test case, incorporating information from 101 native-range and 24 non-native-range sites. The integrated approach was used to estimate ecological impact under current conditions and project potential changes under future climate scenarios for 2050 and 2100. The results demonstrate pronounced context dependence in trophic reliance and niche width, while feeding performance showed a clear thermal optimum, with predation performance changing substantially across temperatures. By integrating these complementary processes, the framework indicates that climate change may not simply increase or decrease invasion impacts, but can redistribute their intensity across space and time. This approach provides a basis for developing more mechanistic and predictive assessments of invasion impacts under environmental change.

Keywords: environmental change, Eurasian perch, invasion impacts, feeding performance

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First Record of *Tubulanus nothus* (Bürger, 1892) (Nemertea: Palaeonemertea) from Tunisian Coastal Waters

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Abstract

Nemerteans, commonly known as ribbon worms, are widespread marine invertebrates characterized by a unique, eversible proboscis used for prey capture. Although primarily benthic organisms inhabiting soft sediments, rocky substrates, macroalgae, and biogenic structures, over 1,300 species have been described globally, with a few adapted to freshwater and terrestrial environments. Despite their ubiquity in marine ecosystems, nemertean diversity remains severely understudied along the Tunisian coastline. This study reports the first record of *Tubulanus nothus* (Bürger, 1892) from Tunisian waters. A single specimen was collected inside its characteristic transparent mucous tube from a sheltered microhabitat between rocky blocks at Errimel Beach (Bizerta, northern Tunisia). Identification was definitively confirmed using an integrative taxonomic approach combining external morphological characteristics, internal histological features, and molecular barcoding.

Keywords: Nemerteans, Biodiversity, Benthos, Mediterranean

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Prodiamesa olivacea as an indicator of heavy metal contamination in upland streams (Drini i Bardhë River, Kosovo)

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Citation: Berlajolli, V., Orqusha, N., Dreshaj, A., Behrami E., Płóciennik, M. (2026). *Prodiamesa olivacea* as an indicator of heavy metal contamination in upland streams (Drini i Bardhë River, Kosovo). MarFresh Symposium., 3: 17.

Abstract

Originating from the Shpella e Bukuroshes së Fjetur cave, the Drini i Bardhë stream in Western Kosovo serves as the primary municipal water source for 25,000 residents in Pejë. While the water quality remains high at the city's intake point, the river's upper reaches and various tributaries are generally impacted by pollution. Benthic invertebrates, specifically Chironomidae, function as effective bioindicators for detecting both natural and anthropogenic environmental stressors. In particular, mouthpart abnormalities in midge larvae serve as a reliable reflection of a freshwater ecosystem's health. During May 2024, researchers analyzed morphological anomalies in 554 *Prodiamesa olivacea* larvae gathered from the Drini i Bardhë. The high frequency of these physical defects may be attributed to elevated levels of Manganese (Mn) and Zinc (Zn), coupled with low dissolved oxygen. Although these findings are preliminary, they highlight critical concerns regarding the water quality of a stream used extensively for local livestock and agriculture. To fully understand the health risks posed to nearby settlements, a comprehensive investigation utilizing a wider array of chemical and biological indicators is essential.

Keywords: mime midges, heavy metals, water quality

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The impact of tectonic processes on the genetic diversity and population structure of Hydrachnidia in river systems of Iran

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Abstract

The relationship between geological processes and biodiversity patterns in aquatic systems is a key research area in evolutionary ecology and population genetics. This study aims to assess the potential influence of Iran's tectonic history on the diversity of water mites in river systems, with particular emphasis on habitat fragmentation and population isolation. Hydrachnidia are strongly associated with lotic environments and exhibit limited active dispersal, making them a useful model for studying population structure and ecological continuity in freshwater systems. Their life cycles and dependence on specific hydrological conditions suggest that changes in river network configuration may influence gene flow and evolutionary differentiation. Planned molecular analyses based on COI gene sequences are intended to evaluate genetic differentiation among populations from different river systems in Iran. It is assumed that observed patterns of variation may be linked to major phases of geological restructuring, including the uplift of the Zagros and Alborz mountain systems. These processes may have contributed to hydrographic reorganization, habitat fragmentation, and the formation of biogeographic barriers, although these relationships require further verification. It is also proposed that river valleys and local aquatic systems may represent areas of limited connectivity, enabling some degree of gene flow between populations. Consequently, the contemporary structure of Hydrachnidia in Iran may reflect the combined effects of geographic isolation and restricted hydrological connectivity, though these interpretations remain preliminary. This research highlights the importance of integrating genetic, hydrological, and geological data in studies of freshwater biodiversity. The findings may improve understanding of how historical environmental changes shape population structure and may inform conservation strategies for river ecosystems under ongoing environmental change.

Keywords: Hydrachnidia, population genetics, tectonics, river systems, aquatic biodiversity

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Threatened Fish of Albania, Conservation Management and Actions Needed

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Abstract

Fishes, both freshwater and marine are among the most threatened vertebrates on Mediterranean, facing accelerating pressures from habitat alteration, hydropower development, overfishing, climate change, invasive species, pollution, and declining water quality. Meanwhile, fisheries and fish biodiversity are essential for food security, livelihoods, culture, and ecosystem function at the wider and local scale within Albania. This contribution aims to bring together interdisciplinary approach that advances understanding of the current state through species status assessments and informs action regarding fish conservation at various aquatic ecosystems. It focuses on: (i) revised baseline extinction risk for each species by integrating recent data, newly-identified threats, and emerging trends since the previous assessment; (ii) identification of priority geographic areas, habitats, and species requiring urgent protection to prevent extinctions and support the achievement of favourable conservation status for Albanian fishes; (iii) assess the principal ongoing threats and review both current and prospective conservation measures in order to address them effectively and (iv) mobilise the compiled data as a foundation for regional conservation planning for fishes and water ecosystems, within the context regional/transboundary biodiversity strategies - thereby contributing to bending the curve of biodiversity loss.

Keywords: red list, ecosystem approach, conservation policy, alien species, aquatic biodiversity

Introduction

The Mediterranean Sea is considered to be a biodiversity “hotspot” characterized by an unusually high species diversity for a temperate sea. It contains around 7% of the total global marine fish species, with a wide range of both temperate and tropical species being present. Of the 519 native marine fish species and subspecies occurring in the Mediterranean Sea, 73 are cartilaginous fish (sharks, rays and chimaeras) and 446 are bony fish. At present, 12% of bony fish and 40% of cartilaginous native fish are regionally threatened with extinction. The Mediterranean region hosts some of the most threatened cartilaginous fish populations in the world (Abdul Malak, et al. 2011). The Mediterranean Sea is considered to be a biodiversity ‘hotspot’ as it has unusually high species diversity for a temperate sea (FAO 2003a and b). It contains around 7% of the total global marine fish species (Bianchi and Morri 2000; Weiss et al., 2018), with a wide range of both temperate and tropical species being present. This high diversity can be explained by geologic and paleogeographic factors, as well as environmental factors including climate, temperature and salinity gradients, and hydrology.

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Within the "Document of Strategic Policies for the Protection of Biodiversity in Albania" (MoE, 2015) there is noted the fact that following references (Poljakov et al., 1958; Rakaj, 1995), there are records of presence of 249 marine and 64 freshwater fishes. So, both numbers are far below from very recent data, particularly with regards to freshwater species, so far listing 103 species (Shumka et al., 2023; Shumka et al., 2024). The Albanian shoreline extends across two seas, the Adriatic Sea in the north and the Ionian Sea in the south. The Albanian Adriatic coast is generally low and flat, formed as a result of continuous and uninterrupted accumulation of sediments from rivers. Only in some hilly areas, which have also created capes on the coast, erosion continues.

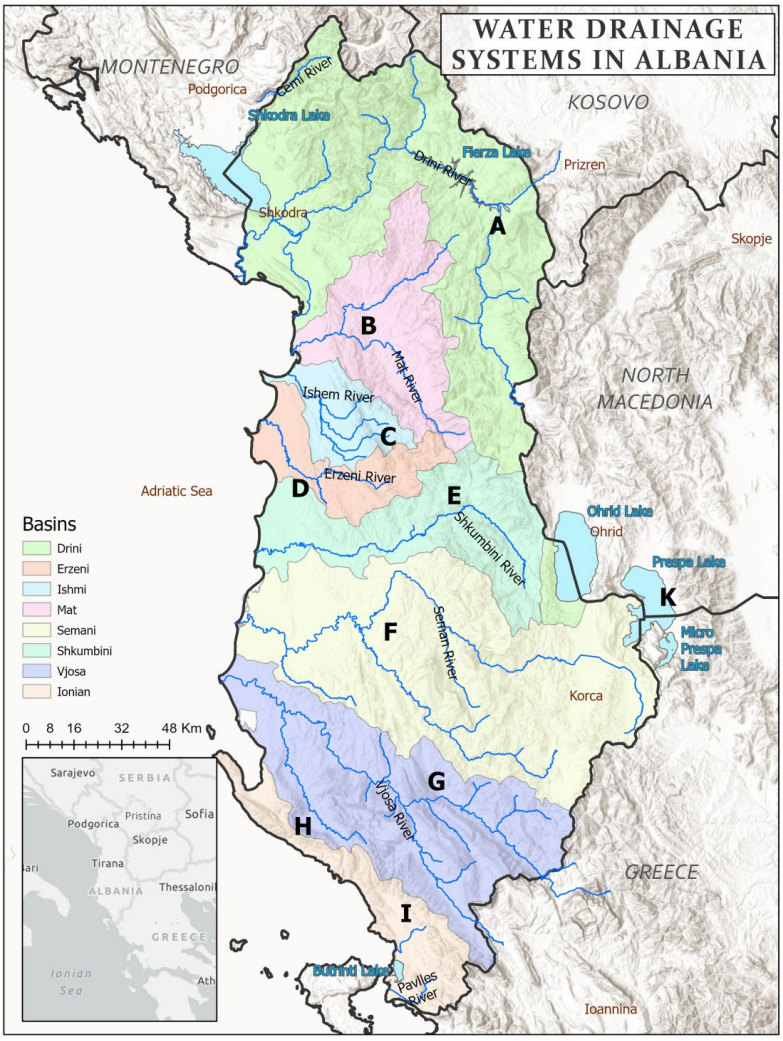


Figure 1. Water drainage systems in Albania (Shumka, 2023)

Albanian territory covers about 65% of a total watershed area of 43,905 km^2 of Drini River with an average height of the hydrographic territory of about 700 m above the sea level (Figure 1). There are more than 152 rivers and streams in Albania, of which the largest area: Buna (41 km), Drini (285 km), Mati (115 km), Ishmi (74 km), Erzeni (109 km), Shkumbini (181 km), Semani (281 km), and Vjosa (272 km), which run southeast to northwest towards the Adriatic coast (Kabo, 1990). Albanian rivers are characterized by a high flow rate: the total annual mean flow is 1,308 m^3s^{-1}, which corresponds to an annual water volume of $42.25x10^9m^3$ of which 30% belongs to subterranean waters. This accounts for more than 13,000 m^3 per capita per year

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(Cullaja et al., 2005). The rivers, fed mainly by precipitation (69%), show a typical Mediterranean regime, with seasonal variation in the flow rate, and high flow from October to May (Cullaja et al., 2005; Kabo, 1990). There are several main contemporarily independent river and lake systems in Albania (Fig. 1). Listed from north to south they are as follows: Ohrid-Drin-Skadar system (including River Buna) (A), rivers Mat (B), Ishëm (C), Erzen (D), Shkumbin (E), Seman (composed of two big inflows—Devoll and Osum) (F) and Vjosë (Aoos in Greece) (G), several short rivers flowing from the Cikes mountains to the southernmost part of the Adriatic Sea and the northernmost part of the Ionian Sea (H), area around Butrint Lagoon (rivers Bistrica and Pavllo) (I) and Lake Prespa (K). Most of the above-mentioned lakes and rivers (A–G) belong to the Adriatic catchment and the southernmost area (I) to the Ionian Sea slope. Area H lies on the border of the Adriatic and Ionian Seas (Figure 3). Whereas there is no surface outflow of water from Prespa Lakes, there are underground connections with Lake Ohrid (Ahmataj et al., 2007). Only a very small area in the northernmost part of Albania, in the Albanian Alps belongs to the Danube basin (J) (Shumka et al., 2017). Discharge of all Albanian rivers is seasonally highly variable, being sometimes more than ten times less in summer than in winter. The beds of the main rivers are usually very wide, as a great amount of gravel and pebbles is deposited around the flow itself (Shumka et al., 2017).

Material and Methods

The conservation status of plants, animals, and fungi serves as a key indicator for evaluating the health of ecosystems and their biodiversity. The Red List is globally regarded as the most authoritative source of information on species' extinction risk. Although Red List assessments are highly valuable for guiding conservation planning and setting priorities, they are not designed to dictate specific policies or function as a standalone framework for biodiversity protection. Furthermore, these assessments must be conducted objectively, free from commercial, emotional, or political influence.

The geographical scope of the Albania Red List spans the entire country both marine and continental areas. The assessment includes the Adriatic and Ionian Sea, river basins, rivers and streams, large and small lakes of different origins.

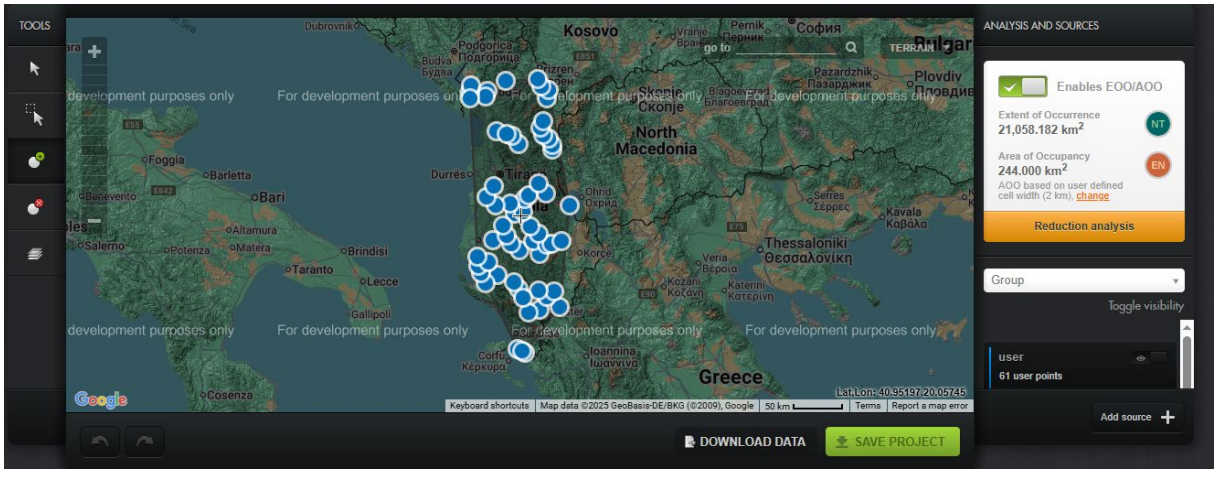


Figure 2. Use of GeoCat (IUCN) for assessing EOO/AOO (Example *P. pictum*)

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The European Red List of Freshwater Fishes assessment follows the IUCN Red List Categories and Criteria (version 3.1, second edition; IUCN 2012a), along with the Guidelines for Using the IUCN Red List Categories and Criteria (version 15.1 ; IUCN, 2022a) and the Guidelines for Application of IUCN Red List Criteria at Regional and National Levels (version 4.0 ; IUCN, 2012b). Proper interpretation of the terms and the application of criteria were ensured through training workshops (Figure 2).

The following data were compiled for all species assessments, depending on availability:

- Taxonomic classification, including notes and common names in countries of occurrence (where available).
- Distributional range and list of countries of occurrence, including Area of Occupancy, Extent of Occurrence and number of locations.
- Population information, including overall population sizes and trends.
- Habitat preferences and primary ecological requirements, including relevant biological details (e.g., generation length, extended larval stage, migratory life cycle, etc.).
- Major threats, including their scope and timing.
- Species use and trade.
- Conservation measures (both current and recommended), including identified research gaps.
- IUCN Red List Category and Criteria at three geographic levels (Global, Europe and, where applicable, EU-27), including rationale.
- Key bibliographic references.

The assessment is based on data reported by EU Member States under Article 17 of the EU HD (for methodology, see Röschel et al., 2020). Additionally, scientific and fisheries reports, national atlases and handbooks, Red Data Books, Red Lists, and peer-reviewed literature were thoroughly reviewed to ensure that the most recent data were considered. The IUCN Species Information Service (SIS) online database was used to store relevant information for each species. Draft assessments were revised following consultations with experts from across the European region. Throughout the project, the assessors and regional experts worked closely - with support from IUCN staff - to discuss species selection, taxonomic issues, distribution maps, and other technical matters. This collaborative approach led to the development of assessments and distribution maps that reflect the current state of knowledge for each species.

Results and Discussions

Diversity Endemism and Assessed fish species

Around 60% of evaluated freshwater fish species species belong to the order Cypriniformes, the largest group of freshwater fishes also in the world. Meanwhile, many species considered endemic to the region, and found nowhere else on Earth, have restricted ranges within specific lakes or rivers. The Valenciidae family (European toothcarps), with one species, is the only one entirely endemic to Balkans, but endemism is notably high in several other families, including Petromyzontidae (northern lampreys), Gobionidae (gudgeons), Cyprinidae (carps), Leuciscidae (true minnows), Cobitidae (true loaches), Nemacheilidae (stone loaches), Umbridae (mudminnows), Salmonidae (salmons, trouts, and whitefishes), and Cottidae (sculpins). In addition, four of the baklans's trout species, belonging to the Salmonidae family, are native to Lakes Ohrid and Prespa (see Table 1).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Table 1. Diversity and endemism in freshwater fish families in Albania and Balkans

Family	Number of species	Endemic Albania	Sub-endemic	Balkan Endemic
Anguillidae	1	0 (0%)	0 (0%)	0 (0%)
Blenniidae	1	0 (0%)	0 (0%)	0 (0%)
Cobitidae	3	0 (0%)	0 (0%)	3(100%)
Cottidae	1	0 (0%)	0 (0%)	0 (0%)
Cyprinidae	5	0 (0%)	1(20%)	4(80%)
Gasterosteidae	1	0 (0%)	0 (0%)	0 (0%)
Gobiidae	3	0 (0%)	3(100%)	0 (0%)
Gobionidae	2	0 (0%)	0 (0%)	2 (100%)
Leuciscidae	20	2(10%)	4(20%)	14(70%)
Nemacheilidae	3	0 (0%)	2(67%)	1 (33%)
Petromyzontidae	3	0 (0%)	2(67%)	1 (33%)
Salmonidae	6	0 (0%)	4(67%)	0 (0%)
Valenciidae	1	0 (0%)	1(100%)	0 (0%)

41 families with 58 marine fish species have been subject of evaluation where larger number of 3 belongs to families *Cetorhinidae* and *Rajidae,* and 4 to *Syngnathidae* (see Table 2).

Table 2. Table Number of marine fish species per family

Family	Number of species	Family	Number of species
Alopiidae	1	*Aphaniidae*	1
Carcharhinidae	1	*Echeneidae*	1
Cetorhinidae	3	*Labridae*	2
Chimaeridae	1	*Luvaridae*	1
Dalatiidae	1	*Molidae*	2
Gymnuridae	1	*Macrouridae*	1
Lamnidae	2	*Mugilidae*	1
Mobulidae	1	*Mullidae*	2
Myliobatidae	2	*Muraenidae*	1
Odontaspididae	1	*Pleuronectidae*	1
Oxynotidae	1	*Sciaenidae*	2
Pentanchidae	1	*Scophthalmidae*	1
Rajidae	3	*Sparidae*	2
Squalidae	1	*Stomateidae*	1
Triakidae	1	*Syngnathidae*	4
Torpedinidae	1	*Synodontidae*	1
Acipenseridae	1	*Trachipteridae*	2
Balistidae	1	*Uranscopidae*	1
Blenniidae	2	*Zeidae*	1
Carangidae	2	*Xiphiidae*	1
Clupeidae	1		

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

At the Albanian level, 56.86 % (39 out of 51 assessed species) of freshwater fish species and 46.55 % (27 out of 58 assessed species) of Marine species are threatened. Out of this referring to freshwater species: 1.98 % (1 species) are EX, 9.9% are CR, 23.5 EN and 21.58 are VU species, while among marine species: 15.51 % (i.e., 9 species) are CR, 18.96 (11 species) are EN and 12.06 (7 species) are evaluated as VU.

23.52% or 12 species among freshwater ones and 10.34% or 6 species among Marine ones were assessed as NT.

By comparison, within the EU-27 region, 41.4% (172 species) of freshwater fish species are threatened, with 9.5% (41 species) CR, 18.8% (81 species) EN, and 11.1% (48 species) VU. A further 17.1% (74 species) are NT. As a result, more than half (56.9%) of freshwater fish species in the EU-27 region are of elevated conservation concern.

Among the EX-taxa, the Scadar nase – Njila e Shkodres (*Chondrostoma scodrense)* have all been confirmed as EX, having formerly been assessed as EX by the Mediterranean Freshwater Fish species.

More than three-quarters of Albania's freshwater assessed fishes (43 species) are endemic to the region. Of these, nearly three-quarters (37 species) are assessed as Threatened or Near Threatened and are of elevated conservation concern.

In the following Figure 3 and 4 is given proportion of Albanian marine and freshwater fish species in each Red List category.

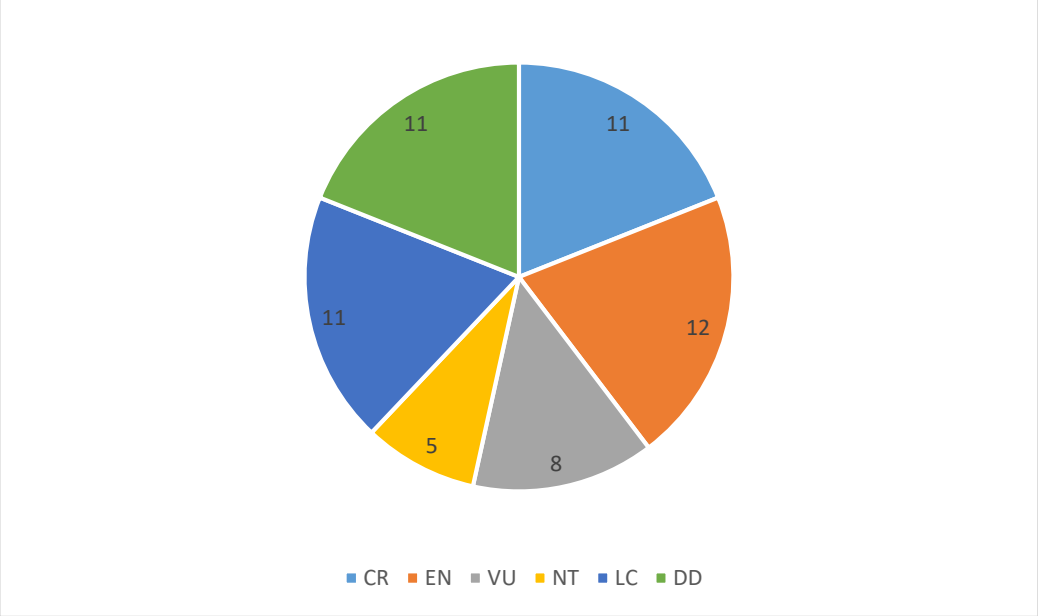


Figure 3. Proportion of Albanian marine fish species in each Red List category

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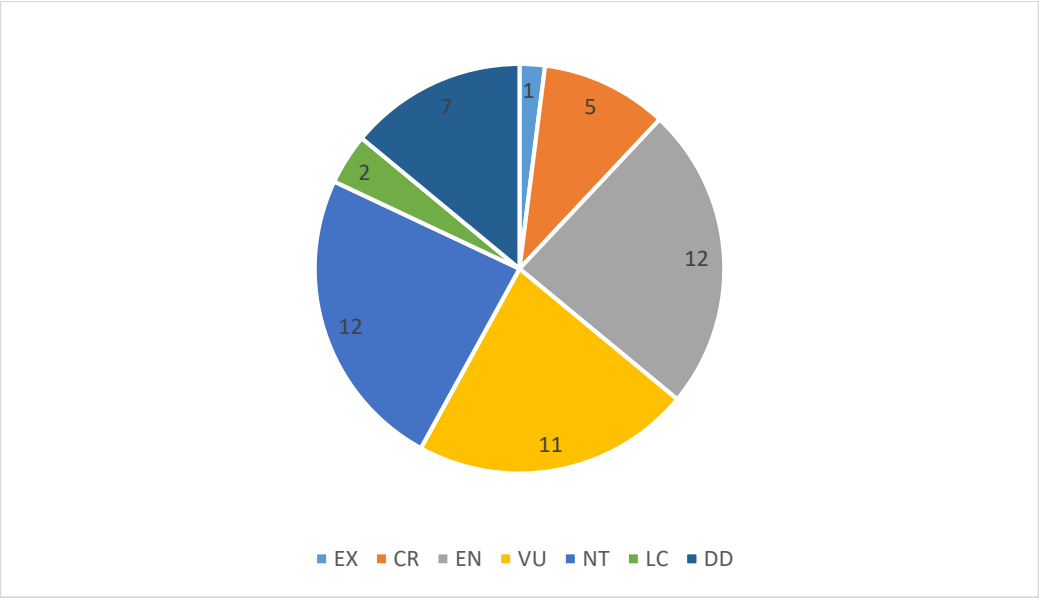


Figure 4. Proportion of Albanian freshwater fish species in each Red List category

Conclusions

Marine & Freshwater fishes represents the most diverse groups of vertebrates Albania, inhabiting ecosystems that are not only essential to their survival but also critical to human development and well-being. As key ecological indicators, they help signal the health of marine and freshwater systems and underpin a broad range of ecosystem services - including food provision, recreation, and cultural heritage.

Despite their significance, over 40% of Albania's freshwater fishes are currently threatened with extinction, and nearly 60% are of elevated conservation concern, being assessed as either threatened or near threatened.

Strengthening the scientific foundations of conservation – through improved monitoring, assessment, and feedback mechanisms - will be essential to support evidence-based management and achieve international biodiversity targets.

Translated into practical conservation and management actions. These must be embedded across policy frameworks at local, national, and regional levels to effectively safeguard Europe's freshwater heritage and reverse the ongoing decline of inland water biodiversity - for nature, for people, and for the planet.

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Current Ecological Pressures, Management Opportunities and the Connectivity Context of Aquatic and Terrestrial Landscape in Butrinti National Park and Unesco Site

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Abstract

Integration of cultural and natural values of a certain coastal site hosting different designations remains a real challenge. Human impact on natural ecosystems is the most important driver of the current state of habitats at the Balkans, extinction of species across larger areas and one of the greatest concerns for biodiversity conservation. The costal Butrinti area lies with core of Balkan Peninsula's exceptional biodiversity of plant and animal species that is a rare ecological and bio-geographical occurrence in Europe. The diversity richness of plants, animals, and ecosystems in this area is incredibly high due to the region's complexity, intricate geological past, and interactions between populations, species, and ecosystems. The great diversity of rare and relict species is highly notable in and of itself, but it also adds to the region's distinctiveness due to its disparate ecological traits, distribution patterns, and places of origin. Further on as a top cultural heritage site within Albania it makes a challenge with regard to integration. With this regard maintenance and restoration of connectivity among ecosystem elements that is the objective of this contribution and processes it is regarded as the most obvious answer to counteract the negative impacts of fragmentation and of the habitats degradation along all water drainage systems of Albania.

Keywords: Climate change, heritage, land use, fragmentation, integration

Introduction

During the past two and a half decades, the understanding of heritage conservation has shifted from “management of change,” as articulated in the Charter of Krakow, to the “management of continuity” (Ripp and Rodwell, 2015; Khafal, 2020) or “management of continuity and change” (Wijesuriya, 2015; 2017), and, more recently, to “management of continuity and compatible change” in scholarly literature (Khafal, 2020). The fundamental aim is to preserve the relevance of heritage sites while allowing for and aesthetically integrating modern modifications that do not negatively affect the significant qualities and functionality of the surroundings.
The importance of PA connectivity is also recognized in global biodiversity targets adopted by the world's governments (Saura et al., 2017). In 2010, the parties to the United Nations Convention on Biological Diversity (CBD) adopted a Strategic Plan for Biodiversity for the

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2011–2020 periods, including the twenty Aichi Biodiversity Targets (CBD, 2010; Shumka et al., 2023). Apart from increasing the PA network to at least 17% it refers to 'effectively and equitably managed, ecologically representative and well-connected systems of protected areas' (CBD, 2010). However, the CBD has neither provided a clear definition of the term 'well-connected', nor guidance on how to measure PA connectivity (Butchart et al., 2016), which has made it difficult to stimulate and track progress towards the Aichi Target 11 connectivity element. In 2017, Saura et al. (2017) used ProtConn to examine the connectivity of PA systems for all terrestrial ecoregions, but a country-level evaluation can provide a more policy-relevant assessment since most political decisions on development and management of protected areas networks are taken at the national level.

The science of connectivity conservation management: The term 'connectivity' is widely used in the literature on landscape change and conservation practice and generally refers to the ease with which organisms move between particular landscape elements, the number of connections between patches of habitat relative to the maximum number of potential connections or the interlinkages of key processes within and between ecosystems (Lindenmayer and Fischer 2007; Pulsford et al., 2015). Landscape connectivity and other connectivity concepts: (i) habitat connectivity; (ii) landscape connectivity; (iii) ecological process connectivity; (iv) evolutionary process connectivity.

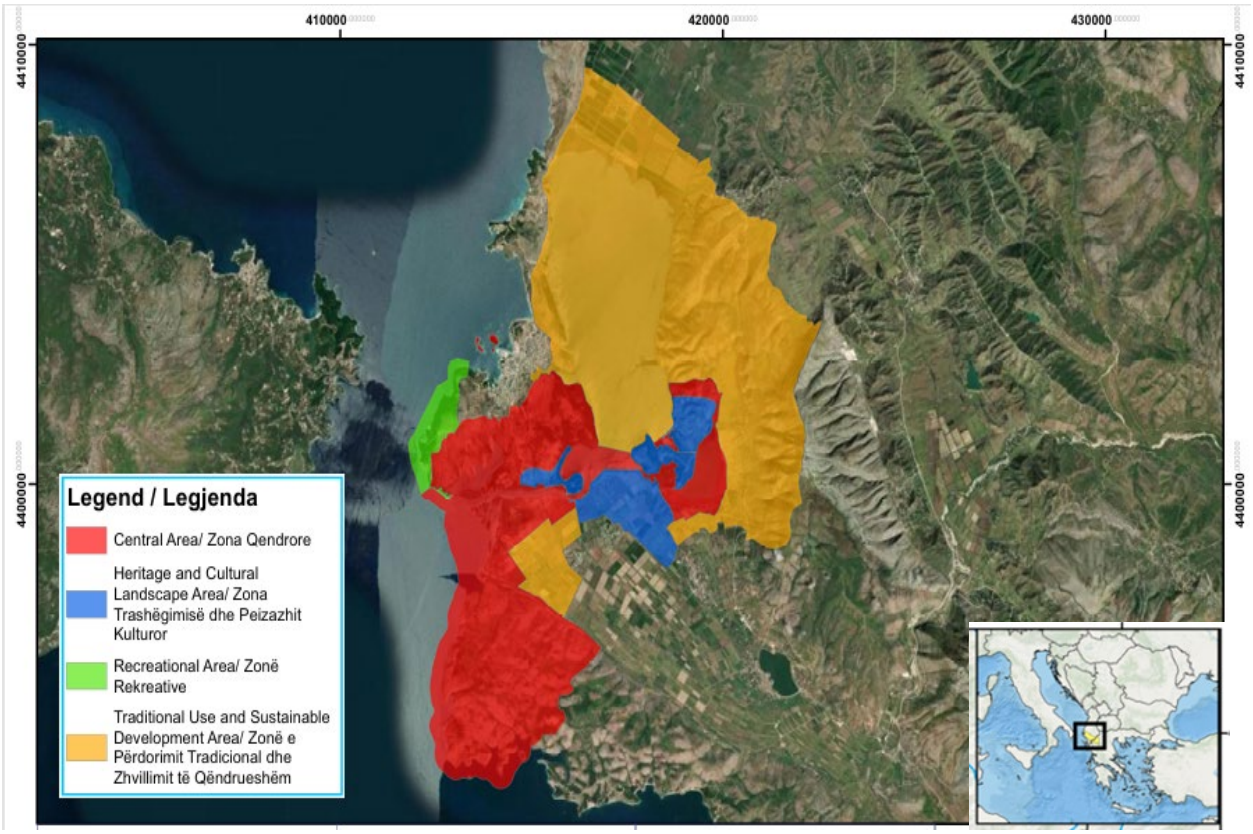


Figure 1. Map of the study area showing different conservation components

This is the challenge with Butrinti heritage site, considering it an integral part of the protected area. A heritage place's attributes are its qualities that communicate its values, and without "connectivity" with other values (in our contexts terrestrial and aquatic natural components) it becomes like a desert. By examining the relationship between continuity, compatibility (or change that is compatible), and integrity in the application of the World Heritage Convention, this report expands integrity of heritage itself within contexts of current and future climate

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driven changes. We are currently facing circumstances where, on a global scale, the 21st century is a time of crisis in the human relationship with the rest of nature (Pulsford et al. 2015), with the climate changing in dangerous ways, and up to one million species currently at risk of extinction (IPBES, 2019). The recent reflection of climate changes in Albania (similarly to other Mediterranean countries) is resulting in significant impacts on the hydrological cycle due to e.g., reduced rainfall and soil moisture, causing and amplifying droughts for weeks to years. Significant climate-driven changes are expected during the coming decades (Cowie et al. 2022; Markovic et al. 2017; WWF, 2022), placing Mediterranean freshwater ecosystems at the top of vulnerability (Shumka et al. 2020; Shumka et al., 3023, European Commission, 2013). Despite the attempts and objectives of international strategies to halt the biodiversity decline (UN Environment Programme, 2022; da Silva et al. 2024), the task is increasingly challenging (da Silva et al. 2024), thus a scientific and technical management support is more than required.

Material and Methods

Desk studies

A list of potential sources of ecological information has been utilized. Different Ecological guidelines provide guidance on the scope of desk study investigations at each stage of the undertaken survey. Various sources were comprehensively reviewed following the research status of BNP, PAs BioNNA, Google scholar and WoS (Web of Science). The literature databases were used as data sources, and the search was conducted with specific subject terms and titles.

Multi-disciplinary walkover surveys

The multi-disciplinary walkover survey conducted during spring time 2024 incorporated habitat mapping and evaluation of standing, transitional, and running flows, and an investigation of riparian habitats at watercourse crossings and downstream. This was combined with a walkover survey to detect the presence or likely presence of a range of protected species. A dedicated survey was undertaken in Butrint Lagoon with the particular aim of identifying both native and non-native species. Different key threat categories were mapped within and around the boundaries of BNP and expressed as a chart of IUCN threat classification scheme, key threats, and current impact and expected future (mid-term) importance in the BNP. Four scores ranging from 0 (no known impact) through 1 (slight impact), 2 (moderate impact) to 3 (severe impact) were used to assess the current impact according to the scoring scheme provided by GIWA (2001). Average scores were calculated for each threat class and maximum values are also given. The level of knowledge regarding these threats is subjectively estimated.

Group-specific and species-specific surveys

Each group-specific approach provides details on the general characteristics of the group, the potential impacts of climate changes and management upon them, and group specific survey techniques and measures. Additionally, detailed information on the protected species is provided, including a brief description of each species, summary information concerning its life-cycle, behavior, and habitat preferences, species-specific survey techniques; optimum survey periods, etc.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Results and Discussions

In order to see how the connectivity function the following analyses reveals bottlenecks and pressure issues. This overview employs a conceptual framework that allows for the assessment of driving factors (D), pressures (P), states (S), impacts (I), and policy responses (R) to determine how environmental issues impacting BNP components may be addressed. The DPSIR technique is based on an idea from the Organization for Economic Cooperation and Development (OECD). Along with EU programs like DITTY (Development of an information technology tool for the management of Southern European lagoons under the influence of river-basin runoff) and EEA (European Environment Agency) and UNEP (United Nations Environment Program), it has been extensively implemented and further improved over the last years.

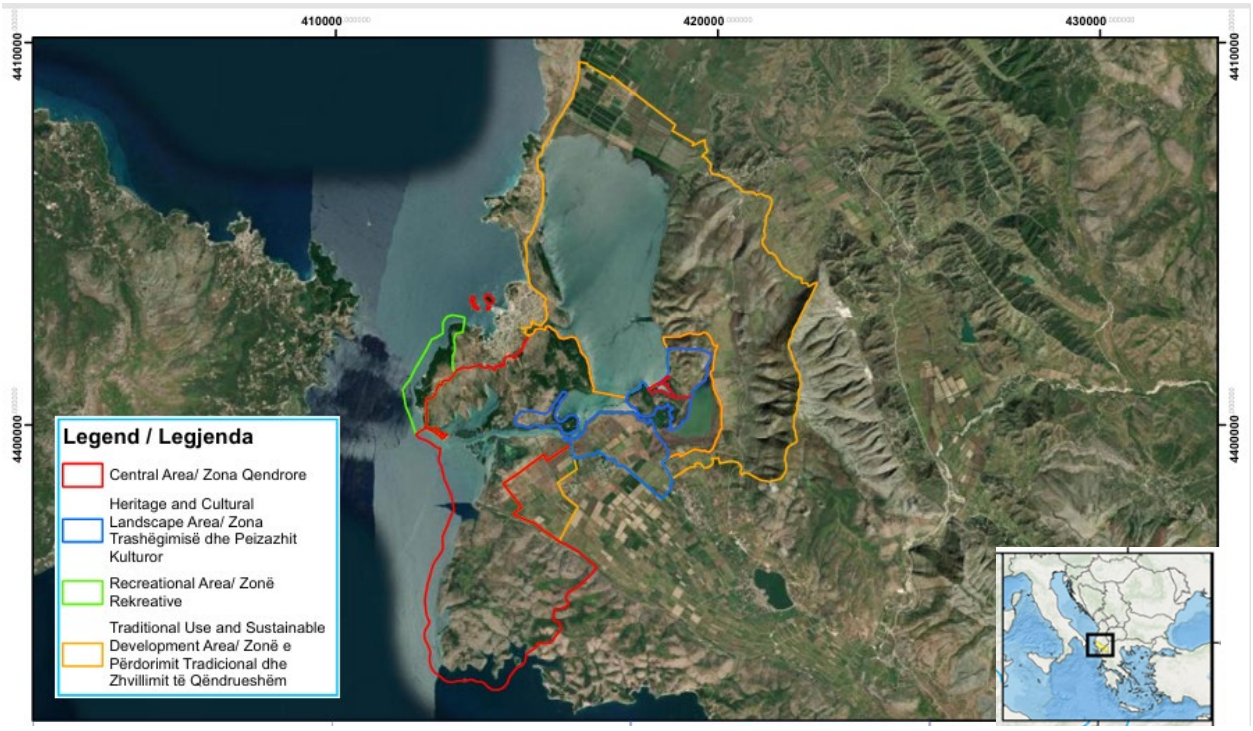


Figure 2. Contours of the protected areas showing central and buffer areas

Numerous other pressures, circumstances, and scenarios relevant to the implementation of EU Directives can be evaluated using the DPSIR framework (Zaldivar et al., 2008). The nomenclature used in the DPSIR has gradually changed in recent years. These days, a state's impact is defined as "Impact on human system" and "impact on human welfare" (Elliott et al., 2006). By treating the coastal ecosystems (mostly semi-enclosed coastal systems) as a Socio-Ecological System, the framework employs an integrated "system approach" (Newton et al., 2013).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Table 1. Major economic activities and drivers in BNP sub-zones (Agri-Agriculture, Aquacul-Aquaculture, Fish-Fishing, Hun-hunting,Tou-Tourism, RiMi-River mining, Landrec-Land reclamation, WastW-Wastewater discharge, IAS-Invasive alien species)

BNP sub-zones	Agri	Aquacul	Fish	Hun*	Tou	RiMi	Landrec	WastW	IAS
Butrinti Lagoon	X	X	X	X	X			X	X
Bufi Lake	X	X	X	X					X
River Pavllo	X		X	X	X	X		X	X
Bistrica and other streams	X		X	X	X			X	X
Vivari Channel					X				X
A3 area					X				X
Coastal semi-natural	X								X
Vrina	X			X			X		X
Vrugu	X			X			X		X
Coastal zone		X	X	X	X				X
Up-land areas				X					X

*There is undergoing a Hunting Ban Moratorium, but illegal hunting is present

Since BNP is dominated by aquatic environments, one example of a direct causal relationship between the DPSIR's constituent parts is eutrophication. Urbanization (home sewage and detergent discharges), agriculture (fertilizers and manure), and industry (organic matter from food processing-in case of BNP milk and mussel processing) are some of the drivers. The pressures that follow—inputs of organic matter, nutrient load, and enrichment—lead to unfavorable changes in the states of the biological and physico-chemical quality elements, such as the composition, abundance, and biomass of phytoplankton and other biota like macro-invertebrates, as well as changes in transparency. Eutrophication is the process that occurs when human-induced nutrient enrichment causes water bodies to fail to reach Good Ecological Status (required by EU WFD). The system's failing state has an effect on human welfare and activity. For example, it closes shellfish harvesting, fisheries catch declines, and tourism money is lost. It also affects tourists since the system produces more toxic algae (considered in following subsections of this survey). Although Albania is not an EU member state, it is on an ongoing integration path and must react by implementing management plans. These plans might include modernizing sewage treatment facilities, increasing oxygenation through the use of other facilities, or replanting riparian areas that border the water (currently almost ineffective due to the discharge of untreated water within Butrint Lake).

Following the analyses performed within this study and current and future visitor projections, it is important to consider the visitors health in line to following components:

(i) Health impacts of extreme events (for example floods and droughts), considering concerns over water level.

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(ii) Diseases and deaths of specific diseases related to extreme temperatures (using data on death certificates, hospital admissions, emergency visits related to cardiovascular problems, and other chronic conditions). This might be connected to the specificity of the lagoon and mussel farms (see section analyses of BNP ecological parameters)

(iii) Heat stress which can make living and working conditions unbearable and increase the risk of cardiovascular, respiratory and renal diseases. The risk is expected to be particularly high in the presence of air pollution and and the projected increased number of visitors particularly in the A3 zone.

(iv) Vector borne diseases and zoonoses**.** Climate change lengthens the transmission season and expands the geographical range of many diseases.

(v) Water related illness (gastrointestinal conditions, outbreaks, parasitic like leptospiroses). Many infectious diseases, including water-borne ones, are highly sensitive to climate condition.

(vi) Potable, surface water and recreational water pollution (bacterial monitoring).

(vii) Food safety and food security (data on food safety pathogens and toxins. Data on food security). Food security, particularly in relation to floods and drought.

(viii) Health conditions data about the most vulnerable populations and those disproportionally affected by extreme weather and climate events (older people, outdoor workers, people with disabilities and chronic conditions, living alone, women, children, those with low access to health services, etc.).

(ix) All the above need to be linked with site and healthcare vulnerabilities and data about adaptation efforts and policies changes.

Conclusions

Integration of cultural and natural values of a certain coastal site hosting different designations remains a real challenge. Human impact on natural ecosystems is the most important driver of the current state of habitats at the Balkans, extinction of species across larger areas and one of the greatest concerns for biodiversity conservation.

The costal Butrinti area lies with core of Balkan Peninsula's exceptional biodiversity of plant and animal species that is a rare ecological and bio-geographical occurrence in Europe. The diversity richness of plants, animals, and ecosystems in this area is incredibly high due to the region's complexity, intricate geological past, and interactions between populations, species, and ecosystems.

Further on as a top cultural heritage site within Albania it makes a challenge with regard to integration. With this regard maintenance and restoration of connectivity among ecosystem elements that is the objective of this contribution and processes it is regarded as the most obvious answer to counteract the negative impacts of fragmentation and of the habitats degradation along all water drainage systems of Albania.

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Pollutants and environmental stresses in aquaculture: Disruptions and the moderating role of medicinal plants from a metabolomics perspective

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Citation: Hajirezaee, S., Khanjani, M.H. (2026). Pollutants and environmental stresses in aquaculture: Disruptions and the moderating role of medicinal plants from a metabolomics perspective. MarFresh Symposium., 3: 35.

Abstract

Pollution caused by heavy metals, petroleum and hydrocarbon derivatives, agricultural pesticides are among the most important categories of pollutants that destructively change the metabolic profile in aquatic animals. Mainly, three groups of metabolites including energy-related metabolites, amino acids and osmolytes have shown significant changes with pollution. The metabolic responses mainly included the reduction of amino acids for the synthesis of detoxifying proteins and glucogenic backbones for gluconeogenesis due to thedepletion of liver glycogen under acute stress; reduction of some osmolytes such as betaine, taurine and hypotaurine, trimethylamine-N-oxide (TMAO) N,N-dimethylglycine as sources of methyl group donors to keep antioxidant reponses; depletion of liver glycogen to provide ATP from glucose; activation of anaerobic metabolism and accumulation of lactate; increases in the level of ketone bodies (acetoacetate, beta-hydroxybutyrate, acetate) under acute stress conditions; reductions in the level of energy shuttles such as phosphocreatine and ATP, and the accumulation of TCA metabolites as a result of mitochondrial disruptions are among the most common metabolic responses recorded in response to environmental stresses in fish and bivalves. Currently, very few studies have investigated the role of medicinal plants in modulating the toxicity of pollutants in aquatic animals from a metabolomics perspective. In rainbow trout, Oncorhynchus mykiss, the medical plant, Ginkgo biloba extract acts as a natural metabolic modulator by restoring osmolytes, improving the amino acid profile (by preventing stress-induced proteolysis), maintaining glucose homeostasis (preserving glycogen stores), reducing the accumulation of ketone bodies (by preventing excessive lipid oxidation), and protecting against cellular damage (by reducing phosphocreatine leakage). The use of metabolomic tools in aquatic toxicological studies and plant-based mitigating matterials can play important role in various fields of modern aquaculture. Areas such as use of herbal supplements according to metabolic needs including stress resistance as well as identification of robust metabolic biomarkers for early warning of contamination.

Keywords: Metabolomics,, Pollutants, Medicinal Plants, Aquaculture

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Oocyte Ageing in fish from a Metabolomics Perspective

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Abstract

Post-ovulation is a sensitive period due to the risk of egg ageing or overripening. Due to delayed spawning or delayed egg retrieval, fish oocytes experience ageing -related biochemical and morphological changes, which ultimately result in reduced fertility and increased embryo mortality. In some fish such as common carp, it may occur within 1-2 h post-ovulation. Many studies have investigated oocyte ageing histologically and biochemically, but the lack of "Omics" studies in this area is a fundamental knowledge gap. Metabolomics studies the metabolome content (metabolites, <500 Da) in biological samples. In this study, an attempt was made to investigate the fish oocyte ageing from a metabolomics perspective. Several groups of metabolites mainly showed fundamental changes during ageing, including: energy metabolites, osmolytes, amino acids and osmolytes. The changes mainly included: progressive decrease in ATP and phosphocreatine (Pcr) content, increase in malondialdehyde as a result of oxidative stress, accumulation of amino acids as a result of activated egg yolk degradation pathways, accumulation of TCA cycle intermediate metabolites (succinate, malate, fumarate) as a result of damage to mitochondrial membrane permeability and their leakage to the outside, activation of apoptosis pathways (decrease in inosine, guanosine, and AMP), and changes in the amounts of some osmolytes such as T-methylamine N-oxide, trimethylglycine, and betaine in response to oxidative stress. From an aquaculture perspective, studying the metabolome during the ageing process can help to a) identifying of metabolite-based biomarkers of egg quality such as MDA and ATP/PCr ratio, (b) Designing artificial storage media for oocytes based on metabolome content information, (c) Using metabolite information to manipulate broodstock diets to maintain post-ovulatory egg quality. For future studies, examining oocyte aging at other omics levels alongside metabolomics and data integration can provide a more comprehensive perspective and understanding of the phenomenon of oocyte aging in fish

Keywords: Oocyte ageing, metabolomics, oxidative stress, energy metabolism, egg quality

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Resource partitioning sustains a multi-endemic threatened barbels assemblage in a Mediterranean stream: evidence from the OLA KBA, Morocco

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Abstract

Morocco harbours one of the most diverse endemic freshwater ichthyofaunas in the Mediterranean basin, yet the functional ecology of its cyprinid assemblages remains largely unexplored. Here, we characterise trophic niche partitioning among five sympatric endemic barbels in the El Abid River, a threatened Mediterranean stream within the Ouled Abdallah Key Biodiversity Area of the Oum Er-Rbia basin, and identify the dietary mechanisms underpinning their coexistence. Fish were collected by backpack electrofishing at four stations across four seasons (2022–2023). A total of 1,234 individuals were examined: *Carasobarbus fritschii*, *Luciobarbus ksibi*, *Luciobarbus zayanensis*, *Carasobarbus harterti*, and *Pterocapoeta maroccana*. Diet was quantified using frequency of occurrence, numerical abundance, and the Absolute Importance Index. Trophic niche breadth was assessed via the Levins standardised index, pairwise dietary overlap via the Pianka and Morisita indices, and prey selectivity via the Ivlev electivity index. Feeding strategies were visualised through Costello diagrams. Multivariate dietary structure was analysed by non-metric multidimensional scaling, principal component analysis, and redundancy analysis, with inter-specific differentiation tested by permutational multivariate analysis of variance. The five species exhibited strongly differentiated trophic profiles spanning a continuous generalist-to-specialist gradient. Carasobarbus fritschii was the most generalist feeder, utilising 17 prey categories with high individual-level dietary diversity, while Carasobarbus harterti and Luciobarbus zayanensis were the most specialised, relying almost exclusively on Diptera–Gastropoda and Diptera–Ephemeroptera pairs, respectively. Trophic resource partitioning, maintained through active prey electivity and seasonal dietary divergence, emerges as the key coexistence mechanism in this assemblage. Each species occupies a functionally unique dietary niche, such that any species loss would represent an irreplaceable reduction in ecosystem function. These findings provide a critical ecological baseline for conservation planning in this freshwater endemism hotspot, and highlight the urgent need to protect benthic macroinvertebrate communities across the full elevational gradient of the Ouled Abdallah Key Biodiversity Area.

Keywords: trophic niche partitioning; endemic cyprinids; freshwater conservation.

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Serum-free Media for Fish Cells in Cultivated Seafood Production

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Abstract

Global seafood demand is climbing fast, and conventional aquaculture systems are starting to buckle under the pressure one reason cellular aquaculture has drawn growing attention as an alternative production route. Yet the field remains constrained by heavy reliance on Fetal Bovine Serum (FBS), which accounts for 85–95% of culture media costs, one of the largest contributors to overall cultivated meat production costs, and raises ethical, regulatory, and food safety concerns. Fish cell lines also require unusually high serum concentrations, up to 20% FBS versus the 10% standard for mammalian systems. Here, we examine the biological basis of this elevated requirement, FBS's core shortcomings, and the main components used in serum-free media (SFM) design: growth factors, protein hydrolysates, vitamins, lipids, trace elements, and algal extracts. Findings so far are encouraging. Fish-specific recombinant growth factors outperform their human counterparts in fish cell culture: the Nile tilapia IGF-2 ortholog showed 1.8-fold higher proliferative activity than commercial human IGF-2, and the Atlantic salmon FGF-2 ortholog was 1.5-fold more active than its human equivalent. Protein hydrolysates from marine invertebrates and insects (Black Soldier Fly, lugworm, oyster, mussel), even at 0.001-0.1 mg/mL, allowed zebrafish embryonic stem cell cultures to maintain growth with serum concentration halved. Microalgal extracts, particularly *Auxenochlorella pyrenoidosa* protein extract (APE), reduced serum requirements to as low as 5% in goldfish muscle cell cultures while influencing key metabolic and signaling pathways. Gaps remain: existing cell lines cover only 1–2% of the ~35,000 Actinopterygian species; chemically defined formulations for commercially important species (salmon, tuna, sea bass, gilthead seabream) are largely missing; and lot-to-lot variability in hydrolysate and algal extract preparations complicates standardization. Future progress will depend on AI-assisted high-throughput screening, omics-guided media optimization, low-cost microbial production of species-specific recombinant growth factors, and systematic translation of classical aquaculture nutrition knowledge into in vitro media design.

Keywords: Serum-free media, cellular aquaculture, FBS alternatives, cultivated seafood, protein hydrolysate, microalgae

Introduction

Global demand for aquatic products is climbing steadily, driven by population growth and rising incomes. Per capita fish consumption has already reached 20.7 kg, and aquatic animal production is projected to grow by another 10% by 2032, according to FAO's *The State of World*

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Fisheries and Aquaculture 2024 report (FAO, 2024a). Yet conventional aquaculture systems are increasingly unable to keep pace with this demand sustainably, hampered by overfishing, habitat loss, pollution, and climate change (FAO, 2024b; Borriello and Pierucci, 2025). These structural constraints make it clear that the sector needs solutions that move beyond current production models.

Cellular aquaculture has emerged as one such alternative protein source, summarized in Figure 1. Cell-based seafood holds real promise on two fronts: food security and environmental sustainability (Çek-Yalnız and Aydın, 2024; Kang et al., 2025). Single-cell proteins (SCP) and related biotechnological approaches help get around the limitations of conventional fishmeal- and soy-based feeds, opening the door to production with a smaller environmental footprint (Chamodi et al., 2025). That said, whether this technology can scale up in an economically viable way depends heavily on one thing: optimizing the cell culture media, which remains one of the most critical bottlenecks in the entire production chain.

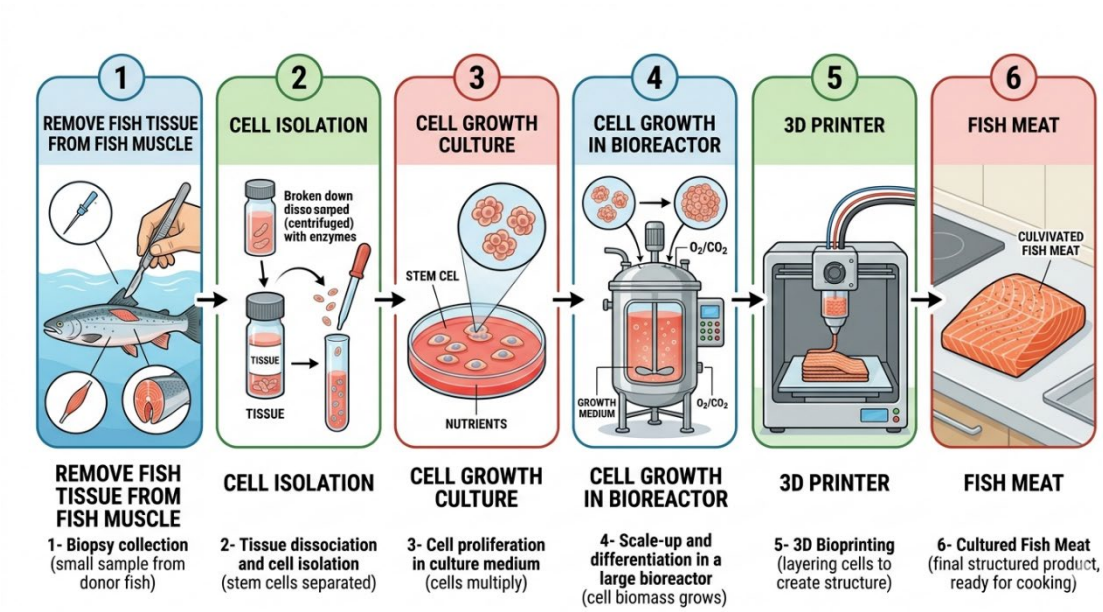


Figure 1. Stepwise Process of Cultivated Fish Meat Production based on Çek-Yalnız and Aydın (2024).

When it comes to culture media, Fetal Bovine Serum (FBS) remains the most widely used component today and arguably one of the biggest bottlenecks in cellular seafood production. It's favored for good reason: it supports cell proliferation and metabolism effectively. But that effectiveness comes at a cost, quite literally. FBS is expensive, raises ethical concerns, faces supply constraints, varies significantly in composition from batch to batch, and simply doesn't hold up from a food safety standpoint (Batish et al., 2022). Given all this, developing serum-free media isn't just a nice-to-have anymore it's become essential, both for economic feasibility and long-term sustainability (Jozef et al., 2025).

This challenge hits fish cell lines especially hard. Fish cell lines used for cultured seafood can require serum concentrations of up to 20%, far higher than what's typical in mammalian systems, and cutting that serum back tends to trigger problems: morphological changes, slower growth, or in some cases the cells stop growing altogether. Some progress has been made IGF-2, algal extracts, and protein hydrolysates have all helped reduce serum dependence to some degree but eliminating serum entirely without harming the cells is still an open problem, and one that continues to sit near the top of the research agenda (Goswami et al., 2024; Ulagesan et

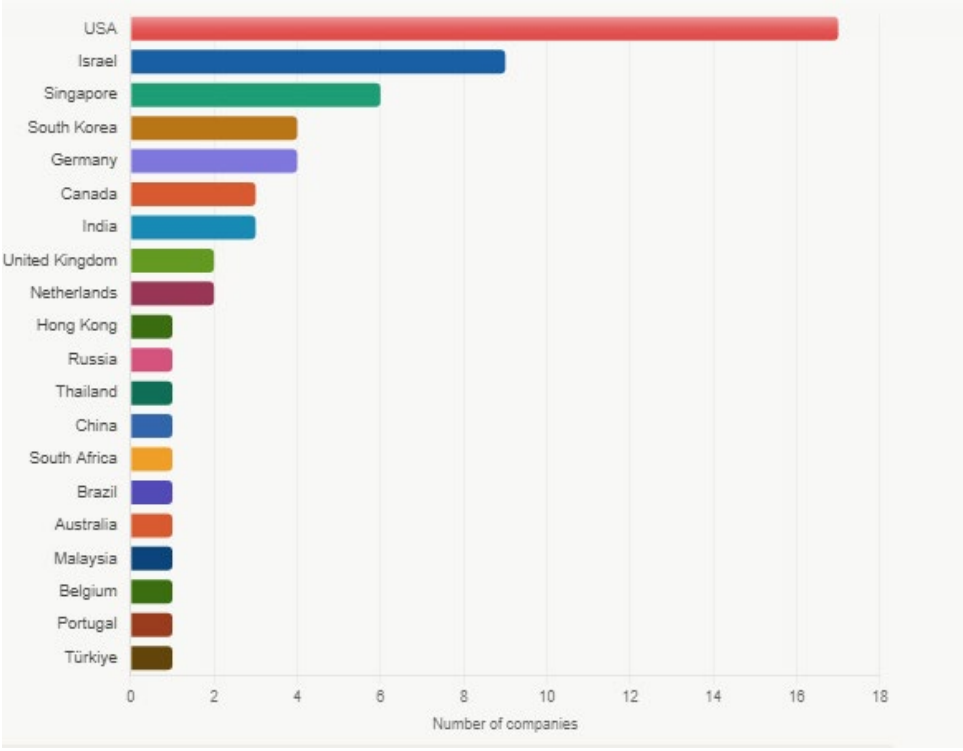
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al., 2026). All of this points to the same conclusion: the field needs a more systematic, holistic look at the issue.

This review's main aim is to take stock of current approaches to developing serum-free or low serum culture media for cellular aquaculture, and to pinpoint where the gaps still lie looking at both fish-specific biology and marine-derived alternatives to conventional serum. Along the way, we examine how classical aquaculture nutrition science might translate into in vitro media design, what marine invertebrate- and insect-derived protein hydrolysates could offer for cell proliferation and differentiation, and where species-specific recombinant growth factors might make serum-free media more cost-effective. Ultimately, the goal is to lay out a scientific basis for serum-free media strategies that make cultured seafood production not just cheaper, but more environmentally sustainable as well.

Cellular Aquaculture and Biological Characteristics of Fish Stem Cell Lines

Cultivated marine and freshwater products are based on the principle of isolating muscle, fat, or fibroblast cells from live aquatic organisms and propagating and differentiating them in vitro. This approach is attracting increasing interest due to its potential to reduce the environmental pressures of traditional fisheries and aquaculture, limit antibiotic use, and provide products free of heavy metals (such as mercury) (Goswami et al., 2024; Goswami et al. 2022, Ural and Topuz, 2024). As of 2024, the global cultivated seafood sector has grown to encompass more than 61 companies; Singapore granted the first commercial approval in 2020, followed by the United States in 2023 (Çek-Yalnız and Aydın, 2024). Figure 2 shows that there are 60 companies worldwide working to cultivate freshwater, marine fish, and crustacean meat. The United States dominates with 17 companies more than double the number in the next country. Israel ranks second with 9, making it a surprising hub for the industry. Singapore is third with 6 companies, reflecting the city-state's strong commitment to alternative proteins. South Korea and Germany are tied with 4 each, followed by Canada and India with 3 apiece (Figure 2).



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Figure 2. List of enterprise working on cell-based fresh water fish , marine fish, crustacean and mollusk meat production. Based on Çek-Yalnız and Aydın, 2024

Despite real progress in tissue engineering, bioprocess optimization, and serum-free media development, cell-based fish meat production still hasn't cleared the technical barriers standing between it and large-scale commercial feasibility. The biggest culprits: no standardized protocols for cell isolation and culture, serum-free growth media formulations that still fall short, and the ongoing struggle to build culture systems that actually scale (Goswami et al., 2024; Çek-Yalnız and Aydın, 2024; Rubio et al., 2019).

That said, fish cells bring some real advantages to the compared to mammalian cells when it comes to in vitro culture. They tolerate hypoxia well, buffer against pH shifts in the culture media more effectively, and can grow at lower temperatures all of which makes them appealing for bioreactor-based scale-up (Shang et al., 2015, 2018;Çek et al., 2016; Çek-Yalnız & Yaraş, 2019; Rubio et al., 2019;Yaraş &Çek Yalnız, 2021). Rainbow trout (*Oncorhynchus mykiss*) cell lines like RTgill-W1, for instance, can be cultured at room temperature and normal atmospheric pressure, with no CO_2 incubator required (American Type Culture Collection, ATCC, 2026). Zebrafish (*Danio rerio*) embryonic stem cells have proven just as useful, emerging as a key model organism in cultured meat research (Batish et al., 2022).

As Figure 3 illustrates, several serum components each play a distinct biological role in keeping fish stem cell lines alive and thriving in culture. Albumin acts as a carrier protein, binding fatty acids, hormones, and metal ions in doing so, it stabilizes the cell membrane, cuts down oxidative stress, and helps nutrients move where they need to go. Insulin, meanwhile, regulates metabolism by controlling glucose uptake and driving cell proliferation, which in turn supports energy metabolism, boosts protein synthesis, and speeds up overall cell growth. Transferrin handles the safe transport of iron ions, supplying what's needed for DNA synthesis and mitochondrial function while also limiting oxidative damage. And lipid carriers ferry essential phospholipids, cholesterol, and fatty acids critical for keeping membranes fluid, providing energy, and participating in signal transduction pathways.

As outlined above, the serum-free medium developed for fish stem cell culture rested on four core components albumin, insulin, transferrin, and lipid carriers which together stand in for the functional roles serum would otherwise play. Radošević et al. (2016) managed to adapt the channel catfish ovary (CCO) cell line to a DMEM/F12-based Ultra-CULTURE medium, supplementing it with insulin, transferrin, and a purified mixture of bovine serum proteins including albumin. More recently, a study in *Marine Biotechnology* found that transferrin and insulin are both critical to fish cell differentiation under serum-free conditions (Bomkamp et al., 2023). A comprehensive review by Quek et al. (2024) compared a range of serum-free formulations and found that DMEM/F12 comes up as the most commonly used basal medium, with insulin and transferrin showing up in nearly every formulation examined. Building on this, Schenzle et al. (2025) introduced food-grade, low-cost alternatives to albumin, cutting medium costs by as much as 73% for certain cell lines. That same year, a study on the RTgill-W1 rainbow trout gill cell line reported something of a milestone: the first systematically developed, fully defined serum-free culture medium for fish cell lines, one that kept cells viable in long-term culture for 35 passages (Jozef et al., 2025).

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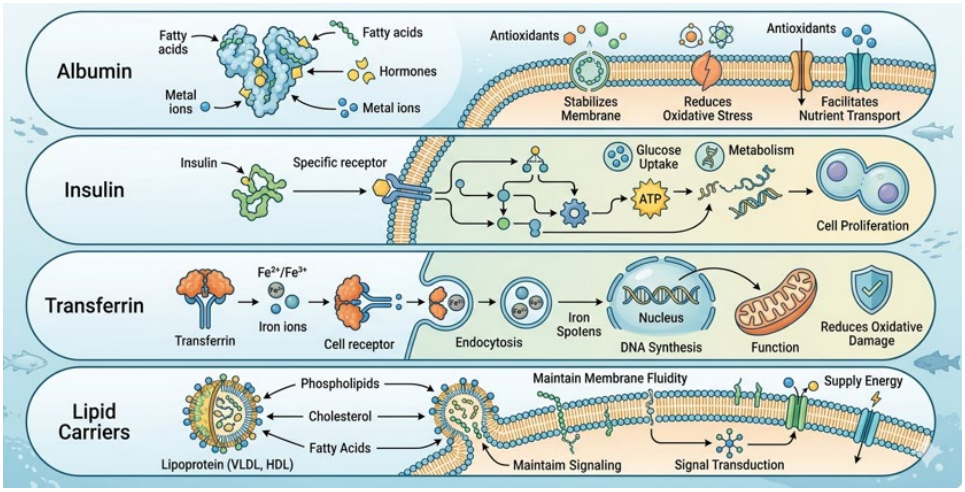


Figure 3. Schematic visualization of biological functions of serum components in fish stem cell lines

That said, fish cell lines come with their own set of serious challenges. Perhaps most notably, developing fish cell lines for cultivated seafood can call for serum concentrations as high as 20% well above the 10% typically used in mammalian systems (Goswami et al., 2024). Cutting back on serum tends to cause trouble: morphological changes, slower growth, or in some cases the cells stop growing altogether. On top of that, cell lines currently exist for only 1–2% of the roughly 35,000 Actinopterygian species out there, which remains a major limitation in itself (Bols et al., 2025). And there's a practical mismatch too commercially available bioreactors are generally built to run at 37°C, while fish stem cells need something closer to 18–22°C, meaning extra cooling is required and energy gets wasted in the process.

FBS: Functions, Limitations, and the Need for Transition to Serum-Free Media

Fetal Bovine Serum (FBS) has served as a near-universal growth supplement in in vitro cell culture for decades now. Its main job is to supply cells with hormones, lipids, micronutrients, and perhaps most importantly mitogenic growth factors that drive cell division (Jozef et al., 2025). Part of why FBS caught on so widely comes down to its broad composition, versatile enough to support a huge range of cell types. In fact, while roughly 260 mammalian cell types can now be cultured in serum-free media, no serum-free formulation has managed to fully replace FBS across the board (Labconscious, 2024).

Still, relying on FBS brings a whole web of interconnected problems. On the economic side, culture media costs make up 85–95% of total cultivated meat production costs, and FBS is a major driver of that figure (Batish et al., 2022). Serum-free media sidestep the ethical baggage that comes with FBS, sure, but recombinant growth factors are pricey enough that current serum-free formulations often end up costing more than FBS itself (Jozef et al., 2025). Growth factors and recombinant proteins alone account for roughly 95% of serum-free media costs (Quek et al., 2024).

Then there's the ethical dimension. FBS is derived from fetal calf blood, which sits awkwardly at odds with the very animal welfare rationale that motivates cultivated meat in the first place. Add to that lot-to-lot variability, the risk of disease transmission, incompatibility with food safety standards, and the greenhouse gas emissions tied to the livestock industry, and it's easy to see why FBS keeps drawing criticism (Batish et al., 2022; Labconscious, 2024).

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Taken together, these limitations make serum-free media development feel less like an option and more like a necessity, both for economic viability and long-term sustainability. Chemically defined serum-free media (CD-SFM) do away with the lot-to-lot variability FBS introduces, offering more consistent performance that fits better with controlled production processes (Jozef et al., 2025). From a regulatory standpoint, serum-free formulations also give food safety assessments a clearer, more verifiable basis to work from. And the fact that more than 260 cell types have already been successfully cultured in serum-free media speaks for itself the technical potential here is hard to deny (Labconscious, 2024).

Serum-Free Media Desingn: Core Components

Growth factors sit at the heart of serum-free media formulations both the most critical component and, unfortunately, the most expensive one, making up over 90% of total medium costs. A recent study by Skrivergaard et al. (2025) captures both the challenge and the opportunity here well. The authors cloned and expressed recombinant FGF-2 variants from bovine, salmon, and cod sources in E. coli, then tested their bioactivity in primary bovine skeletal muscle satellite cells. In a serum-free medium supplemented with fetuin and Insulin Transferrin–Selenium (ITS), all three variants successfully drove cell proliferation. What stood out most was the bovine FGF-2: it hit peak proliferative effect at just 2 ng/mL, performing on par with 5% fetal bovine serum, while also activating the ERK signaling pathway and upregulating key myogenic markers like PAX7, SDC4, ITGB1, and CCND1. Things got even more interesting when bovine FGF-2 was paired with a p38 inhibitor the combination produced a synergistic effect, hinting that pathway-level interactions could be exploited to squeeze better performance out of minimal growth factor concentrations. Altogether, these findings suggest that optimized, species-specific recombinant growth factor production in microbial systems could meaningfully cut medium costs without sacrificing cell performance a real, concrete step toward scalable serum-free media for cultured meat.

Factors such as FGF-2, IGF-1, IGF-2, EGF, and PDGF-BB stand out as key regulators of cell proliferation and differentiation (Skrivergaard et al. 2025; Di Leonardo et al. 2025; Park et al., 2024; Quek et al., 2024; Venkatesan et al. 2022), Figure 4. In a study conducted on the sturgeon-derived AOXla7y cell line, the effects of six different growth factors FGF-2, IGF-1, LIF, IFN-γ, IL-13, and IL-15 were examined following serum reduction (from 10% to 2%). Among these, FGF-2 and IGF-1 were identified as the most effective factors in supporting cell proliferation under low-serum conditions (Di Leonardo et al. 2025).

Beyond their role in cell culture media, members of the FGF family have also been shown to exert important metabolic functions in fish physiology, with direct implications for aquaculture and cell-based production systems. Yu et al. (2024) investigated the therapeutic potential of recombinant FGF-1 (rFGF1) in rainbow trout (*Oncorhynchus mykiss*) fed a high-fat diet, a widely used model for studying metabolic disorders in carnivorous fish. The results demonstrated that rFGF1 significantly reduced serum glucose levels and improved glucose tolerance by inhibiting hepatic gluconeogenesis through upregulation of PDE4D and accelerated cAMP hydrolysis a mechanism distinct from the classical insulin-mediated pathway. In addition, rFGF1 promoted hepatic glycogen synthesis via the AKT/GSK3β axis and reduced liver inflammation by enhancing autophagic flux and downregulating pro-inflammatory cytokines. Strikingly, rFGF1 also reshaped gut microbial composition, increasing the abundance of *Lactobacillus* and elevating short-chain fatty acid levels, particularly acetic and butyric acids. These findings highlight that FGF family members are not only critical components of serum-free media formulations, but also potent regulators of fish metabolism, underscoring the broader biological relevance of species-specific recombinant growth factors in aquaculture contexts.

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The use of species-specific recombinant growth factors is gaining importance due to the limited cross-reactivity between different species. A comprehensive study on recombinant growth factor production demonstrated that the IGF-2 ortholog derived from Nile tilapia (*Oreochromis niloticus*) exhibited 1.8-fold higher proliferative activity compared to commercial human IGF-2. Similarly, the FGF-2 ortholog from Atlantic salmon (*Salmo salar*) showed 1.5-fold higher proliferation activity (7.6-fold increase overall) compared to commercial human FGF-2 (Venkatesan et al., 2022). These findings indicate that fish-specific recombinant growth factors may provide a critical advantage in the performance of serum-free media (Figure 4). In another study, Chen et al. (2000) cloned and expressed recombinant tilapia IGF-I in a bacterial expression system and evaluated its biological activity by injecting the recombinant IGF-I, along with related recombinant IGF polypeptides, into juvenile tilapia. Fish receiving the injections exhibited significant increases in body weight and body length compared to control animals, confirming that the bacterially produced recombinant tilapia IGF-I retained its growth-promoting activity *in vivo*. These findings demonstrate a cost-effective strategy for producing biologically active fish growth factors and support their potential application in aquaculture to enhance fish growth rates.

Strategies to reduce the cost of growth factors include the use of small-molecule mimetics, conditioned media, and production in microbial expression systems. However, the effectiveness of these alternatives for fish cells and their compatibility with food safety standards have not yet been sufficiently investigated.

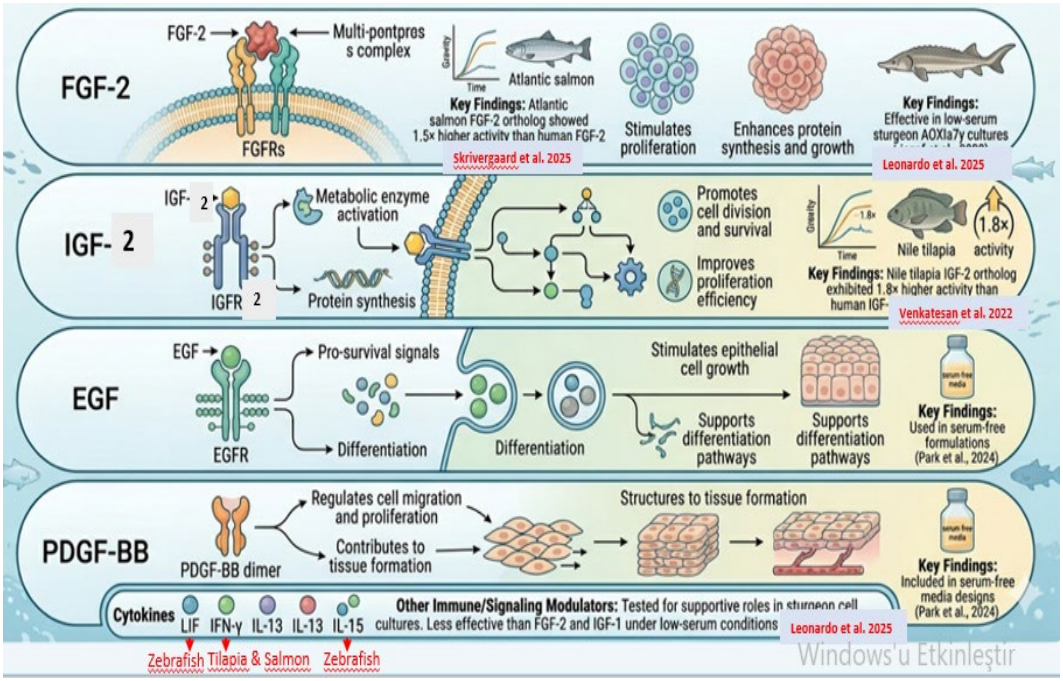


Figure 4. Schematic visualizationof biological funtions of growth factors in dish stem cell lines

Protein Hydrolysates: Marine Invertebrate and Insect-Based Approaches

The push to find sustainable alternatives to conventional fishmeal (FM) and fish oil (FO) in aquaculture feeds has picked up serious momentum over the past decade. Henry et al. (2015) took a systematic look at ten insect taxa yellow mealworms, black soldier flies, silkworms, houseflies among them as potential FM replacements, and found that insect protein content ranges from 50–82% dry matter, with amino acid profiles that line up reasonably well with what fish need nutritionally. Mealworms, silkworms, and maggots emerged as the strongest candidates out of the group. That said, the authors also flagged some real hurdles: chitin can

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limit digestibility, n-3 fatty acids tend to fall short, and there's a risk of toxins building up depending on what substrate the insects were reared on though defatting and better processing can go a long way toward addressing these issues This work ended up laying important groundwork, one that later fed into the use of insect- and marine invertebrate-derived ingredients not just in aquaculture feeds, but in serum-free cell culture media for cellular aquaculture too.

Protein hydrolysates are mixtures of peptides and amino acids produced by breaking down protein sources through enzymatic or chemical hydrolysis. In cell culture media, they can serve double duty as both nutrient sources and growth promoters (Batish et al., 2022). Marine-derived hydrolysates stand out in particular because their amino acid profile tends to be biologically compatible with aquatic cell culture. This has made protein hydrolysates a recurring focus in serum-reduction strategies and alternative nutrient supplementation more broadly, with studies showing that both plant- and animal-derived versions can support cell growth and metabolism. Combe et al. (2026) put together a useful summary of these applications (Figure 5).

One notable study out of Virginia Tech tested protein hydrolysates from Black Soldier Fly (BSF), cricket, oyster, mussel, and lugworm (Arenicola marina) on zebrafish embryonic stem cells (ESC). With degrees of hydrolysis ranging from 18.24% to 33.52%, these hydrolysates used at low concentrations of 0.001–0.1 mg/mL alongside just 1% and 2.5% serum significantly boosted cell growth compared to control groups running at 5% and 10% serum. Even more striking, the researchers achieved equivalent or better performance while cutting serum use in half. And with the exception of cricket hydrolysate, every hydrolysate tested preserved cell membrane integrity, backed up by LDH activity results (Batish et al., 2022).

Interestingly, once concentrations hit 1 mg/mL and above, all the hydrolysates started showing cytotoxic effects, actively suppressing cell growth rather than promoting it. This dose-dependent toxicity lines up with what's been reported before for soy and fish gelatin hydrolysates, and it's a good reminder that concentration optimization needs careful attention during formulation (Batish et al., 2022). Worth noting too, from a circular economy angle: using oyster and mussel waste as a protein extraction source could offer real value to the cultivated meat sector, turning what would otherwise be discarded material into something useful.

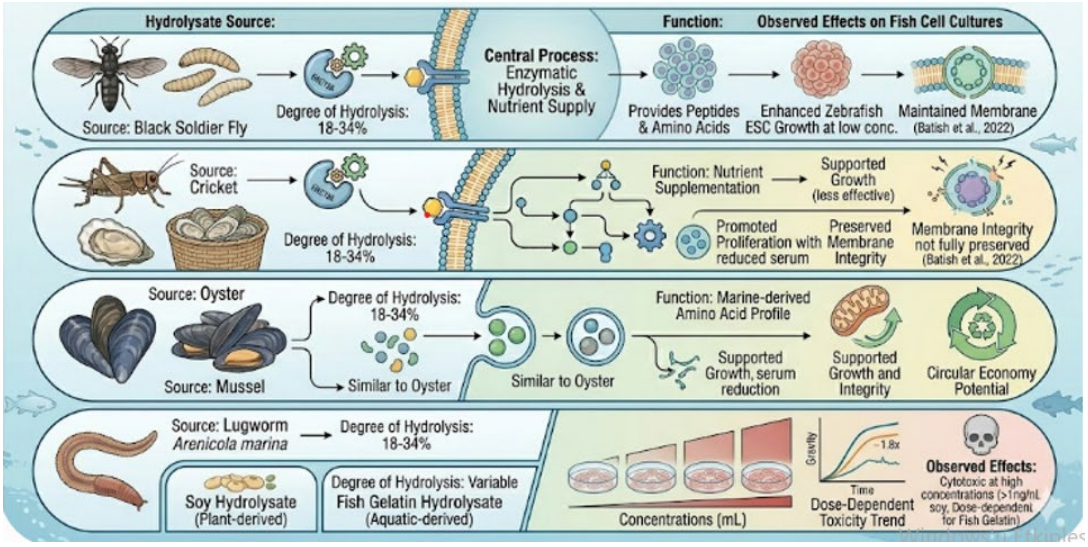


Figure 5. Comparative overview of protein hydrolysate sources on fish esc cultures

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Algal and Microalgal Derived Components

Microalgae have drawn growing interest as a resource for serum-free media development, thanks to their high protein content, omega-3 fatty acids (EPA and DHA), vitamins, and various bioactive compounds (Ng et al., 2020; Yamanaka et al., 2023; Park et al., 2024). In cellular aquaculture specifically, Auxenochlorella pyrenoidosa protein extract (APE) has attracted particular attention. In a study run under low-serum conditions (5% FBS), APE supplementation significantly boosted Carassius auratus muscle (CAM) cell proliferation and increased expression of the myogenic transcription factor Myf5 compared to controls. Combined metabolomic and proteomic analyses showed that APE affected 13 metabolites and 67 proteins, pointing to its influence on specific metabolic and signaling pathways (Dong et al., 2024).

Chlorella vulgaris extracts have also shown promise in several studies, partially substituting for FBS in CHO cells and bovine myoblasts (Ng et al., 2020; Okamoto et al., 2022). Marine microalgal extracts more broadly including Spirulina, Nannochloropsis, and Dunaliella have been successfully worked into serum-free formulations for MDCK and Vero cell cultures when paired with growth factor cocktails (Park et al., 2024). There's also ongoing work looking at native proteins from thermoacidophilic algae like Galdieria sulphuraria as a potential FBS replacement, with encouraging early results (Park et al., 2025). Figure 6 summarizes how microalgal extracts have been applied across CHO, MDCK, and Vero cell serum-free stem cell technologies.

One of the biggest advantages of microalgae-based approaches is that they grow photosynthetically, which makes large-scale production possible at relatively low environmental cost. That said, algal extracts are still chemically undefined in nature, and lot-to-lot variability remains a real obstacle standing in the way of formulation standardization.

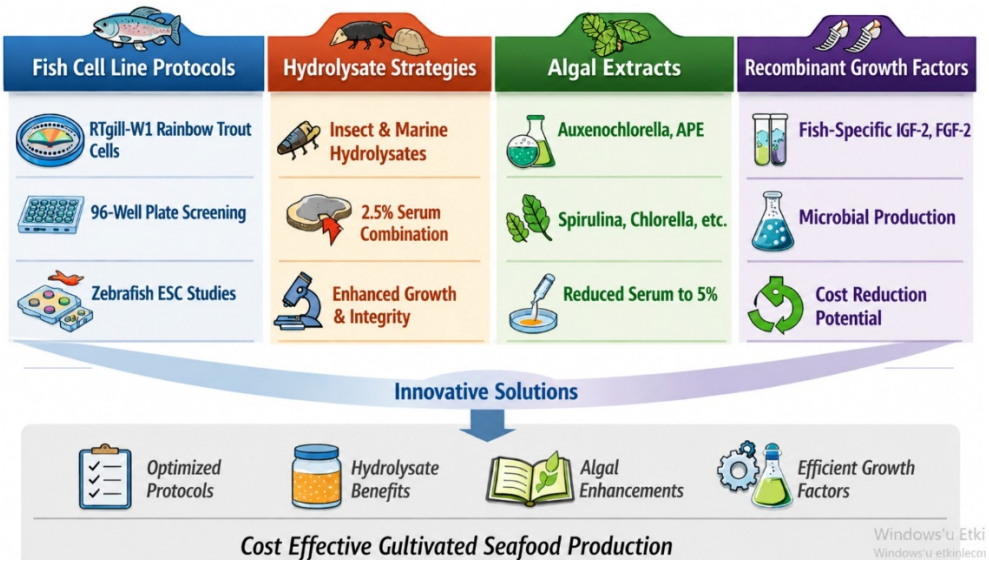


Figure 6. Comparative evaluation of serum free strategies for fish cell culture

Comparative Evaluation of Current Approaches and Research Gaps

Research on serum-free or low-serum culture media for fish cells is still fairly limited, but the progress made in recent years is notable. One of the most thorough efforts in this space involved systematically developing a serum-free culture medium for the RTgill-W1 rainbow trout cell line. The researchers optimized medium components using a 96-well plate screening approach and managed to keep cells growing under serum-free conditions for up to 35 passages. The

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resulting formulation also held up in ecotoxicology tests compliant with OECD Test Guideline 249 (Jozef et al., 2025).

Hydrolysate research using the zebrafish ESC model (Batish et al., 2022) found that BSF and lugworm hydrolysates, combined with just 2.5% serum, matched the cell growth seen in the 10% serum control group while keeping cell morphology intact. On the algae side, Dong et al. (2024) showed that supplementing with Auxenochlorella pyrenoidosa protein extract (APE), a freshwater green algae, allowed serum concentration in goldfish muscle cells to drop as low as 5%.

A broader review looking at cost-reduction strategies for serum-free media (Quek et al., 2024) pointed out that combining species-specific recombinant growth factors with high-throughput screening platforms and media recycling strategies together could meaningfully bring costs down.

Key Knowledge Gaps and Future Perspectives

Looking across the current literature, a few critical gaps stand out in serum-free media development for cultivated seafood production. For one, most research has centered on mammalian cells or a handful of model fish species zebrafish and rainbow trout, mainly while commercially important species like salmon, tuna, sea bass, and gilthead seabream have barely been studied at all. Without standardized cell lines or benchmarking protocols, comparing results across studies becomes genuinely difficult, and that in turn slows down systematic progress in the field (Figure 7).

There's also the ongoing challenge of folding complex components like protein hydrolysates and algal extracts into chemically defined formulations. Because these ingredients suffer from lot-to-lot variability and resist easy standardization, they need extra quality control measures before they can be trusted for reliable industrial-scale use (Fig. 7). Furthermore, the systematic transfer of classical aquaculture nutrition knowledge (species-specific essential amino acid profiles, mineral and vitamin requirements) into in vitro media design remains an underexplored but highly relevant approach.

Looking ahead, the integration of AI-assisted high-throughput screening platforms and omics approaches holds strong potential to accelerate serum-free media optimization. The production of fish-specific recombinant growth factors in low-cost microbial expression systems, combined with the implementation of media recycling strategies, could also significantly reduce costs. In the long term, the commercial sustainability of cultivated seafood production will depend directly on the dramatic reduction of serum-free media costs compared to current values.

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Figure 7. Roadmap for Serum-Free Aquaculture Development: Challenges, Strategies, and Future Outlook

Conclusion

Cellular aquaculture opens the door to a fundamental paradigm shift in production that could sustainably meet global seafood demand. However, realizing this potential depends directly on overcoming dependence on Fetal Bovine Serum (FBS). Current research indicates that fish-specific recombinant growth factors**,** marine invertebrate and insect-derived protein hydrolysates applied at low concentrations**,** and microalgal extracts possess the potential to partially or completely replace FBS.

Nevertheless, species-specific formulation requirements**,** component standardization**, and** scaling challenges remain priority obstacles to be addressed. The integration of classical aquaculture nutrition knowledge such as species-specific essential amino acid profiles, mineral, and vitamin requirements into in vitro media design, along with the strengthening of multidisciplinary research collaborations, are considered strategic steps that could significantly accelerate progress in this field

Author Contribution

ŞÇ conceived the idea, ŞÇ and FA wrote the paper. Both authors read the final version of the review and approved it for publication.

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This is a review article and does not involve any new studies with human or animal subjects performed by the authors.

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Conflict of Interest

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A New Record of Juvenile Shortfin Mako Shark (*Isurus oxyrinchus*) off the Coast of Side, Gulf of Antalya (Türkiye)

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Abstract

Considered one of the dangerous shark species, Shortfin Mako sharks (*Isurus oxyrinchus* Rafinesque, 1810) generally swim near the water's surface and have a cosmopolitan distribution. Juvenile and adult individuals have been reported at various times in different regions along the Turkish coast. It is an important species in recreational fishing. This study reports the accidental capture of a newborn shortfin mako shark by gillnets off the coast of Side in the Gulf of Antalya (Türkiye, Eastern Mediterranean) on February 20, 2026. This species is globally classified as Endangered (EN) on the IUCN Red List. The specimen was a male with a total length of 698 mm and weight of 3688 g. The presence of such individuals in the region suggests the potential existence of a nursery ground in the area. Thus, initial data regarding a juvenile individual have been obtained.

Keywords: Shortfin Mako, shark, *Isurus oxyrinchus*, Side, Gulf of Antalya

Introduction

Shorfin mako *Isurus oxyrinchus* Rafinesque, 1810, is one of the fastest shark in the world (Diez et al. 2015) and epipelagic shark that swims near the surface but also descends to 500 m (Golani et al. 2006). This species is a pelagic species widely distributed in temperate and tropical waters worldwide (Cailliet et al. 2009). The IUCN status of the shortfin mako shark has been assessed as Endangered (EN) (Rigby et al. 2019). It is a species of great value for game fishing and is also a target species in commercial fishing (Golani et al. 2006). *I. oxyrinchus* feeds on a wide variety of fish, some of which are the same size as the shark itself. It is highly dangerous; there have been reported cases of attacks on humans worldwide (Başusta and Başusta, 2024). The meat of the shortfin mako shark is consumed commercially, its fins are used to make shark fin soup and its liver oil is extracted as a source of vitamin A (Cailliet et al. 2009). This study is to give first record of the juvenile shortfin mako from the coast of Side (Gulf of Antalya, Türkiye).

Material and Methods

On February 20, 2026 Juvenile *I. oxyrinchus* sample was accidentally caught by commercial gillnet fishing off the coast of Side (36°44'13.9"N 31°21'24.4"E) in Antalya Bay, Eastern Mediterranean at about 30-35 m of depth. The juvenile shark was transported to the Fisheries Biology laboratory, Faculty of Fisheries, Firat University where they were identified, sexed and

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photographed. Morphometric measurements of fish sample was taken to the nearest 1 mm and digital caliper with 0.01 mm precision. The weight of fish was taken with a digital scale to the nearest 0.01 g. Fish sample was preserved at the Museum of Fisheries Faculty, Firat University.

Results and Discussion

Male shortfin mako shark specimen was examined and its total length and weight were 69.8 cm and 3688 g, respectively (Figure 1).



Figure 1. *Isurus oxyrinchus* captured off Side Coast (Gulf of Antalya), Türkiye



Figure 2. Claspers of juvenile male *Isurus oxyrinchus*

Table 1. Morphometric measurements of *Isurus oxyrinchus*

Date	February, 20-2026
Sex	**Male**
Weight	3688 g
Total length	69.8 cm
Fork length	65.6 cm
Precaudal length	59.3 cm
Pre second dorsal length	53.2 cm
Pre first dorsal length	30.8 cm
Head length	22.6 cm
Prebranchial length	17.6 cm
Preorbital length	5.9 cm
Prepectoral length	20.2 cm
Prepelvic length	43.5 cm

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Preanal length	54.3 cm
Eye length	17.37 mm
Eye height	15.03 mm
Preoral length	62.53 mm
Prenarial length	39.27 mm
Intergill length	53.52 mm
Pectoral length	132.61 mm
Pectoral base	32.01 mm
Pectoral inner margin	41.39 mm
First dorsal anterior margin	80.29 mm
First dorsal base	57.74 mm
First dorsal length	75.69 mm
First dorsal inner margin	12.58 mm
First dorsal posterior margin	59.90 mm
First dorsal heigth	57.01 mm
Dorsal caudal margin	136.81 mm
Clasper inner length	18.07 mm
Clasper outher length	43.66 mm

The shortfin mako shark has occasionally been recorded from all Turkish waters except the Black Sea (Geldiay, 1969;Bilecenoglu et al. 2002; Akyol ve Ceyhan, 2012; Akyol et al. 2013; Kabasakal, 2015; Kabasakal, and Bilecenoglu, 2020). Kabasakal and De Maddelena (2011) documented the presence of the shortfin mako shark in the Aegean Sea (off the coast of Marmaris) using a record dating back 70 years. This species was reported from Urla Bay (İzmir), in the Aegean Sea (Akyol et al. 2010 and Akyol et al. 2011). A few juvenile specimens were recorded from Iskenderun Bay (Eastern Mediterranean) (Erguden et al. 2013) and Saros Bay (Kabasakal and Kabasakal, 2013). More recently, this species was reported from Edremit Bay, Northern Aegean Sea coast of Türkiye (Tuncer and Kabasakal, 2016) and also from Izmir Bay, Gökova and Fethiye Bays (Central and South Aegean Sea, Türkiye) (Bengil et al. 2019). *I. oxyrinchus* generally has a total length of around 200-270 cm. However, Kabasakal and De Maddelena (2011) reported that an individual with a maximum total length (TL) of approximately 585 cm was caught off the coast of Marmaris in the late 1950s. Compagno (2002) reported that the total length of the shortfin mako shark at birth ranges between 60 and 70 cm. Based on the maturity stages proposed by Stehmann (2002) for male cartilaginous fish specifically regarding whether the pterygopodia (claspers) extend beyond or fall short of the tips of the posterior pelvic fin lobes it is evident that the individual is in the late pre-maturation phase or the early maturation phase (Figure 2). This study reports, for the first time, the accidental capture of a juvenile shortfin mako shark off the coast of Side (Gulf of Antalya, Türkiye). The sighting of this shortfin mako shark estimated to be a newborn in the region suggests that the area has the potential to serve as a breeding or nursery ground for the species.

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Ethics Approval
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Conflict of Interest

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Data Availability

The data used in this study can be made available upon request to the corresponding author.

Author Contribution

The authors conceived and designed the study, conducted the fieldwork, collected and prepared the figures, wrote the manuscript, and approved the final version of the manuscript.

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Records of newborn Marbled electric rays (*Torpedo marmorata*) off the coast of Alanya, Gulf of Antalya (Türkiye)

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Abstract

The marbled electric ray (*Torpedo marmorata*) is a species of benthic ray that typically inhabits sandy or muddy bottoms. It feeds on benthic invertebrates, stunning them with an electric shock generated by specialized organs located on either side of its disc. It has no commercial value. This species is globally classified as Vulnerable (VU) on the IUCN Red List. This study reports the accidental capture of a newborn two marbled electric rays by gillnets off the coast of Alanya in the Gulf of Antalya (Türkiye, Eastern Mediterranean) on February 21, 2026. These individuals were male with a total lengths of 16.1 and 16.7 mm and weights of 96.07 and 96.74 g. The presence of both juvenile and adult individuals in the same area indicates that it is a potential nursery area.

Keywords: Marbled electric ray, *Torpedo marmorata*, Juvenil, Alanya, Gulf of Antalya

Introduction

It is a demersal species found at depths ranging from 10 to 50 m and occasionally up to 250 m (Golani et al. 2006). Sexual maturity is reached at a total length of 29 cm in males and 40 cm in females. It feeds almost exclusively on fish; instances of feeding on crustaceans or cephalopods are rare (Fisher et al. 1987). The IUCN status of the marbled electric ray has been assessed as Vulnerable (VU) (Finucci et al. 2021). They caught by trammel nets but lacks commercial value and capable of inflicting strong electric shocks also outside the water (Golani et al. 2006). Studies on Torpedinids are generally quite limited and focus on the length-weight relationships (Filiz and Mater, 2002; Karakulak et al. 2006; Özaydın et al.2007; Başusta et al.,2012; Duman and Başusta, 2013; Eronat and Özaydın, 2014; Şen et al., 2024). Although there is not much data regarding the newborn and juvenile *T. marmorata* species, there are a few studies conducted in Turkish waters; Duman and Başusta (2013) recorded specimens of juvenile marbled electric rays from the Gulf of Iskenderun, while Şen et al. (2024) reported *T. marmorata* embryos from the Sea of Marmara. This study aims to present the first records of newborn marbled electric rays from the coasts of Alanya (Gulf of Antalya, Türkiye) and to report a probable new breeding or nursery area.

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Material and Methods

On February 21, 2026, juvenile marbled electric ray samples were accidentally captured by commercial gillnet fishing off the coast of Alanya (36°29'37.1"N 31°56'08.8"E) in Gulf of Antalya, Eastern Mediterranean at about depth of 25-30 m. The juvenile *T. marmorata* individuals were transported on ice to the Fisheries Biology laboratory, Faculty of Fisheries, Firat University where they were identified, sexed and photographed. Sex determination was based on the presence or absence of the pterygopodium (claspers) (Başusta and Başusta, 2023). Morphometric measurements of the fish specimens were performed using a digital caliper with a precision of 0.01 mm. The weight of each fish was taken with a digital scale to the nearest 0.01 g. Fish samples were preserved at the Museum of Fisheries Faculty, Firat University.

Results and Discussion

Male marbled electric ray specimens were male with a total lengths of 16.1 and 16.7 mm and weights of 96.07 and 96.74 g. respectively (Figure 1).



Figure 1. *Torpedo marmorata* individuals captured off Alanya Coast (Gulf of Antalya)

Table 1. Morphometric measurements of *Torpedo marmorata*

Date	February, 21-2026	February, 21-2026
Sex	**Male**	**Male**
Weight	96.07 g	96.74 g
Total length	16.1 cm	16.7 cm
Disc length	95.85 mm	96.84 mm
Disc width	108.84 mm	111.83 mm
Pre second dorsal length	120.99 mm	127.29 mm
Pre first dorsal length	109.37 mm	109.74 mm
Prebranchial length	36.30 mm	38.87 mm
Preorbital length	15.60 mm	17.41 mm
Eye length	3.60 mm	3.96 mm
Eye height	5.57 mm	5.61 mm
Intergill length	20.31 mm	21.35 mm
Mouth width	9.00 mm	9.97 mm
Base length of first dorsal fin	10.41 mm	10.61 mm

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Base length of second dorsal fin	8.62 mm	8.89 mm
Clasper inner length	5.59 mm	6.25 mm
Clasper outher length	13.44 mm	14.33 mm

The measurements of our samples indicate that the captured individuals were juveniles or newborns. Mellinger (1971) reported that the total length of the marbled electric ray at birth ranges between 10 and 14 cm. However, Abdel-Aziz (1994) reported the mean embryo size for *T. marmorata* as 8.5 cm TL (range: 5.8–10.1 cm TL). Based on the measurements of our samples, it is possible to say that they reproduced very recently. This study reports, for the first time, the accidental capture of a juvenile marbled electric ray off the coast of Alanya (Gulf of Antalya, Turkey). The presence of both adult and newborn individuals in the region indicates that this area has the potential to serve as a breeding or nursery ground for the species in question.

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Ethics Approval
No need to ethical approval for this study.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
The data used in this study can be made available upon request to the corresponding author.

Author Contribution
The authors conceived and designed the study, conducted the fieldwork, collected and prepared the figures, wrote the manuscript, and approved the final version of the manuscript.

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The Role of Ship Stability in Maritime Accidents: A Literature Review

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Abstract

Maritime transportation is one of the most important components of global trade, and ensuring maritime safety is critical to the safe and uninterrupted continuation of international commerce. In this context, ship stability is a fundamental element of maritime safety. Traditional intact stability criteria do not always adequately address dynamic stability problems under realistic operational and environmental conditions. Therefore, the International Maritime Organization (IMO) developed the Second Generation Intact Stability Criteria (SGISC) to assess dynamic stability risks more effectively. This literature review examines the theoretical foundations of ship stability, IMO intact stability criteria, environmental factors, human factors, and bridge simulator training to evaluate the role of ship stability in maritime accidents. It also addresses the evolution of intact stability criteria and the development of SGISC in response to maritime accidents and incidents. Furthermore, it evaluates the role of stability-related deficiencies in maritime accidents by examining common accident types, their causes, and accident investigation reports. The effects of extreme weather events and climate change on ship stability are also examined, with particular emphasis on the increasing risks associated with severe waves, strong winds, and changing sea conditions. Human factors also play an important role in stability-related accidents. Decision-making errors, improper cargo loading, ballast management, and operational deficiencies are significant risk factors. In addition, the role of bridge simulator training in improving ship stability awareness and decision-making skills among maritime students and officer cadets is examined. The study aims to highlight the importance of effective safety strategies and training practices in reducing stability-related accidents.

Keywords: Ship stability, Intact Stability Criteria, Maritime safety, Bridge simulator training, risk factors

Introduction

The maritime industry has historically been one of the key components of international trade and has played a significant role in the global economy. More than 80% of world merchandise

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trade by volume and over 90% by value is transported by sea, highlighting the indispensable importance of the maritime sector in sustaining global commerce (Amuka et al., 2021).

The maritime industry therefore plays a critical role not only in the physical transportation of goods but also in the economic, technological, and legal dimensions of international trade. This foundational role makes the safety and reliability of maritime operations a matter of global priority.

Ensuring an adequate level of safety in terms of ship stability does not depend solely on structural measures implemented at the design stage. Design-based approaches should be complemented by operational measures, while the knowledge, skills, experience, and decision-making capabilities of masters and crew also play an important role in ship safety. Particularly in hazardous dynamic stability phenomena that may arise under the effects of wind and waves, appropriate operational risk control measures and ship-specific operational guidance can make a significant contribution to reducing risks. Furthermore, the inability of existing intact stability criteria to adequately address certain large-amplitude dynamic motions highlights the need for more comprehensive assessment of ship behavior under actual sea conditions. These limitations constitute one of the main rationales for the development of the Second Generation Intact Stability Criteria (SGISC), which take dynamic stability phenomena into account (Bačkalov et al., 2016).

The loss of life, environmental damage, and economic losses caused by maritime accidents demand the continuous improvement of safety measures. Ships must comply with applicable stability criteria established by the International Maritime Organization (IMO) to maintain safe navigation and operations (International Maritime Organization [IMO], 2008).

Ship accidents may result from a convergence of multiple factors, technical failures, and adverse weather conditions. Critically, stability problems can interact with and amplify these contributing factors, significantly increasing the risk of catastrophic outcomes (Latuheru, 2021). Understanding this interplay between stability and other risk factors is essential for both ship design and operational safety management.

This study examines the role of ship stability in maritime accidents based on the literature, taking into account the limitations of existing criteria, human factors, and the importance of bridge simulator training.

Material and Methods

This study presents a literature review examining the role of ship stability in maritime accidents. Relevant academic studies, international regulations, and technical documents were reviewed, covering intact and damage stability, dynamic stability phenomena, the Second Generation Intact Stability Criteria (SGISC), human factors, the development and updating of stability criteria in light of extreme weather events associated with climate change and changing sea conditions, numerical methods, and the role of simulator-based training in enhancing the ability to make decisions and reducing errors arising from human factors. The findings were evaluated to identify key risk areas affecting ship stability and the need for further refinement and updating of existing stability criteria.

Results

Development of IMO Intact Stability Criteria

The IMO's intact stability criteria have evolved significantly over decades, progressively incorporating more comprehensive assessments of ship safety under both static and dynamic conditions.

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The historical development of IMO intact stability criteria (Table 1) demonstrates a gradual transition from primarily static stability requirements toward more comprehensive approaches that also consider the dynamic behavior of ships under realistic sea conditions (Bačkalov et al., 2016). This evolution provides an important framework for understanding the fundamental principles and parameters of ship stability discussed in the following section.

Table 1. Historical Development of IMO Intact Stability Criteria

Year/Period	Development	Main Purpose/Content
1993	Intact Stability Code – Resolution A.749(18)	Established fundamental intact stability principles, including GM, GZ, weather criterion, free-surface effects, icing, and watertight integrity.
1998	Amendments – Resolution MSC.75(69)	Updated and further developed the existing intact stability provisions.
2008	IS Code – Resolution MSC.267(85)	Consolidated mandatory requirements and recommended provisions on intact stability into a more comprehensive framework.
2010	Entry into force of the 2008 IS Code	Relevant mandatory provisions under SOLAS and the 1988 Load Lines Protocol entered into force on 1 July 2010.
2000s-2020	Development of Second Generation Intact Stability Criteria (SGISC)	Developed a more advanced approach for assessing dynamic stability failure modes of ships in waves.
2020	(SGISC) Interim Guidelines	Introduced performance-based assessment of five dynamic stability failure modes, including vulnerability criteria, direct stability assessment, and operational measures.

Fundamentals of Ship Stability

Ship stability refers to a vessel's ability to resist dangerous conditions such as capsizing or excessive heeling and to return to its equilibrium position after being disturbed (Olsen, 2022). A thorough assessment of ship stability requires consideration of hydrostatic characteristics as well as wave effects and dynamic loads under actual sea conditions (Moore, 2010).

The geometric relationship (see Figure 1) between the center of buoyancy, the center of gravity, and the metacenter determines a ship's restoring torque at any given angle of heel (Chen et al., 2019). A ship in static equilibrium has its weight and buoyancy forces balanced, with the metacentric height (GM) governing the initial restoring moment and resistance to small angles of inclination. Under real sea conditions, dynamic forces from waves, wind, and loading changes continuously challenge a vessel's stability, making hydrodynamic assessment equally critical.

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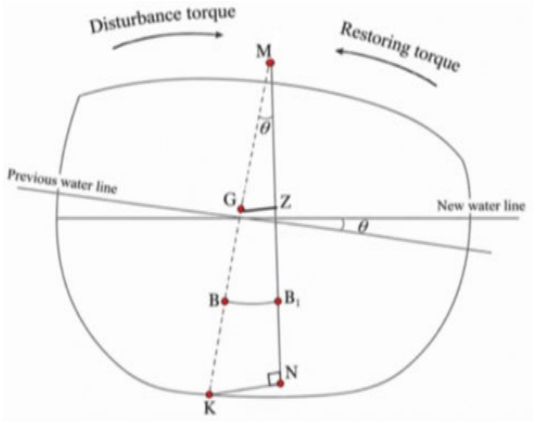


Figure 1. Geometric Relationship Among Buoyancy, Gravity, and Restoring Torque (Chen et al., 2019).

In addition, ship stability is influenced by various hydrostatic parameters as well as operational and environmental factors. In the assessment of intact stability, the ship's structural characteristics, loading conditions, and environmental effects are considered together (Moore, 2010). These parameters and influencing factors are briefly presented in Table 2.

Table 2. Intact Stability Parameters and Influencing Factors

HYDROSTATIC STABILITY PARAMETERS	
GM - Metacentric Height	A fundamental indicator of initial transverse stability.
KG- Height of the Center of Gravity	An increase in KG generally reduces stability.
KB- Height of the Center of Buoyancy	The vertical distance of the center of buoyancy from the keel.
BM - Metacentric Radius	Related to ship geometry and waterplane characteristics.
KN Curves	Provide geometric stability information at different heel angles.
GZ- Righting Arm	Represents the ship's ability to return to the upright position.
OPERATIONAL/ ENVIRONMENTAL EFFECTS	
Load Distribution	Affects the center of gravity and ship stability.
Ballast Management	Affects the center of gravity, trim, and stability.
Free Surface Effect	May reduce the effective GM.
Icing	May raise KG and consequently reduce stability.

Intact stability assessment considers multiple categories of parameters within a comprehensive framework, including: *Structural characteristics:* hull geometry, freeboard, and deck openings
Loading conditions: cargo distribution, ballast, and free surfaces
Environmental effects: wind, wave height, and sea state
Operational factors: speed, course, and dynamic loading changes

Accurate and rapid acquisition of hydrostatic data is essential for ship stability calculations. Traditionally, stability assessments relied on stability booklets and manual calculations, which were time-consuming and prone to human error. Today, Excel-based VBA and MATLAB-based tools enable fast and reliable computation of hydrostatic data, KN, GZ, and GM values

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under varying loading conditions, directly supporting ship masters' decision-making (Shakeel et al., 2022; Abbas et al., 2021; Shahzad et al., 2021).

The GZ Curve and Weather Criterion

Figure 2 shows the GZ curve and weather criterion. The GZ curve represents the ship's righting lever at different angles of heel in accordance with IMO intact stability criteria. Key parameters include the angle of maximum GZ, the range of positive stability, and the area under the curve, all subject to minimum IMO thresholds (IMO, 2008).

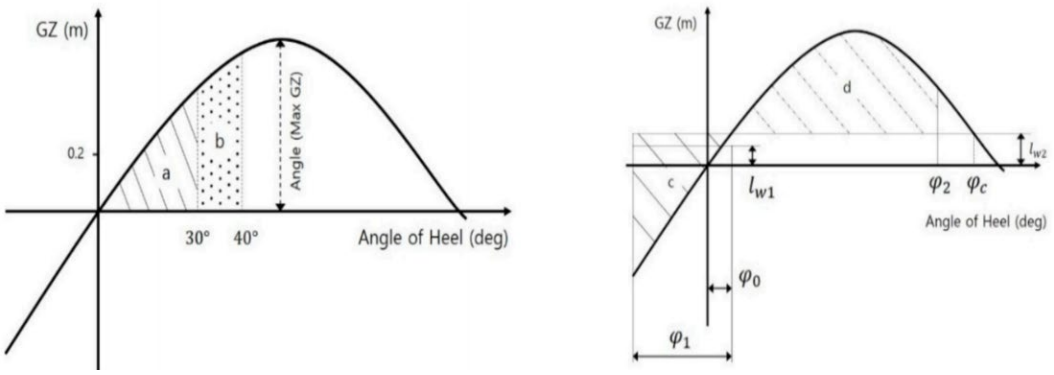


Figure 2. GZ curve (left) and weather criterion (right)

The weather criterion evaluates a ship's stability under combined wind and wave action. Under gust wind conditions, the energy area "d" (restoring) must be greater than area "c" (heeling), ensuring the ship can withstand dynamic environmental forces without capsizing (Woo et al., 2021).

Metacentric Height (GM) and Dynamic Stability

Metacentric height (GM) is a fundamental parameter in determining both the static and dynamic stability of a ship, and its accurate calculation and interpretation are crucial for ship safety and require an interdisciplinary approach.

Ferreiro (2006) demonstrates that the concept of the metacenter developed through the combined contributions of mathematics, physics, and engineering, forming the basis of modern GM calculation methods. Similarly, Lester (1985) examines the effects of GM, the position of the center of gravity, and liquid movements on ship stability from both theoretical and practical perspectives.

Second Generation Intact Stability Criteria (SGISC)

The IMO's Second Generation Intact Stability Criteria (SGISC) were developed to address situations in which existing static rules prove insufficient, providing a more comprehensive assessment of ships' dynamic behavior under wave action (Bulian & Francescutto, 2013). The development of SGISC was driven by the analysis of past maritime accidents that exposed the inadequacy of existing criteria for new-generation ship designs (Begović et al., 2019). Extreme weather events resulting from the effects of the climate crisis and the emergence of high-speed ship forms require the criteria and standards used in the assessment of ship stability to be continuously developed (Taniguchi, 2019; Rosano et al., 2020). In this context, the IMO's Second Generation Intact Stability Criteria are summarized in Table 3.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Table 3. IMO Second Generation Intact Stability Criteria (SGISC)

Failure Mode	Description
Dead Ship Condition	A critical condition in which the ship loses its own propulsion and becomes vulnerable to external effects (Shin & Moon, 2024).
Excessive Acceleration	Sudden and high accelerations induced by wave action, resulting in structural and operational risks (Petacco et al., 2023).
Parametric Roll	May cause sudden and large-amplitude roll motions depending on the wave period (Somayajula & Falzarano, 2019).
Pure Loss of Stability	Occurs when the buoyancy forces that maintain the ship's stability become insufficient (Somayajula & Falzarano, 2019).
Surf-Riding / Broaching-to	Loss of course-keeping ability in following or quartering waves, potentially leading to sudden course changes, excessive roll, and increased risk of capsizing (Spyrou, 1996).

Numerical and Computational Methods in Stability Assessment

The development of the Second Generation Intact Stability Criteria (SGISC) is supported by experimental studies, numerical analyses, and simulation methods (Zhou et al., 2018). As calculation methods become more advanced and additional influencing factors are incorporated, stability assessment becomes more comprehensive, thereby strengthening maritime safety measures (Trimulyono et al., 2023).

Examples of Numerical and Computational Methods: *Statistical and Probabilistic Methods:* Risk quantification across variable sea states and loading scenarios (Piazzola et al., 2020). *Monte Carlo Simulations:* Stochastic modeling of complex, multi-factor stability scenarios (Hu et al., 2019). *Spectral Analysis of Roll Motions:* Frequency-domain characterization of ship response to irregular waves (Moideen et al., 2014). *CFD (Computational Fluid Dynamics) and Time-Domain Simulations:* High-fidelity modeling of fluid–structure interaction and dynamic stability behavior (Visonneau et al., 2022; Zhuang & Wan, 2019). As calculation methods grow more sophisticated, they enable more accurate prediction of stability failure modes that static criteria cannot capture.

Damage Stability

Damage stability refers to a ship’s ability to survive without capsizing or sinking after flooding caused by hull damage. Its assessment considers flooding, watertight subdivision, residual stability, and the ship’s survivability after damage. Both deterministic and probabilistic approaches are used, with probabilistic damage stability forming an important part of the SOLAS framework (Dodman, 2011; Bulian et al., 2019).

As shown in Figure 3, A represents the attained subdivision index, reflecting the ship’s survivability after damage when different damage scenarios are considered, whereas R represents the minimum required subdivision index specified by SOLAS. The criterion, expressed by the IMO as $A \geq R$, is satisfied when the attained index A is equal to or greater than the required index R (IMO, 2020; Kawahara et al., 2011).

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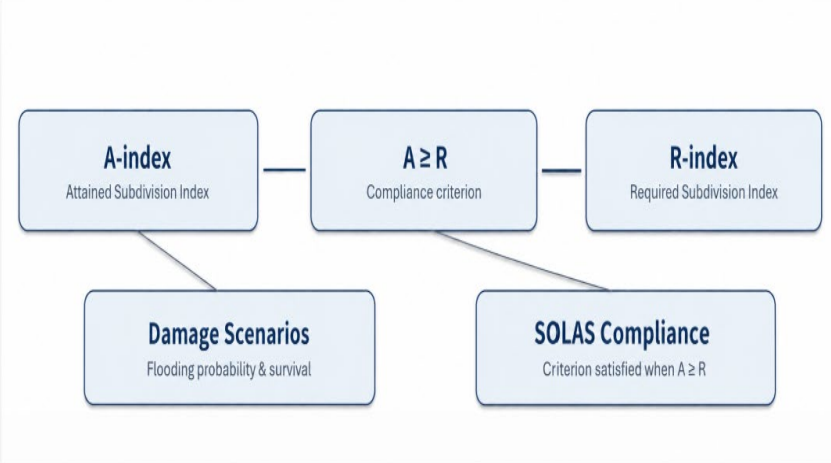


Figure 3. SOLAS Probabilistic Damage Stability Assessment.

Both intact and damage stability are fundamental to ship safety. However, stability-related maritime accidents demonstrate that compliance with static stability requirements alone may not always be sufficient under complex operational and environmental conditions (Begovic et al., 2018).

Stability-Related Maritime Accidents: Case Studies

Stability-related maritime accidents may result in severe consequences such as excessive heel, loss of control, capsizing, and sinking (Andrews, 2021). These accidents can arise from the interaction of hydrostatic and hydrodynamic factors, environmental conditions, ship characteristics, flooding, operational factors, and human factors (Khan & Yang, 2026). Understanding these mechanisms is essential for identifying stability risks and improving maritime safety (Latuheru, 2021).

These case studies are presented according to the main contributing factors involved in the accidents, including ship characteristics, stability conditions, environmental influences, operational factors, and human factors.

Case 1: MV Golden Ray Capsizing (2019),

Incorrect ballast quantities were entered into the stability calculation program, resulting in an incorrect stability assessment and insufficient righting arm (GZ) to counteract the heeling forces during the turn. Inadequate procedures for verifying stability calculations also contributed to the accident (National Transportation Safety Board [NTSB], 2021).



Figure 4. Capsizing of the MV Golden Ray, 2019.

Case 2: Herald of Free Enterprise Capsizing (1987)

The vessel departed Zeebrugge with its bow doors open, allowing large amounts of seawater to enter the vehicle deck. The resulting flooding and free-surface effect caused a rapid loss of stability and capsizing. Human and organizational failures also contributed significantly to the accident (Great Britain, Department of Transport, 1987).

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Case 3: SS Eastland Capsizing (1915)

The SS Eastland capsized while moored in the Chicago River with approximately 2,500 passengers on board. The accident was associated with inadequate stability under loading conditions, influenced by passenger loading, ballast management, and the vessel's design characteristics. The disaster resulted in 844 fatalities (U.S. Congress, House, Committee on the Merchant Marine and Fisheries, 1916).



Figure 5. People stand atop the capsized Eastland steamer in the Chicago River on July 24, 1915. Image: Chicago History Museum, ICHi-040126; Jun Fujita, photographer.

Case 4: m/t Cyranka Capsizing (1956)

The Polish fishing vessel m/t Cyranka capsized in the North Sea under severe following-sea conditions. The accident was associated with dynamic stability loss related to surf-riding and broaching-to phenomena. Subsequent analysis using the IMO Second Generation Intact Stability Criteria (SGISC) identified the vessel as vulnerable to this dynamic stability failure mode (Szozda & Krata, 2022).

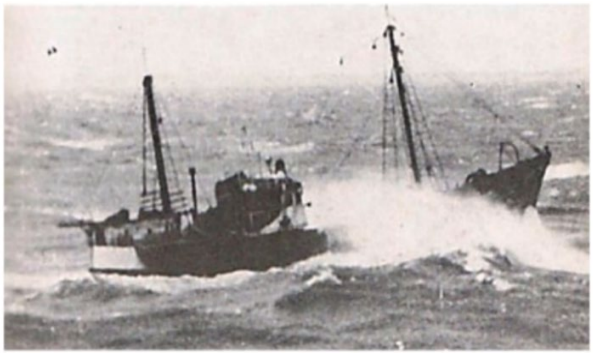


Figure 6. m/t Cyranka, the Motor Trawler That Capsized in 1956. Note. From Szozda and Krata (2022). Original graphic available under a public domain license.

Impacts of Extreme Weather Events and Climate Change

Even before the effects of extreme weather conditions are considered, defects in a ship's hull construction can adversely affect ship stability; however, such deficiencies are largely addressed during the design and construction stages (Ma et al., 2021; Dafermos & Zaraphonitis, 2024).

Extreme weather events associated with climate change expose ship operations to increasingly challenging conditions, including high waves, strong winds, and rapidly changing sea states (Knutson et al., 2021). These environmental conditions can increase roll motions, contribute to parametric rolling and dynamic loads, and increase the risk of loss of stability and capsizing (Mikulić & Parunov, 2023; Peşman et al., 2015). As ships are exposed to increasingly complex

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environmental conditions, stability assessments should consider not only hydrostatic characteristics but also hydrodynamic effects. Therefore, ship stability assessment methods and IMO stability criteria should be continuously reviewed, developed, and updated to address dynamic stability phenomena and emerging operational challenges.

Human Factors Affecting Ship Stability and Simulator Training

Although the technical, operational, and environmental challenges encountered by ships may arise from different sources, their safe management largely depends on the human factor. In a study of ship sinking accidents in Indonesian waters, human error was identified as a contributing factor in 70.5% of the accidents (Waskito et al., 2025). Therefore, particular emphasis should be placed on the human factor and, especially, on the training of seafarers to enhance maritime safety.

Training in stability-related operations and emergency response helps seafarers develop the knowledge and competencies required to respond effectively to challenging situations (Chae et al., 2021). Simulator-based training can further support the development of these skills by providing realistic training environments (de Oliveira et al., 2022).

Simulator-based training contributes to the development of seafarers' situational awareness, decision-making, risk assessment, and ability to cope with challenging situations by allowing them to practice in scenarios that resemble real operational conditions (Bokau et al., 2025). As digitalization and automation continue to make maritime operations more complex, maritime education and training must continuously evolve. In this context, simulator-based training plays a key role in enhancing stability awareness and decision-making skills, while preparing future seafarers for increasingly complex operational challenges (Demirel, 2020; de Oliveira et al., 2022).



Figure 7. Full-Mission Ship Bridge Simulator at Gdynia Maritime University. Note. Photograph by Ciacho5 (2017), Wikimedia Commons, CC BY-SA 4.0.

Conclusion

This literature review demonstrates that ship stability is a fundamental component of maritime safety and that conventional static stability criteria alone may not always be sufficient to address the complex operational and environmental conditions encountered by ships. The development of the IMO Second Generation Intact Stability Criteria (SGISC) represents an important step toward incorporating dynamic stability phenomena into ship stability assessment. The accident cases reviewed in this study further demonstrate that stability-related accidents may result from the interaction of technical, environmental, operational, and human factors.

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Extreme weather conditions and increasingly complex sea states further emphasize the need to consider hydrodynamic effects alongside hydrostatic characteristics in stability assessments. At the same time, human factors remain critical to the safe management of stability-related risks, highlighting the importance of effective maritime education and simulator-based training in improving situational awareness, decision-making, and emergency-response capabilities.

Future research should focus on integrating technological advances, simulator-based training, and human-factor approaches into ship stability education to address emerging operational challenges and enhance maritime safety.

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Ethics Approval

No ethical approval was required for this study, as it did not involve human participants, animals, or personal data.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. All authors have approved the manuscript for publication.

Data Availability

No datasets were generated or analyzed during the current study.

Author Contribution

Özlem DEVECİ: Conceptualization, writing—original draft. Şahnaz TİĞREK: Conceptualization, writing—review and editing.

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A Review of Türkiye's Offshore Renewable Energy Potential

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Abstract

Türkiye's long coastlines along the Black Sea, the Sea of Marmara, the Aegean Sea, and the Mediterranean Sea provide diverse opportunities for offshore renewable energy development. However, the available studies are fragmented across individual technologies, regions, and assessment criteria, limiting an integrated understanding of national priorities. This study therefore presents a systematic review and comparative assessment of Türkiye's offshore wind, wave, and hybrid wind wave energy opportunities. The existing literature along with major institutional reports are examined with respect to resource availability, water depth, distance to shore and grid infrastructure, technology maturity, foundation requirements, environmental constraints, competing maritime uses, and implementation barriers. The evidence is organized within a four-sea comparison framework to identify regional strengths, limitations, and research gaps. The preliminary synthesis indicates that offshore wind currently offers the strongest near to medium term development pathway, supported by substantial technical potential and proximity to major electricity demand centers. Nevertheless, deep waters in many promising areas increase the relevance of floating technologies and introduce additional engineering, cost, and supply chain challenges. Wave energy appears more spatially variable and generally less mature, but may provide complementary value in selected coastal areas and future hybrid systems. The review also highlights persistent gaps in long term measurement data, harmonized resource assessment, environmental impact evaluation, marine spatial planning, and integrated techno economic analysis. The study proposes a staged research and development roadmap for Türkiye, prioritizing site-specific measurements, pilot scale floating offshore wind projects, and coordinated assessment of wind wave hybrid opportunities. The findings provide a consolidated basis for future research, policy development, and sustainable blue economy investment.

Keywords: Offshore renewable energy, wind energy, wave energy

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Bibliometric Analysis of Microplastic Research in Freshwater Ecosystems: Global Trends, Scientific Collaborations, and Emerging Research Themes

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Abstract

Microplastic pollution has become a rapidly expanding research topic in rivers, lakes, reservoirs, and other inland-water systems. This study evaluates the contemporary scientific structure of freshwater microplastic research using bibliometric methods, with emphasis on publication growth, geographical production, international collaboration, and emerging research themes. Records were retrieved from the Web of Science Core Collection using the query TS=((microplastic*) AND (freshwater* OR river* OR lake* OR reservoir*) NOT (marine OR ocean OR sea OR coastal)) and analyzed with Biblioshiny/Bibliometrix. The current dataset contains 2,695 documents published in 368 sources and authored by 8,625 researchers; 2,450 records are articles, 240 are reviews, and 5 are classified as retracted publications. Annual scientific production increased from 207 documents in 2021 to 619 in 2025, while 570 documents were already indexed for 2026 in the partial-year dataset. China dominates national scientific production, whereas the collaboration map reveals dense research links connecting Asia, Europe, and North America. TreeMap and word-cloud analyses identify “microplastics”, “pollution”, “water”, “sediments”, “river”, “toxicity”, “nanoplastics”, and “ingestion” as prominent concepts. Keyword co-occurrence analysis reveals three major conceptual clusters. The thematic map positions “pollution–sediments–water” as a basic theme and “microplastics–environment–fate” as a highly developed central theme. Overall, the field is moving beyond occurrence and abundance surveys toward integrated questions concerning transport, fate, sediment interactions, nanoplastics, toxicity, and ecological risk. Future research should prioritize standardized monitoring protocols, long-term datasets, reliable nanoplastic characterization, and field-based ecotoxicological assessments.

Keywords: Microplastics, freshwater, bibliometric analysis, rivers, lakes, sediments, nanoplastics, scientific collaboration

Introduction

The global increase in plastic production and consumption has resulted in the continuous release of plastic waste into environmental systems, making microplastics one of the most pervasive groups of contaminants worldwide. Microplastics are generally defined as synthetic polymer particles smaller than 5 mm and may enter the environment either as primary particles intentionally manufactured at small sizes or as secondary particles generated through the physical, chemical, and biological degradation of larger plastic debris. The historical scale of

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plastic production and waste accumulation is a major factor underlying the long-term environmental significance of microplastic pollution (Geyer et al., 2017).

Although early microplastic research focused predominantly on marine environments, rivers, lakes, reservoirs, and other freshwater systems have increasingly been recognized both as receiving environments in which contaminants accumulate and as dynamic transport corridors that convey microplastics from terrestrial sources to downstream aquatic systems. Horton et al. (2017) highlighted substantial knowledge gaps concerning microplastics in freshwater and terrestrial environments, while subsequent studies demonstrated that riverbed sediments may serve as important repositories of microplastics and that flooding can remobilize previously deposited particles (Hurley et al., 2018).

Current evidence indicates that microplastics are widespread in freshwater environments and that reported abundances are strongly influenced by factors such as anthropogenic activities, sampling strategies, particle-size ranges, and identification techniques (Zhao et al., 2024). In particular, differences in sampling, extraction, and spectroscopic verification protocols substantially constrain comparability among studies. Koelmans et al. (2019) demonstrated that data quality in freshwater and drinking-water studies is highly dependent on methodological standardization. This challenge persists in recent assessments, in which standardized analytical protocols and transparent, comparable datasets continue to be identified as major research priorities (Zhao et al., 2024; Pastorino et al., 2026).

At the same time, the research focus has expanded beyond documenting the occurrence and abundance of microplastics toward understanding their environmental fate, sedimentation, resuspension, biological uptake, toxicity, and ecological risk mechanisms. Systematic reviews of freshwater environments have indicated that microplastics can accumulate in biological tissues and may act as carrier surfaces for other contaminants (Bhardwaj et al., 2024). Recent risk assessments further emphasize that particle morphology, polymer type, particle size, and environmentally realistic concentrations should be considered jointly when evaluating potential ecological risks (Bae et al., 2025; Morales-Espinoza et al., 2025). Nanoplastics and organism-level mechanisms of toxicity have also emerged as rapidly expanding research areas in recent years (Sha et al., 2025).

The rapid expansion of the literature has made it increasingly difficult to characterize the development of the field through conventional review approaches alone. Bibliometric analysis provides a quantitative framework for simultaneously evaluating the performance, social structure, and conceptual development of a research field through analyses of publications, authors, institutions, countries, and keyword relationships. The Bibliometrix/Biblioshiny framework integrates a range of approaches, including performance analysis, scientific collaboration mapping, co-occurrence network analysis, and thematic mapping (Aria & Cuccurullo, 2017). A recent bibliometric study analyzed 885 Web of Science (WoS) records on microplastic research in freshwater ecosystems published between 2013 and 2023 and identified China, Germany, and the United States as major centers of scientific production (Yildirim et al., 2024). Building upon this earlier assessment, the present study uses a larger and more up-to-date dataset to specifically examine the period of rapid growth between 2021 and 2026.

Accordingly, this study aims to provide an updated bibliometric overview of microplastic research in freshwater ecosystems by evaluating **(i)** temporal trends in annual scientific production, **(ii)** country-level scientific output, **(iii)** patterns of international scientific collaboration, **(iv)** dominant research concepts, **(v)** keyword-based conceptual clusters, and **(vi)** basic and emerging research themes. To achieve these objectives, seven complementary and

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readily interpretable visual outputs were selected. Among these, Word Cloud and TreeMap analyses were incorporated as key visual tools for directly representing the conceptual prominence and frequency of major research terms.

Material and Methods

Data Source and Search Strategy

The bibliometric dataset was retrieved from the **Web of Science Core Collection (WoSCC)**. The search strategy combined terms related to microplastics with terms representing freshwater environments. To minimize the inclusion of studies predominantly focused on marine environments, the terms *marine*, *ocean*, *sea*, and *coastal* were excluded from the search. The complete topic search query was as follows:

TS=((microplastic*) AND (freshwater* OR river* OR lake* OR reservoir*) NOT (marine OR ocean OR sea OR coastal))

According to the Biblioshiny report generated on 1 September 2026, the final dataset comprised 2,695 documents published across 368 sources and involved 8,625 authors. The dataset consisted of 2,450 research articles, 240 review articles, and five records classified as “article; retracted publication.” Temporal trends in scientific production were evaluated for the 2021–2026 period. Because 2026 represented an incomplete publication year at the time of data retrieval, the partial-year status of the 2026 data was explicitly considered when interpreting annual publication trends.

Data Quality and Bibliometric Analysis

According to the missing-data assessment performed in Biblioshiny, no missing records were identified for the fields corresponding to abstracts, authors, DOI, document type, source, language, publication year, title, or total citations. Only one record (0.04%) was missing from each of the institutional affiliation and corresponding-author fields. Author keywords were missing for 149 records (5.53%), whereas Keywords Plus data were unavailable for 204 records (7.57%). In contrast, the *cited references* and *science categories* fields were entirely absent from the Biblioshiny report. Consequently, co-citation and category-based analyses were not included among the principal analyses performed on the current dataset. This methodological decision was made to avoid drawing inferences from bibliographic fields that were not adequately represented in the source data.

Bibliometric analyses were conducted using the Bibliometrix/Biblioshiny framework (Aria & Cuccurullo, 2017). Among the range of analytical outputs available, seven complementary visualizations were selected on the basis of their interpretability and their suitability for presenting the scientific, collaborative, and conceptual structure of the research field: annual scientific production, country-level scientific production, country collaboration mapping, TreeMap analysis, Word Cloud analysis, keyword co-occurrence network analysis, and thematic mapping. TreeMap and Word Cloud analyses were used to visualize the relative frequency and prominence of major research concepts, whereas the keyword co-occurrence network was used to characterize relationships among concepts. The thematic map was used to evaluate the relative development and relevance of research themes according to the dimensions of density and centrality, respectively.

Basic Characteristics of the Dataset

Indicator	Value
Sources (Journal, books, etc.)	2.695

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Document analyzed	368
Authors	8.625
Articles	2.450
Review articles	240
Articles classified as retracted publications	5
Co- authors per document	6,15
International co-authorship	%29,2
Average citations per document	23,22
Authors Keywords(DE)	6.565
Keywords Plus (ID)	3.006

Results and Discussion

Annual Scientific Production

The annual scientific production analysis presented in Figure 1 indicates a strong and continuous growth trend in freshwater microplastic research. The number of publications rose from 207 in 2021 to 352 in 2022, 423 in 2023, 523 in 2024, and 619 in 2025. Accordingly, the annual number of publications approximately tripled between 2021 and 2025, corresponding to a compound annual growth rate of roughly 31.5%. The presence of 570 records for 2026, considering that the year is not yet complete, indicates that research interest remains at a high level.

This rapid growth is consistent with the increasingly recognized role of freshwater systems in transporting microplastics from terrestrial to aquatic environments. Zhao et al. (2024) noted that freshwater microplastic monitoring has expanded rapidly in recent years, although methodological heterogeneity remains a major issue. Pastorino et al. (2026) similarly emphasize that the current freshwater literature spans numerous subthemes, including occurrence, transport, fate, biological effects, modeling, and removal.

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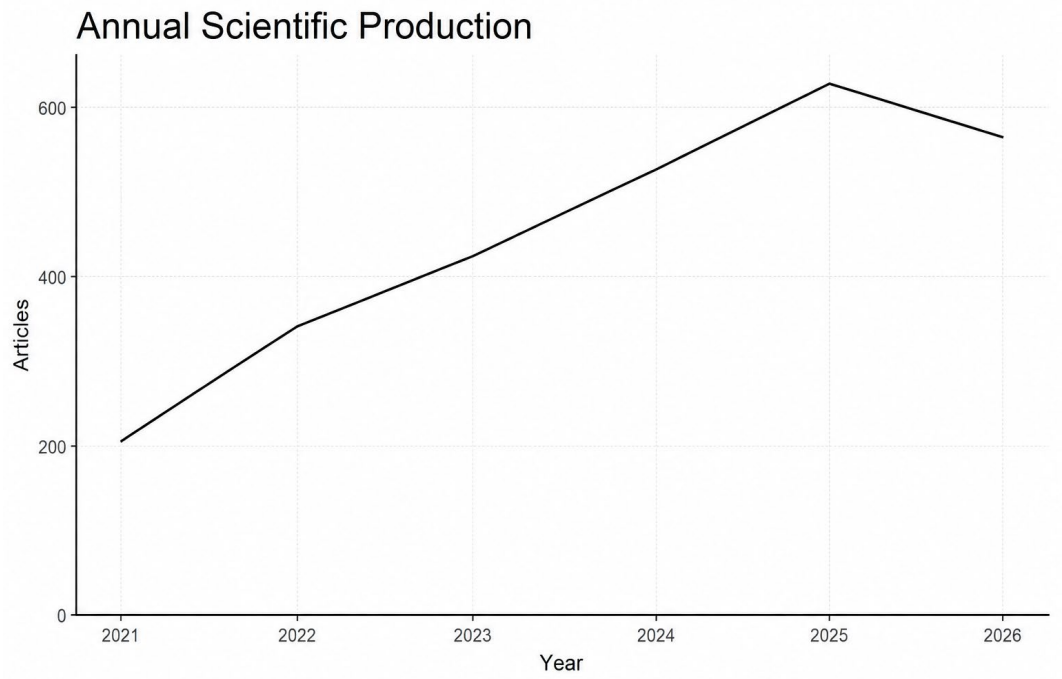


Figure 1. Annual scientific production of microplastic research in freshwater ecosystems.

Scientific Production by Country

The country-level scientific production map presented in Figure 2 shows that the literature is spread across a broad global geography, although production is not evenly distributed. China stands out distinctly as the most intensive production center, while the United States, India, and several European countries form a second strong production tier. Scientific output is also present in South America, Australia, and some African countries, although at lower intensity. This pattern is broadly consistent with earlier bibliometric assessments showing that China, Germany, and the United States are prominent in the freshwater microplastic literature (Yildirim et al., 2024).

Geographical differences in production may affect not only research capacity but also the representativeness of the freshwater systems being studied. Recent global lake assessments show that microplastic concentrations are influenced by local environmental variables such as human activities, shoreline urbanization, lake morphology, and trophic status (Jachimowicz et al., 2026; Liang et al., 2025). Increasing field studies in underrepresented regions would therefore reduce the geographical bias of global risk assessments.

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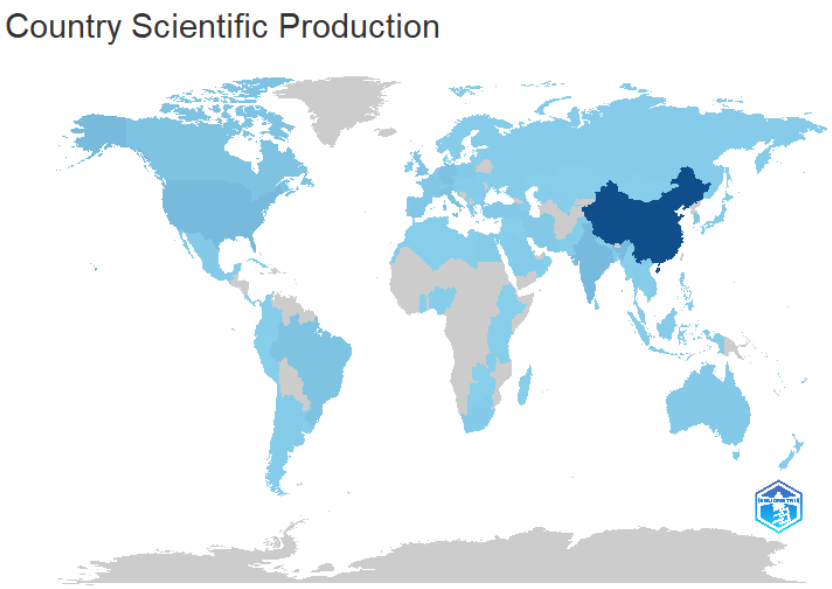


Figure 2. Global distribution of scientific production by country.

International Scientific Collaboration

The country collaboration map presented in Figure 3 shows that microplastic research has become strongly internationalized. The densest connections occur between the China-centered Asian network and Europe and North America, while the United States and Canada have numerous links with Europe and Asia, and Australia is particularly well connected to Asia and Europe. An international co-authorship rate of 29.2% in the dataset indicates that approximately one in every three publications involves international collaboration.

This network structure offers a substantial advantage for methodological standardization in microplastic research, since differences in sampling mesh size, minimum particle size, organic matter removal, polymer verification technique, and reporting units complicate cross-country comparisons (Koelmans et al., 2019; Zhao et al., 2024). International consortia and shared protocols could strengthen the generation of comparable data, particularly in river basins and large lake systems.

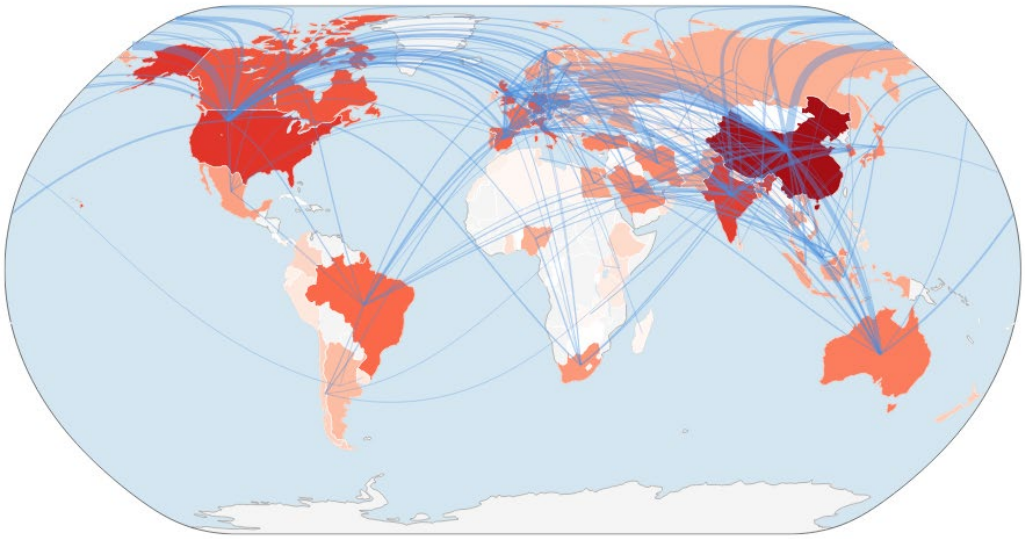


Figure 3. Network of international scientific collaboration.

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TreeMap: Dominant Research Concepts

The TreeMap analysis in Figure 4 directly illustrates the conceptual density of the field and its most frequently used research terms. The most frequent term is "microplastics," ranking clearly first with 1,437 occurrences and a share of approximately 16%. It is followed by "pollution" (578), "water" (360), "sediments" (347), "river" (277), "toxicity" (260), "microplastic" (258), "particles" (249), "ingestion" (215), and "nanoplastics" (208). "Exposure," "sediment," "environment," "abundance," "lake," "adsorption," "fate," "accumulation," "degradation," "fish," "identification," "freshwater," "transport," and "contamination" are also among the high-frequency concepts.

This distribution reveals three main research axes. The first axis concerns studies of environmental occurrence and distribution, represented by the terms water–river–lake–sediments. The second axis concerns biological effects and ecotoxicology research, represented by terms such as toxicity–ingestion–exposure–fish–oxidative stress. The third axis concerns environmental behavior and management research, represented by the terms adsorption–fate–transport–degradation–removal. The presence of "nanoplastics" among the top ten terms indicates a clear research shift toward smaller particle-size fractions, a trend that is also consistent with recent freshwater toxicity and risk literature (Bae et al., 2025; Sha et al., 2025). Furthermore, counting synonymous or formally different terms such as "microplastics/microplastic," "sediments/sediment," and "freshwater/fresh-water" separately illustrates the importance of keyword standardization. Merging these variants using a thesaurus in future advanced network analyses would enable a cleaner and more reliable interpretation of the conceptual clusters.

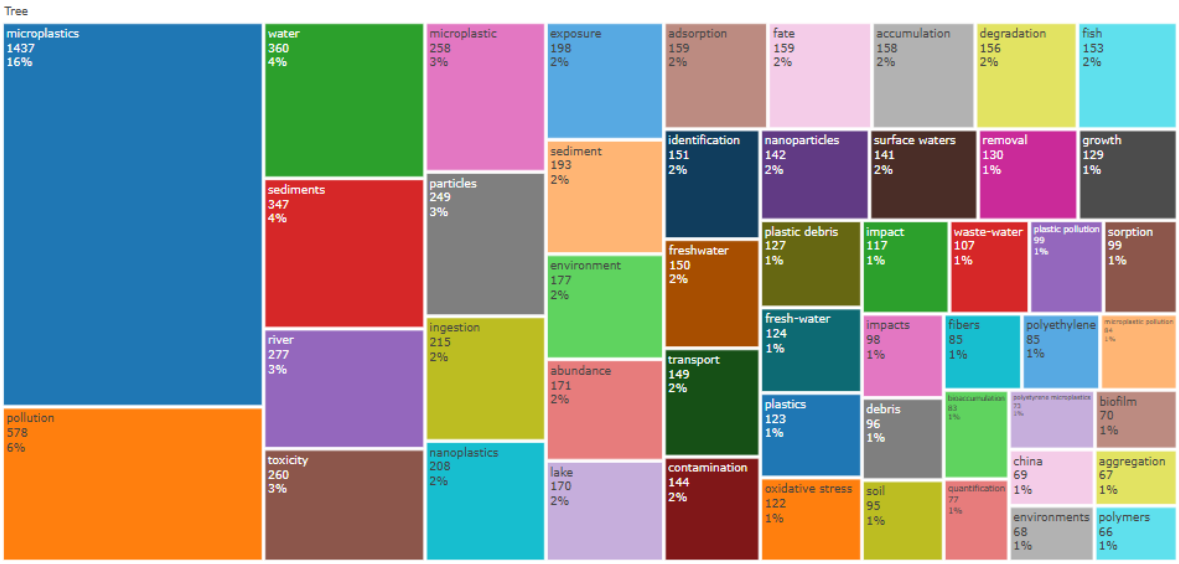


Figure 4. TreeMap representation of the most frequently used keywords.

Word Cloud: Conceptual Overview of the Field

The Word Cloud presented in Figure 5 visually supports the TreeMap findings and rapidly summarizes the dominant terminology of the research field. "Microplastics" is the most dominant concept, surrounded by a dense conceptual field formed by the terms "pollution," "water," "sediments," "river," "toxicity," "particles," "nanoplastics," "ingestion," "exposure," "lake," "fate," and "transport." Although the Word Cloud does not substitute for quantitative

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ranking, it is a powerful complementary visual for conference and full-text presentations, as it rapidly summarizes the field's main research vocabulary.

The co-occurrence of ecotoxicological terms with environmental distribution terms in the word cloud indicates that freshwater microplastic research is moving beyond the question of "where and how much microplastic is present?" toward questions of "through which processes is it transported, which organisms are exposed, and which biological outcomes result?" Recent reviews on freshwater fish and model organisms show that endpoints such as oxidative stress, growth, reproduction, organ function, and gene expression are being examined with increasing frequency (Sha et al., 2025).



Figure 5. Keyword cloud of the freshwater microplastic literature.

Keyword Co-occurrence Network

The keyword co-occurrence network presented in Figure 6 reveals three main clusters. The red cluster is concentrated around "microplastics" and "environment" and includes environmental-interaction concepts such as adsorption. The blue cluster is shaped around the concepts "pollution," "water," and "sediments," representing environmental occurrence, distribution, and water–sediment relationships. The green cluster highlights biological exposure and toxicological effects through the concepts "nanoplastics," "toxicity," and "ingestion." The numerous connections between the clusters indicate that these research subfields are not independent but are strongly integrated with one another.

In particular, the connections between sediment, the water column, and organism exposure reflect the dynamic nature of freshwater systems. The environmental counterpart of this network is formed by the capacity of floods to remobilize microplastics stored in riverbed sediments (Hurley et al., 2018), the role of hydrodynamics and human activities in shaping distribution in lakes (Liang et al., 2025), and the ability of particles to adsorb other contaminants (Bhardwaj et al., 2024).

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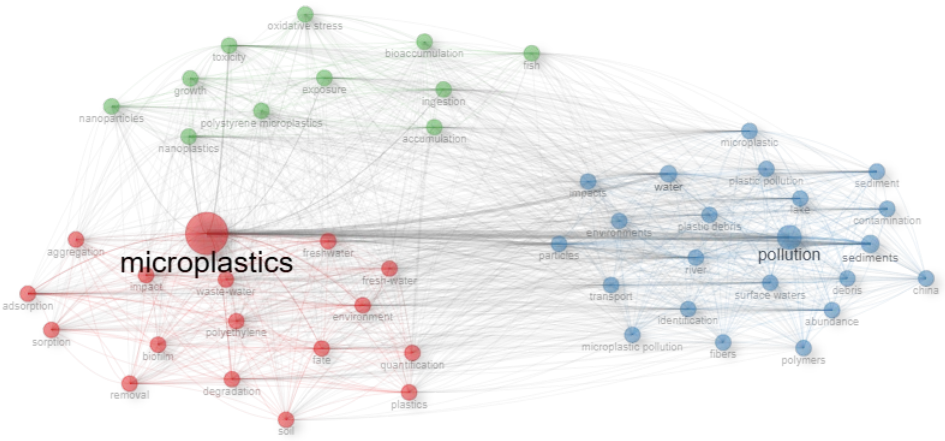


Figure 6. Keyword co-occurrence network and the three main conceptual clusters.

Thematic Map and Emerging Research Themes

The thematic map presented in Figure 7 evaluates the keyword clusters along the axes of centrality (relevance degree) and density (development degree). The "pollution–sediments–water" cluster is located in the Basic Themes region, with high centrality but relatively low density. This position indicates that water and sediment pollution studies form the foundation of the field and are connected to numerous subthemes. The "microplastics–environment–fate" cluster is positioned near the centrality boundary but at high density, indicating a well-developed theme that is connected to the field as a whole.

The "toxicity–ingestion–nanoplastics" cluster, in contrast, is positioned on the low-centrality side, close to the density threshold line. This suggests that the theme has strong specialization potential but is more limited in its connection to the field as a whole compared with the basic water-sediment pollution theme. Because the lower-left region of the thematic map is defined as "emerging or declining themes," it cannot be determined with certainty, on the basis of this single map alone, whether the toxicity–ingestion–nanoplastics theme is rising or declining; this would require a temporal thematic evolution analysis. Nevertheless, the recent increase in publications on nanoplastics and toxicity supports the possibility that this theme is an emerging research area (Morales-Espinoza et al., 2025; Sha et al., 2025).

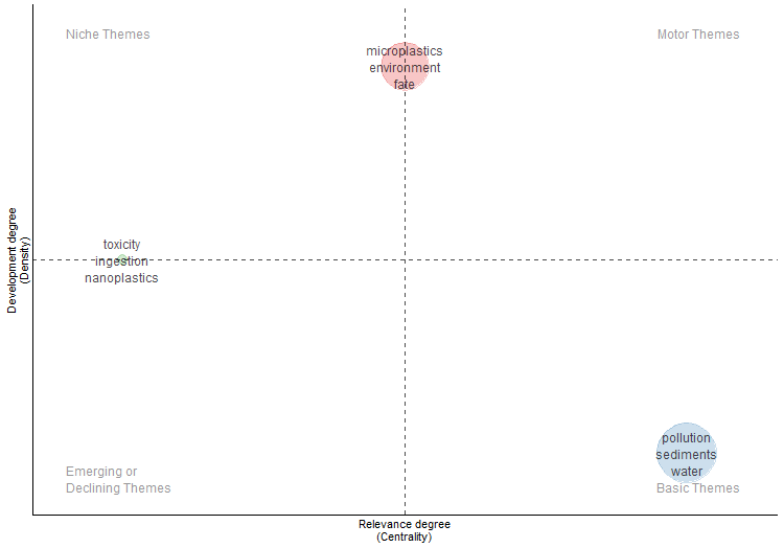


Figure 7. Keyword-based thematic map: centrality and development levels.

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Overall Assessment and Research Trends

When the seven bibliometric visualizations are considered together, freshwater microplastic research is seen to have accelerated markedly after 2021 and to have evolved into an increasingly mature, multidimensional research field. The strong increase in annual production, together with the geographical spread and intensity of international connections, indicates that microplastic pollution has shifted from a local environmental issue to a shared research agenda at the global scale.

The field still relies heavily on environmental occurrence and distribution studies along the "microplastics–pollution–water–sediments–river" axis. However, the high visibility of the concepts "toxicity," "ingestion," "exposure," "nanoplastics," "adsorption," "fate," "degradation," and "removal" indicates that research questions have moved toward a mechanistic level. This shift is also apparent in recent reviews: standardization and data comparability are emphasized in freshwater monitoring studies (Zhao et al., 2024), while risk assessments call for particle morphology and realistic environmental exposure conditions to be addressed more rigorously (Bae et al., 2025; Morales-Espinoza et al., 2025).

Sediments are central to this development. They can serve both as a long-term accumulation compartment for microplastics and as a secondary source during hydrodynamic events. River flooding has been shown to substantially redistribute the microplastic load stored in bed sediments (Hurley et al., 2018). In lakes, factors such as shoreline urbanization, water movement, trophic status, and morphology influence microplastic distribution (Jachimowicz et al., 2026; Liang et al., 2025). It would therefore be more meaningful for future monitoring to treat the water column and sediment not as separate compartments but as an interconnected system.

The appearance of nanoplastics among the top ten TreeMap terms, together with its co-occurrence with the toxicity–ingestion cluster, points to one of the most important research axes for the field going forward. However, the reliable sampling and characterization of nanoplastics remain more difficult than for microplastics. Emerging analytical methods must therefore not only detect smaller particles but also produce results that are comparable across different laboratories.

Although the international collaboration network is strong, the concentration of scientific production in a limited number of countries remains an important limitation. Because freshwater systems vary considerably in climate, land use, population density, waste management, and hydrological characteristics, standardized field research needs to be expanded in underrepresented regions such as Africa, South America, and parts of Asia. Such geographical expansion would strengthen the representativeness of global microplastic risk assessments.

Finally, the keyword variants present in the dataset indicate a need for conceptual standardization. Merging variants such as "microplastic/microplastics," "sediment/sediments," and "freshwater/fresh-water" using a thesaurus would allow future bibliometric analyses to more accurately reflect the network structure. Similarly, ensuring that critical fields such as cited references are fully captured in bibliographic exports is necessary for co-citation and intellectual-structure analyses to be performed reliably.

This study has characterized the current bibliometric structure of freshwater microplastic research based on 2,695 records retrieved from the Web of Science Core Collection. The rise in the annual number of publications from 207 in 2021 to 619 in 2025, together with 570 publications already reached in the incomplete portion of 2026, indicates that the field continues to exhibit a strong growth trend.

Although scientific production is widespread at the global scale, China stands out by far as the strongest production center. An international co-authorship rate of 29.2% and the dense

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connections among countries indicate that the field possesses a strong international research network. Nevertheless, geographical imbalances in representation persist.

Conceptual analyses have shown that the core backbone of microplastic research consists of the concepts "microplastics–pollution–water–sediments–river." The strong visibility of terms such as "toxicity," "ingestion," "nanoplastics," "exposure," "fate," "adsorption," "degradation," and "removal" reveals that research has moved beyond mere pollution detection toward a stage focused on processes, effects, and risk. The co-occurrence network shows strong connections among environmental distribution, water–sediment processes, and biological toxicity.

Based on these findings, future studies are recommended to focus on four priorities: (1) international standardization of sampling and polymer identification methods, (2) long-term, multi-compartment field studies that track the water column–sediment–biota linkage, (3) reliable analysis of nanoplastics and their ecotoxicological assessment at realistic environmental concentrations, and (4) the development of monitoring networks using shared protocols in underrepresented geographical regions. In this way, freshwater microplastic research can progress from measuring occurrence and abundance toward a more mature scientific framework that holistically explains the source–transport–fate–exposure–effect–risk–management chain.

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Research on Endemic Fish Species in the Orontes River: A Bibliometric Approach

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Abstract

The Orontes River is one of the most important rivers in the Middle East. It is also the second-richest basin in the Mediterranean region in terms of endemic freshwater fish species diversity. From a scientific perspective, access to all relevant data can facilitate conservation efforts in these basins. This study uses bibliometric methods to analyze research on the discovery of endemic fishes in the Orontes River, identifying key trends, influential publications, authors, institutions, and emerging research areas. Data were retrieved from the Web of Science database, covering original articles, reviews, books, and book chapters published between 1989 and 2025. After collecting relevant bibliographic and citation data, keywords, citation networks, and co-citations, the analysis was performed using VOSviewer software. A total of 90 articles were retrieved from the Web of Science database. The articles were cited a total of 2.424 times (2.441 times excluding self-citations). The highest number of citations occurred in 2025 with 233 citations in 3 publications. The Hirsch index is 25. Among 39 countries, the USA led in the number of published articles (n = 32). Türkiye ranked 5th with 6 articles. Out of a total of 12 research areas, the most active research area was "Zoology" (n = 52). The most prolific publisher was “Magnolia Press” (n = 17). The journal with the most published articles was “Zootaxa” (n=17). In conclusion, this bibliometric analysis provides a general overview of the region’s research history and contributes to our understanding of the various stress factors associated with global change, including endemic species diversity, the introduction of invasive species and habitat loss. Furthermore, the study offers valuable insights into identifying future research priorities, such as assessing the potential impact of these pressures on ecosystems, and developing effective national and international conservation policies.

Keywords: Bibliometric analysis, Orontes River, Endemic Fishes, conservation policies

Introduction

Endemic species, in particular, are vulnerable to environmental changes, both natural and anthropogenic, and face serious threats due to their limited geographic ranges and high dependence on specific environmental conditions. These species are threatened by a variety of factors, which often act in combination. The primary threats are drought, excessive water extraction, and habitat loss or degradation caused by pollution, eutrophication, sewage, and water infrastructure projects (Dudgeon et al., 2006; Kottelat & Freyhof 2007; Reid et al., 2019; Vári et al., 2022). In addition, introducing and spreading alien and invasive species into natural ecosystems, as well as the simultaneous or sequential occurrence of these threats, can further increase the risk of extinction by reducing the resilience of endemic species, particularly those

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with small, isolated populations, to environmental changes (Özcan, 2009; Bellard et al., 2016; Reid et al., 2019).

In addition, introducing and spreading alien and invasive species into natural ecosystems, as well as the simultaneous or sequential occurrence of these threats, can further increase the risk of extinction by reducing the resilience of endemic species, particularly those with small, isolated populations, to environmental changes (Balian et al., 2008; Reid et al., 2019; Xiong et al., 2023; Yousefi et al., 2024).

A total of 390 species of freshwater fish belonging to 37 families have been identified in Türkiye's inland waters. Of these, 367 are native species and 23 are naturalised non-native taxa. 202 of these species (55%) are endemic to Türkiye (Kaya et al., 2025). The Orontes River and its watershed have been recorded as home to more than 50 different species of fish (Krupp & Schneider, 1991; Fricke et al., 2007; Yalçın-Özdilek, 2007; Özcan, 2013). The Orontes River and its basin, which is located along the Mediterranean coast, is the second-richest in terms of endemic freshwater fish species diversity. It is second only to the Po River Basin in Italy, which has between 11 and 17 species (Smith & Darwall, 2006).

Bibliometric analysis provides a quantitative approach to examining scientific literature and understanding the evolution of research on endemic fish species in the Orontes River (Xie et al., 2021). It helps identify major research trends, themes, influential studies, and emerging areas of interest. This study aims to systematically analyze the global academic literature on endemic fish species in the Orontes River using bibliometric methods. Specifically, it identifies key research areas, shifts in research focus, and major contributors to the field.

Materials and Methods

Research Design and Search Methodology

A bibliographic (retrospective) review was conducted using the bibliometric analyses method proposed by Zhang et al. (2021). The Web of Science Core Collection (WoSCC) an electronic database widely used by researchers due to its provision of comprehensive, consistently exportable data was used to create the literature dataset. The WoSCC was selected as the primary data source for this study due to its long-standing reputation as a reliable source of biomedical research data, and because it provides a solid foundation for research. Particular emphasis was placed on its capacity to deliver extensive citation data.

Data Collection

The data search terms were "Endemic Fishes" AND "Orontes River" (Research on Endemic Fish in the Orontes River: A Bibliometric Approach). To eliminate bias and account for frequent updates to the WoSCC database, the literature search was performed on a single day (July 30, 2026). The research was conducted between 1989 and 2025.

Standards for Study Selection

A comprehensive search in WoSCC using the terms "Endemic Fishes" AND "Orontes River" (Research on Endemic Fish in the Orontes River: A Bibliometric Approach) resulted in the identification of a total of 100 studies. Subsequently, by selecting original articles from the document type tab, the search yielded 90 records.

Data collection

The primary data points were publication titles, publication years, names of journals in which the research was published, author names, keywords, names of the institutions to which the authors were affiliated and countries of origin and were extracted in plain text format. The analysis considered the total number of articles and total citations retrieved from the WoSCC

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database. WoS publications were saved as .txt files and subsequently exported to Microsoft Office Excel 2019 (Los Angeles, CA, USA) for further analysis.

Network analytics

In this bibliometric study, VOSviewer (version 1.6.10, Leiden University, Netherlands) was used to import data and identify trends, collaborations, and key findings. The software was also used to collect and analyze data related to authorship, links, keywords, citations, and thematic words. The program aided in the analysis of keywords, concurrent uses, citations, co-authorship, and co-citations.

Results

During the study period from 1989 to 2025, publications were produced in two languages. The most common language used was English (Table 1).

Table 1. The languages in which articles were published.

Languages	Record Count	% of 90
English	89	98,889%
Portuguese	1	1,111%

A total of 39 countries/regions have been published in the period from1989 to 2025. The ten countries with the most publications are shown in Table 2.

Table 2. Most productive countries

Countries/Regions	Record Count	% of 90
USA	32	35,556%
BRAZIL	15	16,667%
INDIA	7	7,778%
SWITZERLAND	6	6,667%
TÜRKİYE	6	6,667%
IRAN	5	5,556%
GERMANY	4	4,444%
SINGAPORE	4	4,444%
ARGENTINA	3	3,333%
MEXICO	3	3,333%

Over the course of the study, which ranged from 1989 to 2025, 12 independent research areas were discovered. Table 3 shows the top 10 most extensively researched areas.

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Table 3. Most active research areas

Research Areas	Record Count	% of 90
Zoology	52	57,778%
Marine Freshwater Biology	21	23,333%
Environmental Sciences Ecology	16	17,778%
Fisheries	9	10,000%
Evolutionary Biology	8	8,889%
Biodiversity Conservation	6	6,667%
Biochemistry Molecular Biology	4	4,444%
Life Sciences Biomedicine Other Topics	4	4,444%
Genetics Heredity	3	3,333%
Water Resources	3	3,333%

Showing first 10 out entries

In the years between 1989 and 2025, a total of 194 affiliations have been published. Table 4 presents the affiliations of the ten journals with the highest number of publications.

Table 4. Most prolific affiliations

Affiliations	Record Count	% of 90
SAINT LOUIS UNIVERSITY	7	7,778%
PONTIFICIA UNIVERSIDADE CATOLICA DO RIO GRANDE DO SUL	5	5,556%
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO	5	5,556%
NATIONAL UNIVERSITY OF SINGAPORE	4	4,444%
RECEP TAYYIP ERDOGAN UNIVERSITY	4	4,444%
AUBURN UNIVERSITY	3	3,333%
AUBURN UNIVERSITY SYSTEM	3	3,333%
CONSEJO NACIONAL DE INVESTIGACIONES CIENTIFICAS Y TECNICAS CONICET	3	3,333%
LEIBNIZ INSTITUT FUR EVOLUTIONS UND BIODIVERSITATS FORSCHUNG	3	3,333%
NATIONAL UNIVERSITY OF LA PLATA	3	3,333%

Between 1989 and 2025, ten entries were detected, representing 29 different publishers. Table 5 lists the most prolific publishers.

Table 5. Most prolific publishers

Publishers	Record Count	% of 90
Magnolia Press	17	18,889%
Wiley	16	17,778%
Pensoft Publishers	7	7,778%
Springer Nature	6	6,667%
Elsevier	5	5,556%
Soc Brasileira Ictiologia	4	4,444%
Verlag Dr Friedrich Pfeil	4	4,444%
Amer Soc Ichthyologists & Herpetologists	3	3,333%
PLEIADES PUBLISHING LTD	3	3,333%
AMER SOC ICHTHYOLOGISTS HERPETOLOGISTS	2	2,222%

Showing first 10 outentries

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A total of 45 journal articles have been published on this topic. Table 6 includes the top 10 journals with the most publications.

Table 6. Journals publishing the most articles

Journals	Record Count	% of 90
ZOOTAXA	17	18,889%
JOURNAL OF FISH BIOLOGY	6	6,667%
COPEIA	4	4,444%
ICHTHYOLOGICAL EXPLORATION OF FRESHWATERS	4	4,444%
NEOTROPICAL ICHTHYOLOGY	4	4,444%
ZOOKEYS	4	4,444%
ZOOSYSTEMATICS AND EVOLUTION	4	4,444%
MOLECULAR PHYLOGENETICS AND EVOLUTION	3	3,333%
RAFFLES BULLETIN OF ZOOLOGY	3	3,333%
AQUATIC CONSERVATION MARINE AND FRESHWATER ECOSYSTEMS	2	2,222%

Showing 10 out of entries

The evaluated articles generated a total of 2.484 citations (2.441 of which were self-citations), with an average h-index of 25. The highest number of citations (233) and publications (3) were found in 2025 (Figure 1).

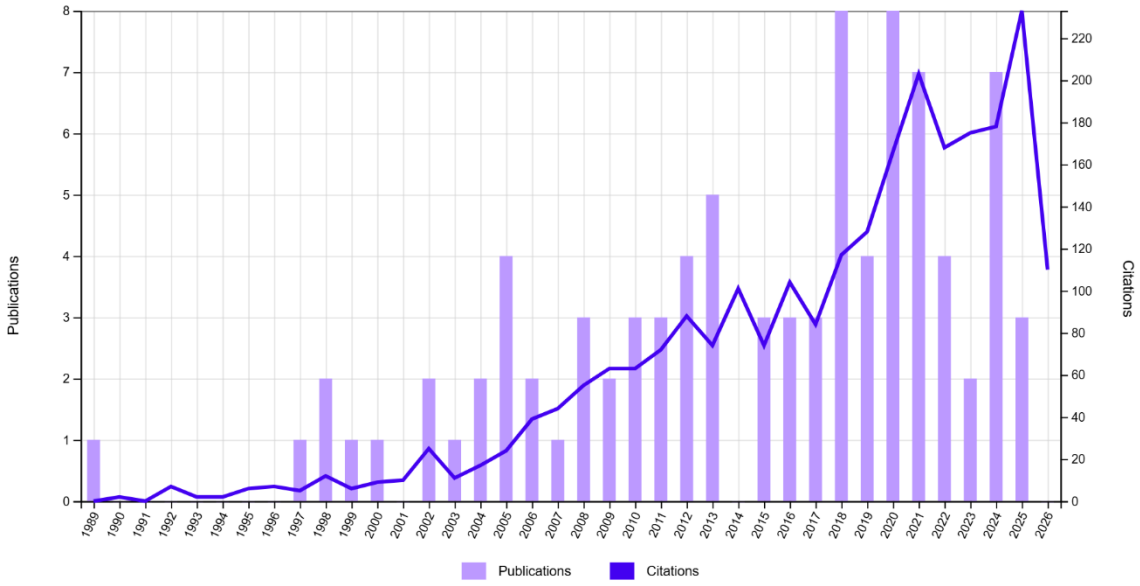


Figure 1. Publication and citation frequency

As shown in Figure 2, our bibliometric study has revealed the most frequently used keywords. It was found that the font size of the keywords was closely proportional to the frequency with which they were repeated in the articles.

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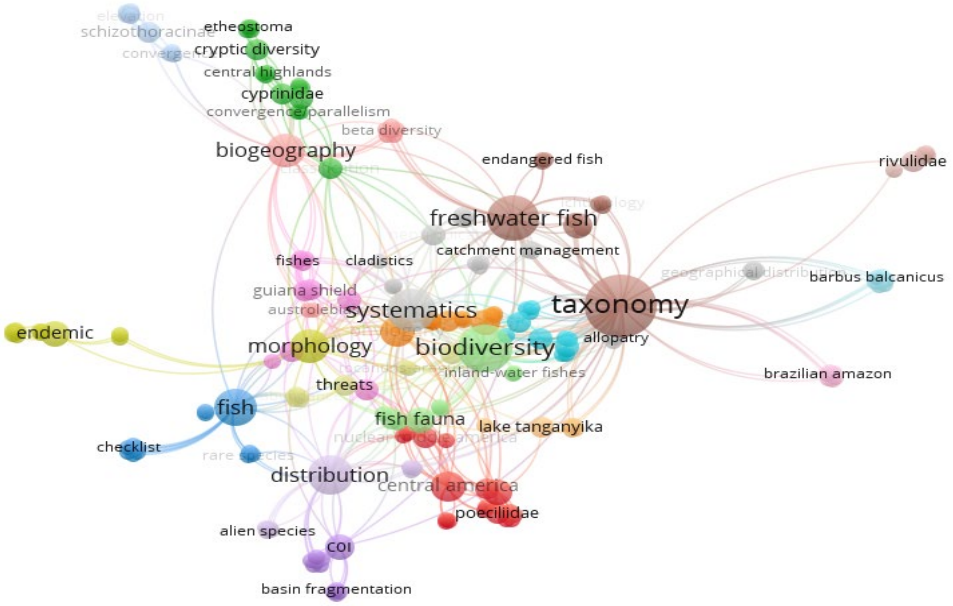


Figure 2. KeyWord visualization map of articles

The size of the bubbles was proportional to the amount of research done in each country in the network representation of bibliographic coupling between nations. The width of the lines connecting the countries indicates the degree to which they are linked, while the colours of the lines indicate the cluster to which each country is assigned. For this study, we have included at least one document and one citation from each country (Figure 3).

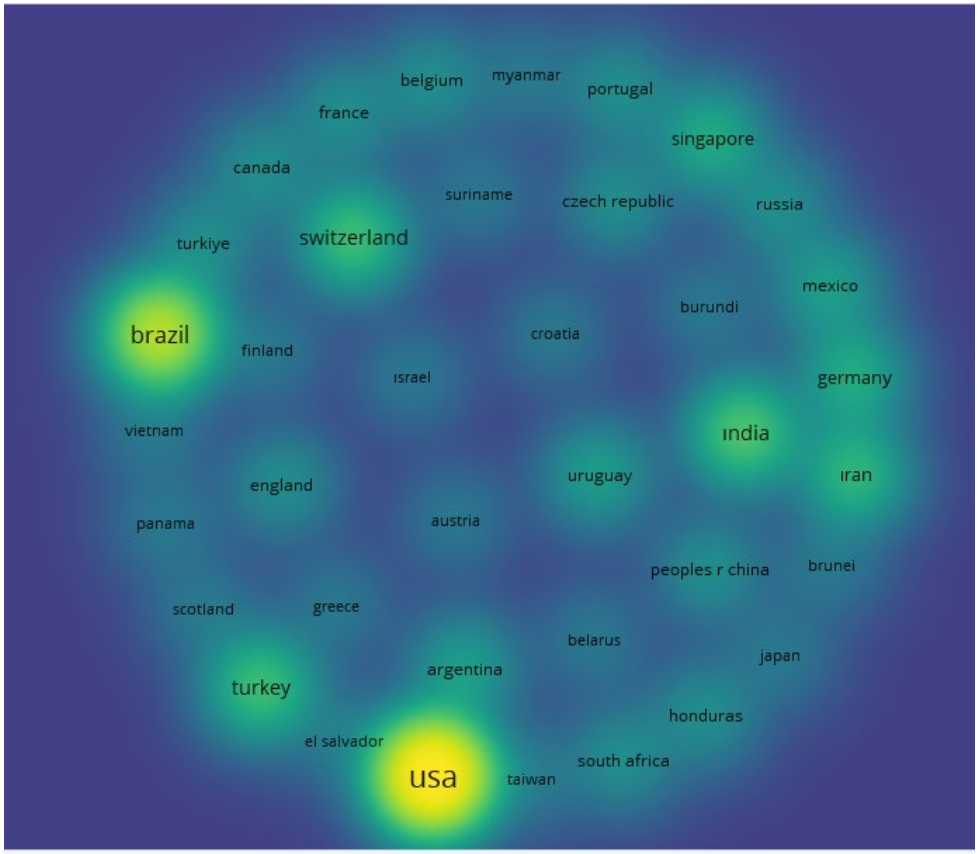


Figure 3. Network visualisation of bibliographic coupling between countries

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

The relationship between authors with at least one publications and at least one citations is shown in Figure 4.

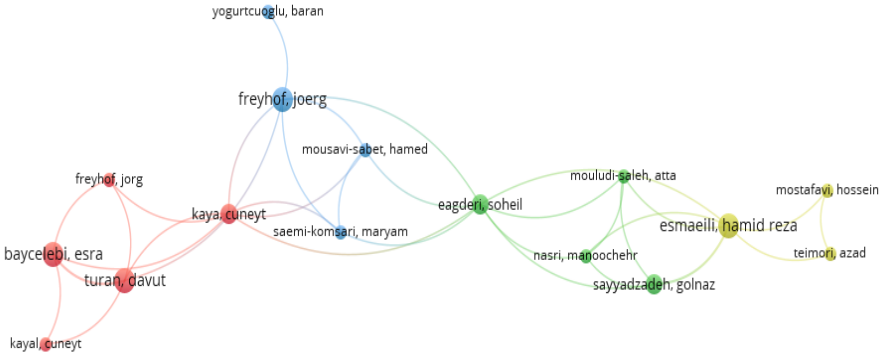


Figure 4. The relationships between authors

Universities that have published at least one article and received at least one citation are shown in Figure 5.

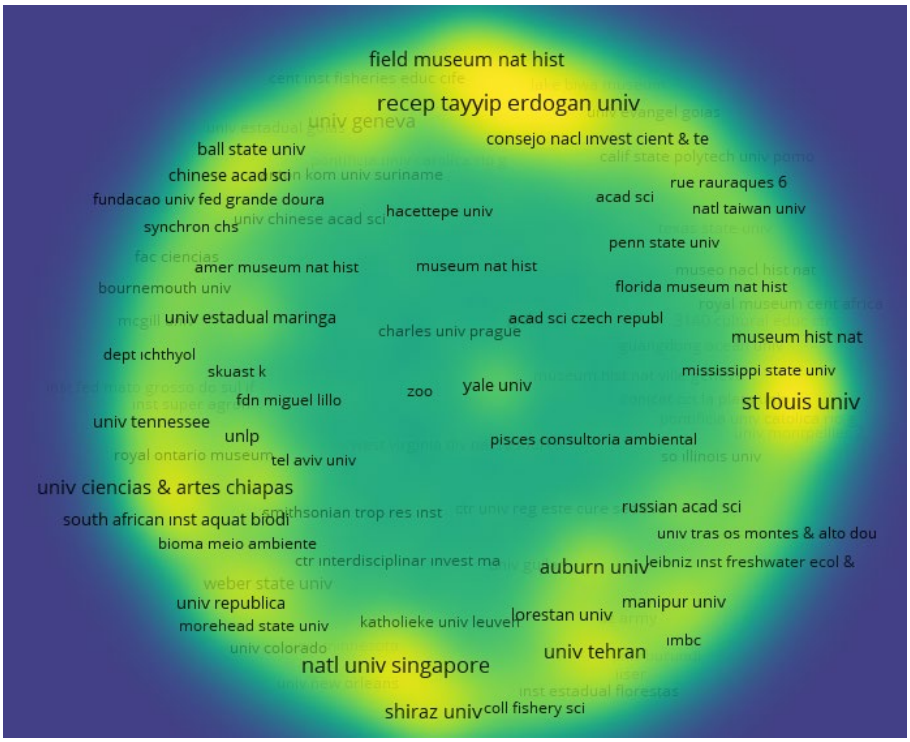


Figure 5. Universities with publications and citations

Discussion

The Asi River (Orontes) basin is the second richest in the Mediterranean in terms of freshwater fish biodiversity and endemism. Taxonomic and ecological studies conducted in the basin over the past 25 years have revealed a rich and unique ichthyofauna. Bayçelebi et al. (2021) described *Garra orontesi* from the Asi River drainage, while Freyhof and Turan (2019) described a new species of minnow, *Alburnus magnificus*, from the northern Asi drainage. These discoveries have drawn attention to the basin's unique phylogenetic and endemic structure. Furthermore, Freyhof and Yoğurtçuoğlu (2023) re-examined the species *Mystus*

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misrai, confirming its validity. They noted that, as the species is only found in a single source lake within Türkiye borders, it faces a high risk of extinction and may be critically endangered.

Studies of the current distribution of fish species in the basin, as well as efforts to conserve biodiversity, provide important baseline data. Bayçelebi (2020) determined the taxonomic classification and drainage distribution of fish species in the Seyhan, Ceyhan and Asi river basins. Özcan and Altun (2015), on the other hand, determined the length-weight relationships and condition factors of the endemic and threatened species *Alburnus orontis*, *Capoeta barroisi* and *Chondrostoma kinzelbachi* in the Asi River. This provides important data for monitoring populations of endemic species, managing fisheries and developing conservation strategies.

This study provides a comprehensive bibliometric overview of research conducted on the endemic fish species of the Asi River (Orontes), the second most important freshwater system in the Mediterranean basin in terms of biodiversity, and synthesises the basin's current taxonomic and ecological status.

It was found that publications issued between 1989 and 2025 received a total of 2,484 citations, indicating a growing momentum in this field of research (h-index of 25). Türkiye's fifth-place ranking, behind the United States, which leads in publication output, indicates ongoing global and local academic interest in the region's fauna.

The fact that a large proportion of the research is concentrated in the field of 'Zoology' and that the most productive journal is Zootaxa confirms that the species diversity of the basin is still in the early stages of exploration. The presence of newly introduced species, such as *Garra orontesi* and *Alburnus magnificus*, and critically endangered species with updated status, such as *Mystus misrai*, highlights the basin's potential for endemism and phylogenetic isolation. Studies on the condition and length-weight relationships of threatened endemic species, such as *Alburnus orontis*, *Capoeta barroisi* and *Chondrostoma kinzelbachi*, provide crucial reference points for fisheries management.

These endemic species, with their limited geographical distribution, are under severe pressure from successive and simultaneous stressors, such as drought, excessive water extraction, infrastructure projects and the introduction of invasive species.

Future research should focus on interdisciplinary studies modelling the effects of global climate change and anthropogenic pressures on ecosystem resilience, rather than being limited to taxonomic identification and inventory studies. In light of this scientific data, the conservation and sustainability of the Asi (Orontes) River's unique freshwater biodiversity will only be possible through the urgent implementation of national and international transboundary water conservation policies.

In conclusion, the Asi (Orontes) River's freshwater fish fauna is notable for its rich endemic species and biodiversity, as well as its highly threatened species. To ensure the sustainability of biodiversity, it is crucial to continue taxonomic studies, monitor the ecological status and population dynamics of existing species, and implement action plans for habitat conservation.

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Ethics Approval

No need to ethical approval for this study.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

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The data supporting the findings of this study were derived from the Web of Science Core Collection database. The bibliometric dataset generated and analysed during the current study is available from the corresponding author upon reasonable request due to privacy reasons.

Limitations

One limitation of this study is its reliance on the Web of Science database. This database may have excluded some relevant articles from other databases or grey literature, thus overlooking important perspectives on the Discovery of Endemic Fish Species in the Orontes River research. The exclusion of case reports and other document types may have led to underrepresentation of certain research trends or reviews that could complement the primary research findings. Furthermore, since the study only covers publications in English and a few other languages, significant contributions from non-English speaking regions may have been excluded. The use of citation counts and h-indexes as baseline measures may not fully reflect the qualitative impact of the research. These measures tend to favor older and more widely cited studies compared to newer and more innovative studies.

Author Contribution

The authors conceived and designed the study, collected and analyzed the data, prepared the figures, wrote the manuscript, and approved the final version of the manuscript.

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Application of the Integrated Beach Quality Index (BQI) for selected semi-urban, semi-rural, and urban types in Albania

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Abstract

One of the most crucial elements in drawing tourists and fostering economic development is the state of coastal beaches. For countries with considerable coast line like Albania, where "beach and sun" tourism is a major economic driver, ongoing beach quality development is a key objective. In terms of planning and management there are series of challenges linked with intention for enhancing the quality of the visitor experience and safeguarding the ecosystem of beaches. To assess "Environmental Quality" and "Human Welfare & Health," an integrated Beach Quality Index (BQI) was established. The index also provided an environmental engineering-based framework for evaluating coastal sustainability, ecosystem protection, and onsite environmental management practices. On the Albanian Adriatic coast, it was used on three distinct kinds of beaches: semi-urban, semi-rural, and urban. Implemented BQI enabled to assess the overall beach state and quality; separated components of beach quality on three types of the selected beaches; dominant strengths and weaknesses of the study beaches and provided possibility to draw priorities for future appropriate management. Following the implemented overall quality was evaluated as “Excellent” at the Lalzi Bay (urban), Tale beach (semi-urban) “Good” and the same quality at Vain (semi-rural) beach. Nonetheless, there is room for improvement through onsite management, as some of the specific beach quality factors had lower values.

Keywords: Sustainability, planning, coastal development, conservation, environmental management

Introduction

Beaches serve as useful connections between the water and the land. They are the primary draw for people to coastal regions and have a significant impact on the Western Mediterranean's tourism potential. Physical characteristics like local geology and geomorphology, ecological characteristics like flora and fauna, and socioeconomic characteristics like recreational amenities, access, safety, and landscape can all contribute to the allure of coastal places. For

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the numerous coastal regions of the world that experience extremely complex interactions and repercussions, planning the integrated management of these aspects is crucial.

The concept that the beach is an integrated system where several interrelated processes take place gave rise to this essay. The project's main focus was on analyzing these procedures as well as the administrative and physical environment in which they take place. The procedures for the chosen beaches on Albania's Adriatic coast, which is in the country's west, were examined during the study's creation. This region is an excellent illustration of a coastal area where tourism dynamics have a significant impact. Several significant flaws were found in the legal and administrative framework. There isn't a single beach policy that covers all fundamental aspects. As a result, only a portion of the information is provided. Within the scope of the Integrated Coastal Zone Management (ICZM) strategy, national and regional strategies are now being created, incorporating indicators of processes that have not yet been included in monitoring programs. This could alter the current state of ignorance.

The Beach Quality Index (BQI) evaluates coastal zones using recreational, natural, and protective functions. In Albania, comprehensive academic or standardized BQI scoring across all typologies is limited; instead, water quality and environmental pressures are tracked by the European Environment Agency and the national environment authorities, showing that roughly 30% of monitored coastal sites meet "excellent" standards, with urban areas facing higher degradation (Bici and Shumka, 2026). Field applications across diverse settings show that urban and semi-urban beaches score high in recreation and infrastructure, while semi-rural beaches excel in natural conservation but may lag in amenities.

The direct connection with BQI is the spatial planning. Albania has a history of troubled complex spatial planning that dates from the last century (1950) and has experienced a dramatic transformation due to changing political and economic regime in 1990. Spatial planning in Albania has territorial basis, legal and institutional (Nikolli, 2014). Planning institutions at central and local level are essential elements in the organization and functioning of the planning activity. Currently, the law no. 10119, dated 23.04.2009, "On Territorial Planning" harmonize policies and territorial planning law principles of territorial planning of the EU (inclusive territorial planning that combines all the political issues at all territorial levels). The new planning system in Albania lists a number of principles that comply with the European philosophy of planning and include almost all the features of other models. The law does not stop only to territory planning, but pays significant attention to the development of the territory and its control.

A more comprehensive and integrated management approach known as integrated coastal zone management (ICZM) has been developed in response to the complexity of coastal ecosystems, while in case of Albanian Adriatic zone in many extends this has been partly completed.

It involves coordinated planning and analysis across multiple disciplines, with sustainability as the primary goal. Proactive management of people and environmental resources is necessary for ICZM procedures. Goals must be set in accordance with the roles that coastal areas are allocated (De Groot 2002), and planning must be promptly adjusted to meet specified goals. To deal with the uncertainty involved in managing complex systems, management transparency and the active participation of all stakeholders are essential (Barragán 2003).

Material and Methods

Systems for rating performance and performance standards: Before analyzing their current application in the South Adriatic coastal region, an evaluation of the most significant quality criteria employed now in the management of beach ecosystems has been considered. The evaluation was based on a number of publications from organizations in charge of those ecosystems (ACA 2002, FEE 2004, Bici and Shumka, 2025; Shumka et al., 2023) as well as the body of existing literature (Jiménez & Van Koningsveld 2002, Villares 1999, Yepes 2005).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

The fourteen criteria that were ultimately chosen were broad guidelines that make an effort to compile all the data needed for the proper, environmentally sound management of those ecosystems.

The Beach Quality Index (BQI) evaluates coastal areas using three core functional dimensions: the **Recreational Function Index (RFI)**, the **Natural/Ecological Function Index (NFI)**, and the **Protective Function Index (PFI)**. The used approach Calculations of Beach Quality Index following (), is presented in Table 1, while considered study area is shown in Figure 1.

Table 1. Beach Quality Index approach

BQI= p1(A, B) (RFI)+p2(A, B) (NFI)+p3(A, B) (PFI)

RFI= α [t1(IC)+t2(IEQ)+t3(ISerF)+t4(IAct)+t5(IAcPar)+t6(IComf)+t7(IS)+t8(IBS)]
NFI= u1(IN)+ u2(IWSP)+ u3(IPQ)
PFI= IPP

RFI	**p1A**	**p1B**
NFI	**p2A**	**p2B**
PFI	**p3A**	**p3B**

BQI	Nën – indekset	Indekset e pjesshme
BQI **Beach Quality Index**	**RFI** **Recreational Function** Funksioni rekreativ	**α: Microbiological Water Quality** **IC: Beach Crowding** **IEQ: Environmental Quality** **ISerF: Services and Facilities** **IAct: Activities** **IAcPar: Access and parking** **IComf: Comfort Quality** **IS: Surrounding Area Quality** **IBS: Beach Safety**
	NFI **Natural function** Funksioni natyror	**IN: Natural Conditions** **IWSP: Water-Sand Pollution** **IPQ: Physical Quality**
	PFI **Protective Function**	**IPP: Protection**

The calculation of the BQI was based on data collected during beach assessments and laboratory analyses conducted in this study. Initially, individual indicators were grouped into sub-indices according to their respective categories, and these were subsequently combined to construct the final index. To enable comparison between parameters with different units of measurement, all values were normalized on a scale from 0 to 1, where 0 represents the least favorable condition and 1 the optimal condition.

The relative importance of the included indicators was also taken into account when constructing the index. Consequently, parameters directly linked to microbiological quality and public health risks were assigned greater weight in the final assessment. This approach aims to better reflect the actual impact of key factors on beach quality. The use of the BQI enabled an integrated assessment of the environmental and sanitary conditions of the studied beaches, providing a useful tool for comparing different areas and supporting decision-making in coastal management. However, the results should be interpreted with the understanding that the index's performance depends on the selected indicators, the weighting method, and the normalization procedures employed.

Results and Discussions

Beach use and waste/litter production are two particularly significant beach management activities that need to be examined, as evidenced by the information available and the features of the researched location. The findings show that beaches are generally inundated. Some

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beaches have exceeded the 5 m^2 criterion for urban beaches specified in the literature. Over the course of more than 20 years, crowding has been a continuous problem. One such issue is the high generation of garbage, which accounts for a significant portion of the total amount produced in a beach town during the summer (9.50%). mechanical cleaning techniques and putting environmental education initiatives into action.

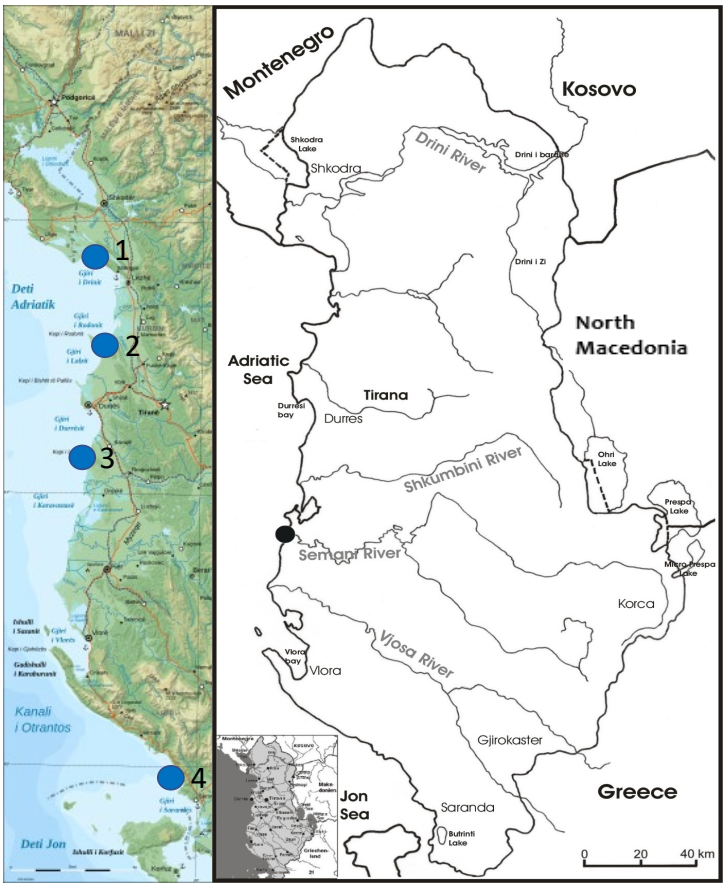


Figure 1. Four selected coastal area along the Adriatic Sea

Semi-Urban Beaches are situated in suburban or outlying residential zones with moderate population density and balanced land use. Their strength lies in fact of having good balance of amenities and visitor services combined with preserved open space and moderate crowding, while weakness lies at vulnerable to seasonal spikes in visitor pressure and localized infrastructure strain.

Semi-Rural Beaches are located in low-density or peripheral zones with restricted public transit access and natural surroundings with a strength based on high ecological integrity, minimal artificial modification, and strong natural protective functions and weaknesses linked with limited commercial facilities, reduced user amenities, and occasional gaps in on-site management. The value of index application is connected not only with increased number of visitors, but also with climate changes associated impacts that are also including beach zones (Sekovski et al, 2014; Semeoshenkova and Newton, 2015) and increased pollution of different types (Bojar and Klapec, 2018; Bolívar-Anillo et al., 2023).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

The linear aggregation of sub-indices enabled the construction of a composite assessment system capable of integrating heterogeneous environmental, microbiological, and functional components into a single beach quality indicator. Similar approach has been found by Ariza et al. (2010), Ansari et al (2014) etc. The sub-indices were calculated based on relevant indicators following the methodology of Ariza et al., while standardization and final aggregation were carried out during the final stage of developing the Beach Quality Index (BQI) to integrate these diverse components into a single composite index. The final BQI was standardized on a scale of 0 to 1, where higher values represent superior beach quality and more favorable environmental and recreational performance. This approach facilitates comparisons between beaches, the identification of high-risk beaches, and inter-annual performance analysis, while supporting decision-making for the integrated and sustainable management of coastal zones. By integrating microbiological, ecological, recreational, and sanitary-hygienic parameters, the BQI serves as a modern, innovative tool for assessing beach quality and protecting public health at Albanian coastal beaches.

Table 2. Values of the recreational function index (RFI) components for the studied beaches during 2024- 2025

	Year	α	IC	IEQ	ISerF	IAct	IAcPar	IComf	IS	IBS
1	2023	1	0.16	0.38	0.05	0.2	0.6	0.94	0.85	0.58
	2024	1	0.16	0.32	0.04	0.4	0.64	0.78	0.11	0.75
2	2023	0.8	0.2	0.15	0.045	0.4	0.35	0.94	0.15	0.58
	2024	1	0.2	0.32	0.04	0.4	0.39	0.78	0.18	0.75
3	2023	0.8	0.16	0.55	0.054	0.2	0.42	0.94	0.14	0.66
	2024	0.8	0.16	0.26	0.12	0	0.21	0.78	0.2	0.75
	2024	1	0.36	0.7	0.06	0.4	0.75	0.94	0.083	0.58
4	2023	0.8	0.3	0.01	0.01	0.2	0.42	0.94	0.2	0.58
	2024	0.8	0.3	-0.05	0.01	0.2	0.39	0.94	0.2	0.75

Table 3. Assessment of the partial sub-indices RFI, NFI, PFI, and BQI

Beach	1	2	3	4
RFI	0.496	0.36	0.44	0.33
NFI	0.6	0.94	0.94	0.85
PFI	0.64	0.33	0.29	0.56
BQI	**0.55**	**0.33**	**0.36**	**0.36**
RFI	0.45	0.45	0.36	0.35
NFI	0.86	0.86	0.86	0.86
PFI	0.64	0.33	0.29	0.56
BQI	**0.53**	**0.45**	**0.31**	**0.37**

In 2025, BQI values showed significant variability across the beaches. Beach 2 exhibited the highest performance (BQI = 0.79), driven primarily by high values for natural function (NFI = 1.09) and protective function (PFI = 0.66), reflecting the preservation of ecological integrity and the physical characteristics of the coastline. Relatively favorable values were also recorded in beach 1 (0.55) and 3 (0.47), where the natural component was the dominant contributor.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Conclusions

The majority of beach management procedures are still based on performance standards and rating systems. Nevertheless, no single performance evaluation metric takes into account every factor thought to be pertinent to beach management. The majority of the criteria used in performance standards and rating systems are related to the recreational function of beaches, and only a small number of them are related to the two other significant beach functions, which are the protective and natural functions. Beach management objectives are constrained and static since commonly employed performance assessment metrics do not take proactive and dynamic planning into account.

The use of the BQI enabled an integrated assessment of the environmental and sanitary conditions of the studied beaches, providing a useful tool for comparing different areas and supporting decision-making in coastal management. However, the results should be interpreted with the understanding that the index's performance depends on the selected indicators, the weighting method, and the normalization procedures employed.

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Comparative Assessment of Nutritional and Organoleptic Properties of Sun-and Oven-Dried Small Prawns in Bangladesh

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Abstract

The main purpose of this study was to evaluate and compare the nutritional and organoleptic qualities of dried small prawns prepared using conventional sun- and oven-drying methods. Organoleptic attributes (colour, odour, appearance, texture, and overall acceptability) were assessed using standard sensory evaluation protocols, and proximate composition (lipid, protein, moisture, ash, crude fibre, and carbohydrate), along with selected minerals (calcium and iron), were determined by established analytical methods. The results demonstrated that oven-dried prawns exhibited significantly higher sensory scores (colour, appearance, and texture) with overall acceptability reaching 7.8±0.67 compared to 7.13±0.83 in sun-dried samples. Proximate analysis revealed that oven-dried prawns contained significantly higher levels of protein (57.69±0.98%), lipid (13.65±0.59%), and ash (11.01±0.32%) compared to sun-dried samples. Sun-dried samples had significantly higher moisture content (17.27±0.64%) and carbohydrate (1.28±0.11). pH levels in dried prawns did not differ significantly between drying methods. The calcium and iron contents ranged from 5.67±0.72 mg/100g (sun-dried) to 23 6.48±1.46 mg/100g (oven-dried) and 0.69±0.26 mg/100g (sun-dried) to 1.51±0.43 mg/100g 24 (oven-dried), respectively. These findings provide practical guidance for processors to adopt improved drying techniques for producing higher-quality consumer preferred products as well as enhance the knowledge regarding improved dried fish products for the consumer.

Keywords: Oven drying technology, food preservation, small prawn, organoleptic properties

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Adaptation of clupeid visual systems in aquatic habitats

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Abstract

While the current status and dynamics of fish stocks -influenced by fishing, climate-driven habitat changes, and invasive species- are being actively studied, fascinating adaptations in fish sensory organs have evolved over longer timescales in aquatic ecosystems, often receiving little attention; examples include the optimization of retinal functional morphology for hunting and predator avoidance. Species-specific adaptations of photoreceptors and pigment epithelial cells to habitat conditions and the visually guided behavior have resulted in distinct visual capabilities at the histological, neuroanatomical, and ultrastructural levels. Within the order Clupeiformes (specifically in a subgroup of anchovies), a contrast system unique among vertebrates for the specific detection of polarized light has evolved, originating from "normal" trichromatic contrast vision (Fineran & Nicol 1978 PTRS Lond B 283: 25; Heß et al. 2006 J Morphol 267: 1356). Polarization-contrast vision offers a significant advantage in situations where intensity and color contrast contribute little to object recognition -such as detecting transparent plankton swarms, particularly in turbid water- because the depolarizing cuticular surfaces of planktonic crustaceans stand out against the partially polarized background light. What initially appears to be an exotic specialization gains significance when one considers the sheer number of anchovies, which e.g. far exceeds that of humans. We have currently reached the stage of mapping and analyzing neuronal connectomics, i.e. reconstructing the neural network underlying retinal information processing to better understand polarization vision in anchovies. Analysis of horizontal cell connectivity has already revealed that this system functions as a modified color-contrast calculator (Guder et al. 2025, BMC Biol 32: 137). Ongoing investigations are focusing on bipolar, amacrine, and ganglion cells. In the long term, the project will be extended to include other Clupeiformes species with different (color) contrast systems; this aims to incorporate an evolutionary perspective into connectomics research and thereby uncover further secrets of aquatic biology.

Keywords: teleost, retina, polarization vision

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Zebrafish as a model organism in probiotic and postbiotic research for aquaculture

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Abstract

In aquaculture, the control of infectious disease and the maintenance of fish health are addressed through both therapeutic and preventive approaches. Because antimicrobial use can lead to resistance and to residue accumulation in farmed fish, probiotics and, more recently, postbiotics form an expanding area of aquaculture research that addresses growth performance, intestinal histology, microbiota composition and immune-related gene expression in fish. Zebrafish are inexpensive to maintain, develop rapidly and have a short generation time, and germ-free and gnotobiotic conditions can be used to examine host–microbiota interactions. This review examines the use of zebrafish (*Danio rerio*) as a model organism for investigating the mechanistic responses to probiotics and postbiotics, covering immune and inflammatory regulation, oxidative stress and antioxidant defence, intestinal barrier integrity and histomorphology, gut microbiota composition, pathogen resistance, growth and reproduction. The extent to which findings obtained in zebrafish can be applied to aquaculture species is also discussed, in view of the differences reported between zebrafish and farmed fish and between laboratory and farm conditions. Further studies are needed to characterise dose–response relationships, compare live and inactivated forms of the same strain, and examine pathogens other than the *Aeromonas* species used in most challenge studies to date. The potential use of preparations that give consistent responses in zebrafish could then be assessed in target farmed species.

Keywords: *Danio rerio*; immune regulation; oxidative stress; intestinal barrier; gut microbiota; pathogen resistance

Introduction

Infectious disease is a major challenge in intensive aquaculture. The antimicrobial treatments used to control it have been linked to the emergence and spread of antimicrobial resistance (Reverter et al., 2020; Mushtaq et al., 2026), to residues in farmed fish and the associated food safety risks (Ljubojević Pelić et al., 2024), and to the environmental dissemination of resistant bacteria and resistance genes (Mushtaq et al., 2026). Preventive approaches that reduce the need for therapeutic antimicrobials have been investigated for many years, and functional feed additives are among the strategies examined.

Among these additives, probiotics and, more recently, postbiotics have become an expanding area of aquaculture research, encompassing freshwater and marine fish as well as crustaceans,

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and addressing growth performance, intestinal histology, microbiota composition and immune-related gene expression (Yu et al., 2023; Lalitha et al., 2025; Pérez-Gómez et al., 2025; Ponomareva et al., 2025; Wiratama et al., 2025).

Probiotics are live microorganisms administered in amounts sufficient to benefit the host (Hill et al., 2014). Postbiotics are preparations containing inanimate microorganisms or their components, such as inactivated cells, cell fractions and culture supernatants (Salminen et al., 2021). Production and storage reduce the viability of probiotic cells, and the resulting non-viable cells can also benefit the host (Aydın, 2023). The stability of postbiotics during industrial processing and storage is one reason for the interest in them. Many probiotic organisms are sensitive to oxygen and heat, and a long shelf life is more easily maintained with inanimate microorganisms. Limited cold chain infrastructure and high ambient temperatures restrict the storage of live microorganisms in some regions, and postbiotics may be more suitable than probiotics in such settings (Salminen et al., 2021).

The application of inactivated microbial preparations requires further research into the selection of suitable probiotic species and strains, effective inactivation methods, and the stability and functionality of the resulting products (de Almada et al., 2016). Despite increasing interest in postbiotics, live probiotics remain a subject of research. Recent studies have examined growth performance, immune responses and antioxidant status (Alghamdi, 2026; Ebrahimi et al., 2026; Elayaraja et al., 2026; Shovon et al., 2026), as well as water quality and resistance to pathogen challenge (Nassar et al., 2025; Elayaraja et al., 2026).

Zebrafish offer several advantages as a model for investigating biological processes. They are readily available and relatively inexpensive to maintain. Fertilisation takes place externally and the embryos are optically transparent, allowing developmental processes to be observed directly. Development is rapid and the generation time is short (Çek & Aydin, 2016; Dede & Çek Yalnız, 2018). In aquaculture, the zebrafish has been used as a well-characterised model for studies of growth, reproduction and disease biology, supported by the availability of functional genomics and molecular biology data. It has been used as a model for bacterial infections in fish (Aydın & Yalnız, 2023). It has also contributed to the development of vaccines and therapies (Alestrom et al., 2006).

Most studies of fish intestinal microbiota have focused on microbial structure and diversity, while work on the function of commensal microbes remains scarce (Zhang et al., 2020). The gnotobiotic zebrafish model, in which fish are reared under axenic conditions or with defined microbial lineages, is used to identify molecular interactions between the intestinal microbiota and the host, and its colonisation conditions and applications in aquaculture have been reviewed (Zhang et al., 2020). Rawls et al. (2004) developed methods for rearing germ-free zebrafish, identified genes in the digestive tract regulated by the microbiota, and showed that some host responses depend on the bacterial species used for colonisation. Among the uses of gnotobiotic models is the evaluation of the protective function of probiotics and other additives (Zhang et al., 2020).

Zebrafish have been used in studies examining host responses to probiotic and postbiotic administration. This review summarises the mechanistic responses reported in this model, including immune regulation, oxidative stress and antioxidant defence, intestinal barrier integrity, gut microbiota composition, pathogen resistance, and growth and reproduction. The studies considered are listed in Table 1. The limitations of the model and the extent to which the findings can be transferred to farmed species are also considered.

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Table 1. Studies of probiotic and postbiotic administration in the zebrafish model.

Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
Zebrafish fed a practical diet; no pathogen challenge	Ethanol-inactivated (70%, v/v), freeze-dried *Vibrio proteolyticus* DCF12.2 (CECT 9300) at 1% of diet	21 days	Final body weight and mortality; gut microbiota (16S rRNA V3–V4) and predicted function (PICRUSt2); intestinal transcriptome (RNA-seq, ORA and GSEA)	Final body weight ↔ and no mortality in either group. Alpha and beta diversity and phylum- and genus-level composition ↔; significant differences only at ASV level, with variants affiliated with *Aeromonas*, *Crenobacter*, *Delftia*, *Plesiomonas*, *Ralstonia*, *Shewanella*, *Stenotrophomonas* and ZOR0006 ↑. Predicted microbial function ↔. RNA-seq: 201 of 25,015 genes differentially expressed, 161 ↓ and 40 ↑ in the supplemented group. GO ORA: lipid transport and metabolism, regulation of glucose transport and fibroblast proliferation ↑; immune-related processes ↓. KEGG ORA ↔. GSEA: cytokine–cytokine receptor interaction pathway ↓; nutrient-metabolism, oxidative-phosphorylation and cellular-biosynthesis pathways ↑. The authors describe this as an altered immunometabolic state and raise the possibility of immunosuppression.	García-Márquez et al., 2026
Adult zebrafish; intraperitoneal viral haemorrhagic septicaemia virus (VHSV) challenge after the feeding period	Heat-inactivated *Lacticaseibacillus paracasei* HA-108, Lactiplantibacillus plantarum HA-119 or *Lactobacillus helveticus* HA-122, each at 6×10^6 cells/g feed; fed at 4% of biomass	21 days feeding; 10 days post-challenge	Expression of 16 immune-related genes in gut and kidney tissue at day 21 (pre-challenge; fold change ≥ 1.5); qualitative immunoblotting of five immune proteins (Tnf-α, Ifn-γ, Il10, Alp, Hsp70) in gut and kidney; intestinal goblet cell density and villus height; kidney histology; post-challenge survival; kidney viral copy number	Gene expression profiles differed among strains and between tissues. All three treatments raised gut expression of *tnf-α*, *ifn-γ*, *mx*, *defβ1* and *muc5.1*, and kidney expression of *tlr5b*, *il-1β* and *cd8a*; downregulation occurred only in the kidney, where *cat* ↓ with all three strains. In the gut, *ccl34a.4* ↑ with HA-108 and HA-122 and *cxcl18b* ↑ with HA-119 and HA-122. HA-119 upregulated 13 of 16 genes in the gut. Immunoblotting (qualitative): Ifn-γ and Il10 ↑ in gut and kidney with all three strains; HA-108 also ↑ Tnf-α and Alp in both tissues (Hsp70 not ↑ in either tissue); HA-119 ↑ Alp and Hsp70 only in gut and Tnf-α only in kidney; HA-122 ↑ Alp in both tissues, and Tnf-α and Hsp70 only in gut. Goblet cell density and villus height ↔ (numerically lower with HA-119, not significant); no structural, inflammatory or toxicity-related change in kidney histology. Survival at 10 days post-VHSV challenge: challenged control 43.3%, HA-108 60.0%, HA-119 83.3%, HA-122 70.0%; survival curves differed from the challenged control for HA-119 ($p < 0.01$) and HA-122 ($p < 0.05$) (log-rank test). Kidney VHSV copy number in survivors ↔ among challenged groups.	Nikapitiya et al., 2026
Zebrafish subjected to four weekly immersion challenges with *A. hydrophila* (10^7 CFU/mL, 48 h per exposure)	Engineered *Bacillus subtilis* 168 secreting tilapia-derived piscidin-1 (BS168-sfGFP-piscidin), hepcidin (BS168-sfGFP-hepcidin), or both peptides in tandem (BS168-sfGFP-PH), at 10^8 CFU/g; 1.5% xylose was added to the feed to induce peptide expression; empty-vector strain (BS168-con) as control	7 days feeding before the first challenge; feeding continued through four weekly challenges; survival followed for 33 days	*tlr4*, *nlrb1*, *myd88*, *trif*, *mapk*, *fos*, *jun*, *traf6*, *ripk2*, *ikbkb*, *tnf-α*, *il-1β*, *il-10*, *il-22*; intestinal *A. hydrophila* copy number; anterior intestinal histology; survival	Survival after four weekly *A. hydrophila* immersion challenges ↑ with the tandem strain vs. empty-vector control and both single-AMP strains. Intestinal *A. hydrophila* copy number ↓ with the piscidin strain and the tandem strain, lowest with the tandem strain ($p < 0.0001$). Dual-AMP strain only: *tlr4*, *nlrb1*, *myd88*, *fos*, *jun*, *traf6* and *ikbkb* ↓; *il-10* ↑ and *tnf-α* ↓; *il-22* showed an upward and *il-1β* a downward trend; *trif*, *mapk* and *ripk2* ↔. The two single-AMP strains had no significant effect on these pattern recognition receptors. Muscularis thickness ↑ vs. empty-vector control.	Zhang et al., 2026
Juvenile zebrafish	Dietary *Bacillus subtilis* HBB 493® at 6.5×10^9, 1.3×10^{10}, 2.6×10^{10}	100 days	Growth performance and survival; gonadal histology (spermatogenesis and oogenesis stages); intestinal histology	Weight gain and daily growth ratio ↑ vs. control at the two highest levels. At the highest level final weight and specific growth rate ↑ and FCR ↓, each differing from all other groups. Survival ↔.	Dede & Çek, 2025

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
	and 3.9 × 10^{10} CFU/g; unsupplemented control			Gonads: testicular stages advanced with dose, free spermatozoa in the lumen at the highest level; exogenous yolk formation in groups III–V, absent in control; oogenesis stages 4–7 present in groups IV and V. Intestine: control showed ruptured villi and an enlarged lumen. Villi and goblet cells were intact at all levels. No hypertrophy, villus fusion or mucosal degeneration was seen. Villus length tended to be higher than in the control.	
Zebrafish fed probiotic diets and subsequently challenged with *A. hydrophila*	T1: live *Lactobacillus plantarum*, 1 × 10^{8} CFU/g feed; T2: live *Lactobacillus reuteri*, 1 × 10^{11} CFU/kg feed; T3: both combined at the same doses	8 weeks feeding; 14 days post-challenge	Growth performance and survival; digestive enzymes; lysozyme activity, total immunoglobulin and total protein in tissue; ACH50, lysozyme activity, total immunoglobulin, IgM, ALP, AST, ALT, CAT, GPx, SOD, MDA and T-AOC in mucus; *cat*, *gpx*, *sod*, *il-1*, *lyz* and *tnf-α* expression; post-challenge mortality	Final weight and weight gain ↑ vs. control in T3 only; FCR ↓ vs. control in T2 and T3; specific growth rate and survival ↔. Amylase ↑ in all supplemented groups; lipase ↑ in T3 only; protease, pepsin, chymotrypsin and trypsin ↔. Tissue: lysozyme activity ↑ in T3 only; total immunoglobulin ↑ in all supplemented groups; total protein ↔. Mucus: ACH50, lysozyme activity and total immunoglobulin ↑ in T2 and T3; IgM ↑ in all supplemented groups. ALT ↑ in T2 and T3; ALP and AST ↔. GPx and T-AOC ↑ in T2 and T3; CAT ↑ in T3 only; SOD and MDA ↔. *gpx*, *sod* and *tnf-α* expression ↑ in all supplemented groups, *gpx* and *tnf-α* highest in T3; *cat* and *lyz* expression ↑ in T2 and T3; *il-1* expression ↑ in T3 only. Post-challenge cumulative mortality lower in all supplemented groups and lowest in T3; no statistical test reported for the challenge.	Esfahani et al., 2025
Adult male zebrafish exposed to permethrin (1 µg/L)	*Lactobacillus casei* LC89 at 10^{7}, 10^{8} and 10^{9} CFU/g diet; heat-killed cells at the medium dose as a negative control	30 days	Gut *L. casei* colonisation; survival (SR) and growth (WGR, SGR); testicular organ coefficient and histology; testicular ROS, MDA, T-AOC, SOD, CAT and GSH-Px; *il-1β*, *il-6* and *tnf-α* transcripts and IL-1β, IL-6 and TNF-α protein; testicular Fe^{2+} and *gpx4* and *slc7a11* transcripts	Gut *L. casei* counts ↑ with dose; not detected with heat-killed cells. SR ↔ in all groups. Permethrin ↓ WGR and SGR; in exposed fish, viable *L. casei* ↑ both dose-dependently, ↔ vs. control at 10^{8} and 10^{9} CFU/g. Permethrin ↓ testicular organ coefficient; histology showed disorganised spermatogenic tubules, fewer spermatozoa and a wider interstitial space. At 10^{8} CFU/g, *L. casei* ↑ organ coefficient (still ↓ vs. control). *L. casei* ↑ sperm concentration (histological observation). Permethrin ↑ testicular ROS, MDA and Fe^{2+}, *il-1β*, *il-6* and *tnf-α* transcripts and IL-1β, IL-6 and TNF-α protein, and ↓ T-AOC, SOD, GSH-Px, CAT and *gpx4* and *slc7a11* transcripts. Viable *L. casei* attenuated these changes dose-dependently. At 10^{9} CFU/g all ↔ vs. control except ROS (↓) and *slc7a11* (↑); at 10^{7} CFU/g T-AOC, SOD, GSH-Px, CAT, *gpx4* and IL-1β and IL-6 protein ↔ vs. the permethrin group. Heat-killed cells in exposed fish: growth, oxidative, inflammatory and ferroptosis parameters ↔ vs. the permethrin group, except SGR (↑ vs. the permethrin group, ↔ vs. control).	Huo et al., 2025
Adult zebrafish supplemented with graded probiotic doses and challenged with *A. hydrophila* by immersion	*Lysinibacillus sphaericus* HY3 added to the culture water at 10^{5} (ZF5), 10^{6} (ZF6) and 10^{7} (ZF7) CFU/mL, re-administered every two days with 50% water change; the strain was undetectable in water at that interval	33 days; challenge monitored for 7 days	Growth performance and survival; hepatic antioxidant and immune enzyme activities; digestive enzymes; intestinal morphology; gut microbiota; non-targeted metabolomics of intestinal contents; challenge test	Survival, WGR and SGR ↔ among groups, with a numerical increase as concentration rose. Hepatic SOD and CAT showed a significant quadratic rising trend with concentration (lowest values at 10^{5}, highest at 10^{7}), but neither differed from the control in pairwise comparison; MDA ↓ linearly, below control at the two higher concentrations; LZM ↑ at 10^{7} only; GPT ↑ at the two higher concentrations; GOT ↔. Trypsin ↑ at 10^{7} only; lipase ↑ linearly,	Qiu et al., 2025

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
				above control at the two higher concentrations; α-amylase ↔. Villus height and crypt depth ↑ at all three concentrations, highest at 10^7; goblet cell number per villus ↑ linearly; villus height to crypt depth ratio ↔; control villi were short, narrow and sparsely distributed with a thin muscle layer, and at 10^7 the villi were evenly distributed and the muscle and submucosal layers better developed. ACE, Chao1 and Shannon ↔; Fusobacteriota ↑ and Acidobacteriota ↓ at 10^6 only; Planctomycetota ↓ at 10^5 and ↑ at 10^6; *Cetobacterium* and *Aeromonas* ↑ at 10^6; *Paracoccus* ↑ at 10^6 and 10^7; *Bacillus* ↓ at all three concentrations; *Rhodobacteraceae* ↓ at 10^6 and 10^7; *Gemmobacter* ↓ at 10^5; beta diversity differed from control (PERMANOVA $p = 0.002$). Metabolomics, relative to the control: 560 metabolites ↑ and 417 ↓ at 10^5, 517 ↑ and 279 ↓ at 10^6, 484 ↑ and 499 ↓ at 10^7. Challenge: control fish began dying on day 3 and reached 100% mortality by day 6; mortality started later as concentration increased; survival at 10^7 CFU/mL ↑ vs. control ($p < 0.001$, log-rank); all groups reached 100% mortality by day 7.	
Adult male zebrafish exposed to PFOS (0.3 mg/L) and/or PFOA (30 mg/L) in water	*Lactobacillus rhamnosus* ATCC 53103, 1×10^8 CFU/g	21 days	Intestinal and hepatic SOD, CAT, MDA and GSH; intestinal histology; gut microbiota (16S rRNA) and predicted function (PICRUSt2)	Hepatic SOD ↓ in all exposure groups at all sampling points; intestinal SOD ↓ from day 14 and ↑ under PFOS at day 7. *L. rhamnosus* ↑ hepatic SOD in the PFOA and combined-exposure groups at days 14 and 21; ↔ in the PFOS group. Intestinal CAT ↑ in the combined-exposure group given *L. rhamnosus*. Intestinal MDA ↓ under PFOA; *L. rhamnosus* ↑ MDA from day 14. Hepatic MDA ↔ under PFOA, ↑ under PFOS and the combined exposure at day 7, ↔ by day 21. Intestinal and hepatic GSH ↓ in all exposure groups; *L. rhamnosus* ↔. Intestine: pollutant exposure ↓ goblet cell number and caused epithelial shedding and vacuolisation. Damage was least severe under PFOA and most severe under the combined exposure. *L. rhamnosus* partially reduced these changes. Relative to the pollutant-exposed groups the probiotic ↑ Firmicutes and Actinobacteria and ↓ Proteobacteria. At genus level *Leucobacter* ↑ and *Rhodobacter*, *Gordonia* and *Rhodococcus* ↓; *Microbacterium* ↑ under PFOA and PFOS and ↓ under the combined exposure. Chao1 ↑ in the supplemented groups vs. control; PCoA separated supplemented from unsupplemented groups. Predicted function: the contribution of *Rhodococcus* to PWY-7315 rose from 2.75% to 73.49% under PFOA and fell to 7.31% in the supplemented group.	Wang et al., 2025
Adult zebrafish fed for 1 week, immersed in *A. hydrophila* AH40 (1×10^8 CFU/mL) for 12 h, then held 5 days in freshwater on the basal diet	*Lactobacillus plantarum* SHY21-2 at 1×10^8 CFU/g feed	1 week feeding; sampling 5 days post-challenge	Mortality and body weight; hepatic SOD, POD, CAT, GSH-Px and MDA; muscle and intestinal *tnf-α*, *il-6*, *il-1β* and *lamp2*; intestinal *claudin-2*, *occludin* and *zo-1*; intestinal histology; gut microbiota	Mortality 4.4% in the control, 24.4% with AH40 and 13.3% with AH40 + SHY21-2; SHY21-2 alone ↔ vs. control. Body weight ↔ among groups. Infection ↓ hepatic SOD (1.81-fold), POD (2.37-fold), CAT (1.44-fold) and GSH-Px (2.42-fold) and ↑ MDA (1.54-fold). In infected fish SHY21-2 ↑ SOD vs. the infected group; the value remained below control. POD, CAT, GSH-Px and MDA ↔ vs. the infected group. In uninfected fish SHY21-2 ↑ SOD and POD vs. control. Muscle: AH40 ↑ *tnf-α* (2.05-fold), *il-1β* (1.30-fold) and *il-6* (1.85-fold). Intestine: AH40 ↑ *tnf-α* (1.59-fold), *il-6* (1.77-fold)	Jiang et al., 2024

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
				and *lamp2* (3.09-fold). In infected fish SHY21-2 lowered all six; muscle *il-1β* and intestinal *tnf-α* and *il-6* returned to control level. In uninfected fish SHY21-2 ↓ muscle *il-6* and *il-1β* and intestinal *il-6* vs. control. Infection caused structural breaks in the intestine and villi and ↓ *claudin-2* and *zo-1*; *occludin* ↔ vs. control. SHY21-2 attenuated the histological damage; in infected fish *zo-1* returned to control level and *claudin-2* rose above the infected group but remained below control. In uninfected fish SHY21-2 ↑ *claudin-2* and *occludin* vs. control, with denser, more intact villi. Chao1 and Sob ↑ in all three treatment groups vs. control; Shannon ↔. Proteobacteria and Bacteroidota ↓ and Firmicutes ↑ in both SHY21-2 groups; Verrucomicrobiota ↑ in the infected group only. Unclassified Parachlamydiaceae, *Rhodococcus*, unclassified Microbacteriaceae and *Bosea* ↑ in the infected group only; *Lactiplantibacillus*, *Leucobacter* and *Gemmobacter* ↑ and *Achromobacter* ↓ in both SHY21-2 groups.	
Zebrafish (3–4 months old) injected with *Pseudomonas aeruginosa* LPS (25 mg/mL), live *Flavobacterium psychrophilum* (1.5×10^8 CFU/mL) or PBS after the feeding period	Sodium butyrate 0.5 g/kg; yeast prebiotic 2 g/kg; yeast probiotic 10 g/kg; black soldier fly larvae meal 100 g/kg (fishmeal reduced from 300 to 223 g/kg in that diet)	63 days; sampling 24 h after challenge	Survival and weight gain; hepatic expression of *tnf-α*, *il-1β*, *hepcidin*, *myd88*, *caspase-b*, *pgrp*, *saa*, *angptl4*, *nf-κb/p65* and *cxcl8*	Survival (77–91%, $p = 0.355$) and weight gain ↔ among diets; no mortality after either challenge; males and females were reared together. In the control diet, *nf-κb/p65* ↑ after both challenges vs. PBS ($p = 0.017$); the other nine genes ↔. Twenty-four hours after the pathogen challenge, sodium butyrate ↓ hepatic *tnf-α*, *il-1β*, *hepcidin*, *saa*, *nf-κb/p65*, *pgrp* and *caspase-b*; black soldier fly larvae meal ↓ *nf-κb/p65* ($p < 0.001$). The two yeast diets ↔ vs. control. In unchallenged (PBS) fish, *nf-κb/p65* ↑ with black soldier fly larvae meal as a trend ($p = 0.063$). In the LPS subgroup, *angptl4* ↓ with the yeast probiotic as a trend ($p = 0.070$); all other comparisons ↔.	Gao et al., 2024
Adult female zebrafish (4 months, AB strain) exposed to microcystin-LR at 0, 2.2 and 22 µg/L in water, with or without probiotic supplementation	*Lactobacillus rhamnosus* GG (GDMCC 1.2223), 10^8 CFU/g in the feed	28 days; cumulative spawning counted over 21 consecutive days from day 7	Cumulative spawning, fertilisation and hatching rates; gonadosomatic index; egg weight, protein and lipid content; ovarian histology (oocyte stage distribution); microcystin-LR accumulation in the ovary; ovarian vitellogenin; hepatic *vtg1* and *vtg2* expression; ovarian transcriptome	Ovarian microcystin-LR accumulation ↓ under co-administration at both exposure concentrations. At 22 µg/L: cumulative spawning ↓ vs. control from day 13; fertilisation and hatching rates ↓; primary oocyte proportion ↑ and cortical alveolar and late/mature oocyte proportions ↓; early vitellogenic oocyte proportion ↔. *L. rhamnosus* GG ↑ cumulative egg production vs. the microcystin-only group. Fertilisation and hatching rates ↔ control under co-administration, and the oocyte stage distribution returned to control values. *vtg1* and *vtg2* ↓ in all treated groups and lowest under the combined exposure, whereas ovarian VTG protein ↓ at 22 µg/L and partly recovered under *L. rhamnosus* GG. The ovarian transcriptome showed no significant functional or pathway enrichment under microcystin-LR alone. Gonadosomatic index, egg weight and egg protein and lipid content ↔ in all groups.	Liu et al., 2024
One-month-old zebrafish (AB strain); immersion and feeding	*Lactobacillus rhamnosus* GG (ATCC 53103) and its pilus-deficient mutant PB22 at 10^7 CFU/mL by immersion;	7 or 14 days (bacterial immersion); 3, 7	Intestinal histopathology; serum LPS; cleavage of Caspase-3, GSDMEa, Caspy2 and GSDMEb; gut microbiota	Wild-type LGG immersion: intestinal injury and serum LPS ↑; pilus-deficient PB22 caused less injury, and its serum LPS was lower than under wild-type LGG at day 14. Of the three pilin	Zhang et al., 2024

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
trials; germ-free, *casp3a*$^{-/-}$ and knockdown models also used	recombinant SpaA, SpaB or SpaC at 10 µg/mL; dietary SpaC at 0.5 and 1.0 mg/g and LPS at 0.2 mg/g	or 14 days (pilin immersion); 3 weeks (feeding)	(16S rRNA); receptor identification; pyroptotic rate in ZF4 and Caco-2 cells	subunits, only SpaC raised serum LPS. Dietary SpaC ↑ serum LPS at both doses and ↑ intestinal injury score at both doses, and the score at 1.0 mg/g did not differ from that under the LPS diet. Dietary SpaC cleaved Caspase-3, GSDMEa, Caspy2 and GSDMEb; the LPS diet cleaved Caspy2 and GSDMEb only. At 1.0 mg/g SpaC, Proteobacteria (24.6% → 39.4%) and Enterobacteriaceae ↑, as did OTUs with predicted LPS-producing capacity; the microbiota did not change at 0.5 mg/g. LGG immersion shifted the microbiota in the same direction and did not change total bacterial abundance. The shift was smaller under PB22. Gut microbiota from SpaC-fed fish transferred to germ-free fish cleaved Caspy2 and GSDMEb. SpaC ↑ pyroptosis in ZF4 cells in a concentration-dependent manner and ↔ in human Caco-2 cells. SpaC bound zTLR4ba and hTLR2 but not hTLR4.	
Adult zebrafish; *Aeromonas hydrophila* challenge at 1.5×10^8 or 1.5×10^4 CFU/mL for 5 days	*Lacticaseibacillus casei* ATCC 393, suspension at 1.5×10^8 CFU/mL mixed into the feed	30 days; 5-day challenge (days 30–35)	*il-1β* and *tnf-α* expression; intestinal villus height; intestinal histopathology; goblet cell number	Pre-challenge: *il-1β* and *tnf-α* ↓ vs. control at days 15 and 30. Post-challenge (day 35): both genes ↑ vs. control in basal-diet fish at both bacterial concentrations. In probiotic-fed fish the increase was smaller at 1.5×10^8 CFU/mL; at 1.5×10^4 CFU/mL *tnf-α* ↔ and *il-1β* ↓ vs. control. Villus height ↑ in both probiotic-fed groups at days 15 and 30. Day 35: severe necrosis, submucosal oedema, inflammatory cell infiltration and epithelial shedding in basal-diet fish at 1.5×10^8 CFU/mL; variable epithelial necrosis and goblet cells ↑ in probiotic-fed fish at the same concentration; villi intact and goblet cells ↑ (not significant) in probiotic-fed fish at 1.5×10^4 CFU/mL.	Alavinejad et al., 2023
Zebrafish fed a high-fat diet supplemented with a postbiotic, followed by *Aeromonas veronii* Hm091 challenge	Dried cultured supernatant mixture (SWFC) of *Lactococcus lactis* ZHY1 and *Cetobacterium somerae* XMX-1 at 0.3 and 0.4 g/kg of a high-fat diet	6 weeks; 48 h post-challenge	Growth performance and survival; liver histology, TAG and MDA; hepatic lipid metabolism, antioxidant and inflammatory gene expression; intestinal immune and inflammatory gene expression; gut microbiota; survival after challenge	WGR, FCR and survival during feeding ↔. Liver, both doses: TAG and MDA ↓; vacuolar fatty lesions ↓, described as less marked at 0.4 g/kg (histological observation); *ho-1* and *tgf-β* ↑; *nf-κb*, *cebpa*, *dgat2* and *acc1* ↓; *tnf-α*, *il-1β*, *nrf2*, *sod*, *gstr* and *ucp2* ↔. Liver, 0.3 g/kg only: *keap1b*, *pparγ* and *il-6* ↓; *pparα*, *cpt1* and *il-10* ↑. Liver, 0.4 g/kg only: *keap1a* ↓. Intestine, both doses: *nf-κb*, *tnf-α* and *il-1β* ↓; *il-10* and *defbl2* ↑; *il-6*, *tgf-β*, *muc2* and *hepcidin* ↔. Intestine, 0.3 g/kg only: *lysozyme*, *c3a* and *defbl1* ↑. Microbiota: Firmicutes ↑ at both doses, and an unclassified *Rhodobacteraceae* taxon ↑ at 0.4 g/kg; at 0.3 g/kg Proteobacteria and Cyanobacteria ↓, Acidobacteria and Chloroflexi ↑, *Novosphingobium*, *Shewanella*, *Gemmobacter*, *Aeromonas* and the same *Rhodobacteraceae* taxon ↓, Shannon and Simpson indices ↑, ACE and Chao1 ↔, and principal coordinate analysis separated this group from the control and the 0.4 g/kg group. Post-challenge survival ↑ at 0.3 g/kg ($p < 0.01$); increase at 0.4 g/kg not significant.	Li et al., 2023
Two-month-old male zebrafish in a feeding trial; second trial challenged with spring viremia of carp virus (SVCV) by	Stabilised culture of *Lactobacillus rhamnosus* GCC-3, 1% of diet (1×10^7 CFU/g diet)	4 weeks (first trial); challenge survival monitored for 14 days	Growth performance and survival; intestinal *occludin1*, *zo-1a*, *zo-1b* and *hif-1α* transcripts and HIF-1α protein; *gpx1a*, *cu/zn sod*, *mn sod*, *cat* and *gst* transcripts;	Final weight and weight gain ↑ and FCR ↓ vs. control; survival ↔. Intestinal *zo-1a* and *zo-1b* ↑; *occludin1* ↔. *hif-1α* ↑ at transcript and protein level. *gpx1a*, *cu/zn sod*, *mn sod* and *gst* ↑; *cat* ↔. T-AOC ↑, SOD ↑ and MDA ↓. Serum LPS ↓. Villus atrophy and lamina	Liang et al., 2023

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
intraperitoneal injection of 5×10^6 copies			T-AOC, MDA and SOD; serum LPS; intestinal histology and histopathological score; *il-1β*, *il-6* and *il-10* transcripts; gut microbiota (16S rRNA); splenic *ifnφ1*, *ifnφ2* and *mxc* transcripts; SVCV replication; post-challenge survival	propria thickness reduced; histopathological score ↓. *il-1β* ↓; *il-6* and *il-10* ↔. Proteobacteria ↓; Firmicutes and Actinobacteriota ↑. At genus level *Lactobacillus*, *Ralstonia* and *Mycobacterium* ↑ and *Herbaspirillum* ↓. After SVCV challenge: survival ↑ at 14 days; splenic *ifnφ1* and *ifnφ2* ↑ at day 2; *mxc* ↑ and SVCV replication ↓ at day 4.	
Gnotobiotic zebrafish larvae (3 dpf), co-exposed to *Aeromonas hydrophila* or *Edwardsiella tarda* at 0.1 OD (10^2 cells/mL) and the probiotic	*Bacillus subtilis* TLDK301120C24 isolated from tilapia, applied by immersion (10 µL of a 0.6 OD suspension, 10^7 cells/mL)	3 days	Colonisation by RFP-tagged probiotic and GFP-tagged pathogens (fluorescence microscopy, PCR, plating); pathogen LD50 by immersion; competitive inhibition measured as fluorescence intensity (ImageJ, fluorimetry)	TLDK301120C24 colonised the larval gut. During co-exposure with *A. hydrophila* or *E. tarda*, pathogen-associated GFP fluorescence ↓ and probiotic-associated RFP fluorescence ↑ between 4 and 6 dpf. No mortality was recorded in the co-exposed larvae. Pathogen LD50 by immersion was 1×10^2 cells/mL for both pathogens. The authors describe the result as semi-quantitative evidence of competitive exclusion.	Nayak et al., 2023
Healthy adult male zebrafish reared in a freshwater recirculating aquaculture system	Live *Lactobacillus helveticus* HA-122 (LHPro); heat-inactivated *L. helveticus* HA-122 (LHPost); heat-inactivated *Lactiplantibacillus plantarum* HA-119 (LPPost), all at 6×10^6 cells/g feed	5 weeks	Villus length; lamina propria width; goblet cell density and coverage; mucin chemotype; IELs; lysozyme and cathepsin L; *zo-2a* and *muc2.1* expression; innate immune markers (*tnf-α*, *il-1β*, *il-17a*, *ifn-γ*, *il-22*, *tgf-β*); $CD4^+$ and $CD8\alpha^+$ T cells	Posterior intestine. LHPost: lamina propria width ↓ vs. control. LHPro and LPPost: villus length ↑ vs. control. LPPost: goblet cell density and goblet cell coverage ↑ vs. control; LHPro: goblet cell coverage ↓ vs. control. Acidic mucin proportion ↑ in both heat-inactivated groups. IEL abundance ↑ in all three groups, largest with LHPost. *cathepsin L* and *zo-2a* expression ↑ vs. control in all treatments. *lysozyme* expression and lysozyme protein level ↑ with LHPost and LPPost. LPPost: *ifn-γ* ↑ vs. control ($p < 0.05$) and vs. all other groups, *il-22* ↑ vs. control ($p < 0.01$). $CD8\alpha^+$ T cell proportion ↑ in LPPost vs. all other treatments ($p < 0.01$). *tnf-α* ↔ vs. control in all treatments, but ↑ with LHPro vs. LHPost. *il-1β*, *il-17a* and *tgf-β* ↔.	Rawling et al., 2023
Zebrafish fed probiotic diets; all groups challenged with *Aeromonas hydrophila* (1.3×10^8 CFU/g) on day 56	*Lactobacillus delbrueckii* (G2), *Lactobacillus acidophilus* (G3), or both strains combined (G4); each diet contained 1×10^8 CFU/g	60 days (sampling at days 0, 28, 56 and 60)	Immune gene expression (*il-1β*, *tnf-α*); detection of probiotic strains in intestinal tissue; villus length and goblet cell number in proximal, middle and distal segments; intraepithelial cells; growth (weight, length); survival	Intestinal *L. acidophilus* signal ↑ vs. control in G4 at days 28, 56 and 60 and in G3 at day 56; *L. delbrueckii* signal ↑ vs. control in G2 and G4 at all three time points. Body weight ↑ vs. control in G2 at days 28 and 56; G3 and G4 ↔; height differed only from the day-0 baseline. Intestinal *il-1β* and *tnf-α* expression highest in the untreated control at all three sampling points; on day 56 *tnf-α* was lower than the control in G2 and G4; no other comparison with the control was significant, including after challenge. Survival highest in G4 and lowest in the untreated control; no statistical test reported. Villus length measured separately in three segments: longest proximally, shortest distally. At days 28 and 56, G4 villus length ↑ vs. G2 and G3 in all three segments. Goblet cell number ↑ in all segments vs. control at days 28 and 56, highest in G4 and higher in the middle segment than proximal or distal. Three days post-challenge (day 60), villus length ↓ in G1; probiotic-fed groups retained more length, most with G4; goblet cell number ↓ in G3 and G4 vs. control.	Ehsannia et al., 2022
Germ-free zebrafish larvae (6 dpf) challenged with multidrug-resistant	*Weissella confusa* DD_A7 powder at 2.5×10^7, 5×10^7 or 1×10^8 CFU/mL	12 h pre-treatment, 12 h in E3 media, 6 h bath exposure,	*nf-κb-p65*, *tnf-α*, *il-1β* and inos transcripts (RT-PCR); COX-2 (ELISA); nitric oxide (Griess); ROS (DCFH-DA);	Infection ↑ *nf-κb-p65* (2.2-fold), *tnf-α* (2.02), *il-1β* (2.13) and *inos* (2.45) transcripts (all $p < 0.001$), COX-2 (853.24 vs. 30.01 pg/mL in germ-free larvae; $p < 0.001$) and nitric oxide (65.05 vs. 5.1 µM; $p <$	Dey & Kang, 2020

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Zebrafish model / Exposure	Probiotic / postbiotic and dose	Duration	Parameters assessed	Key findings	Reference
Escherichia coli O157:H7 (1×10^8 CFU/mL) by bath immersion		then transferred to fresh E3 media and monitored to 3 dpi	bacterial CFU count; heartbeat rate and physical characteristics; survival; whole-mount immunohistochemistry (*NF-κB-p65, TNF-α*)	0.001). ROS ↑ at all four pathogen concentrations vs. germ-free larvae ($p < 0.001$), increasing with concentration. DD_A7 pre-treatment ↓ *nf-κb-p65* (1.11; $p < 0.001$), *tnf-α* (0.91; $p < 0.01$), *il-1β* (0.88; $p < 0.05$) and *inos* (0.73; $p < 0.05$) vs. infected larvae. COX-2 ↓ at all three DD_A7 concentrations (375.2 pg/mL at 1×10^8 CFU/mL). Nitric oxide ↓ at 5×10^7 ($p < 0.05$) and 1×10^8 CFU/mL (40.74 µM; $p < 0.01$) and ↔ at 2.5×10^7 CFU/mL. Survival: infected larvae and larvae pre-treated at 2.5×10^7 CFU/mL ↓ vs. germ-free ($p < 0.001$); at 5×10^7 CFU/mL ↓ vs. germ-free ($p < 0.01$); at 1×10^8 CFU/mL ↔ germ-free. DD_A7 alone ↔ germ-free for survival at all three concentrations. Whole-mount immunohistochemistry: NF-κB-p65 and TNF-α ↑ with infection and ↓ with DD_A7 pre-treatment. At 3 dpi, drastic changes in physical appearance and cardiac function in exposed larvae. CFU per larva ↑ with *E. coli* O157:H7 vs. DD_A7 ($p < 0.05$).	
Larval zebrafish: Nile red intestinal motility model (wild-type AB, 5 dpf) and TNBS-induced intestinal inflammation model (Tg(MPO:GFP), 3 dpf, 0.0125% TNBS)	*Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis subsp. lactis* Bi-07 at 55.6, 166.7 and 500 µg/mL, and *Lactobacillus rhamnosus* HN001 at 222, 667 and 2000 µg/mL, applied by immersion	24 h probiotic treatment (motility model); 48 h TNBS induction, then 48 h in fish water, then 48 h probiotic treatment (inflammation model)	Intestinal Nile red fluorescence as an inverse index of motility; intestinal neutrophil number; intestinal area as an index of mucosal damage; gut histopathology; *il-6* and *tgfβ-1* expression (qPCR)	All three strains ↑ intestinal motility at the middle and high concentrations and ↔ at the lowest. Neutrophil recruitment ↓ under HN001 at the middle and high concentrations and under Bi-07 at all three; NCFM ↔ at every concentration. Mucosal damage ↓ under HN001 and Bi-07 at the highest concentration only; NCFM ↔. Histopathology: intestinal dilatation and mucosal structure recovered under HN001 and Bi-07, and sections from NCFM-treated fish resembled the inflammation model. *il-6* ↓ under HN001 at the middle and high concentrations and ↔ under Bi-07; *tgfβ-1* ↑ under HN001 at the middle and high concentrations and under Bi-07 at all three. In the tolerance trial NCFM caused 73.3% mortality at 1500 µg/mL and 100% at 2000 µg/mL, and Bi-07 caused 76.7% mortality at 1000 µg/mL and 100% at 1500 and 2000 µg/mL; HN001 caused no abnormality up to 2000 µg/mL.	Wang et al., 2020

↑ increased, ↓ decreased and ↔ no significant difference, relative to the control group unless another comparison is stated. Gene names are given in lower-case italics and protein names in roman type.
ACE, abundance-based coverage estimator; ACH50, 50% haemolytic activity of complement; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMP, antimicrobial peptide; AST, aspartate aminotransferase; ASV, amplicon sequence variant; CAT, catalase; CFU, colony-forming unit; COX-2, cyclo-oxygenase-2; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; dpf, days post fertilisation; dpi, days post infection; ELISA, enzyme-linked immunosorbent assay; FCR, feed conversion ratio; GFP, green fluorescent protein; GO, gene ontology; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; GSEA, gene set enrichment analysis; GSH, glutathione; GSH-Px and GPx, glutathione peroxidase; GST, glutathione S-transferase; HIF-1α, hypoxia-inducible factor 1α; IEL, intraepithelial leukocyte; IFNφ, interferon phi; IgM, immunoglobulin M; KEGG, Kyoto Encyclopedia of Genes and Genomes; LD50, median lethal dose; LGG, *Lactobacillus rhamnosus* GG; LHPost, heat-inactivated *Lactobacillus helveticus* HA-122; LHPro, live *Lactobacillus helveticus* HA-122; LPPost, heat-inactivated *Lactiplantibacillus plantarum* HA-119; LPS, lipopolysaccharide; LZM, lysozyme; MDA, malondialdehyde; OD, optical density; ORA, over-representation analysis; OTU, operational taxonomic unit; PBS, phosphate-buffered saline; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; PERMANOVA, permutational multivariate analysis of variance; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PICRUSt2, phylogenetic investigation of communities by reconstruction of unobserved states 2; POD, peroxidase; RFP, red fluorescent protein; RNA-seq, RNA sequencing; ROS, reactive oxygen species; sfGFP, superfolder green fluorescent protein; SGR, specific growth rate; SOD, superoxide dismutase; SR, survival rate; SVCV, spring viremia of carp virus; SWFC, cultured supernatant mixture of *Cetobacterium somerae* and *Lactococcus lactis*; T-AOC, total antioxidant capacity; TAG, triacylglycerol; TCID50, 50% tissue culture infectious dose; TNBS, 2,4,6-trinitrobenzene sulfonic acid; VHSV, viral haemorrhagic septicaemia virus; VTG, vitellogenin; WGR, weight gain rate.

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Immune regulation and inflammatory response

In aquaculture, immune regulation involves maintaining a balance between an effective response to pathogens and the control of excessive or prolonged inflammation. Probiotics and postbiotics may influence this balance by modulating the expression of cytokines, innate immune effectors, and genes associated with intestinal barrier function. However, the immune responses elicited by these interventions may vary depending on the strain used, the inactivation or preparation method (Yang et al., 2014), the administered dose (Li et al., 2008), the tissue examined (Wang et al., 2022), and whether the fish were subjected to a pathogen challenge (Chomová et al., 2025).

Rawling et al. (2023) compared live and heat-inactivated *Lactobacillus helveticus* HA-122 with heat-inactivated *Lactiplantibacillus plantarum* HA-119 in healthy zebrafish. In the posterior intestine, the inactivated *L. plantarum* preparation raised *ifn-γ* expression above all other groups and increased *il-22* expression by 65% relative to the control. The same preparation produced a higher proportion of $CD8\alpha^{+}$ T cells than any other treatment. Expression of *tnf-α* did not differ from the control under any treatment, but the live *L. helveticus* preparation gave higher *tnf-α* expression than its inactivated form. Expression of *il-1β* and *il-17a* did not change.

Nikapitiya et al. (2026) fed adult zebrafish postbiotics from three heat-inactivated lactobacilli and profiled 16 immune-related genes in gut and kidney tissue. Each strain produced a different expression profile, and the two tissues responded differently. All three treatments raised gut expression of *tnf-α*, *ifn-γ*, *mx*, *defβ1* and *muc5.1*, and kidney expression of *tlr5b*, *il-1β* and *cd8a*. Downregulation occurred only in the kidney. In the gut, expression of *ccl34a.4* increased with HA-108 and HA-122, and that of *cxcl18b* with HA-119 and HA-122. *Lactiplantibacillus plantarum* HA-119 upregulated 13 of the 16 genes in the gut, the largest number among the three strains. After viral haemorrhagic septicaemia virus challenge, survival curves in the *L. plantarum* HA-119 and *Lactobacillus helveticus* HA-122 groups differed significantly from the challenged control.

In Alavinejad et al. (2023) dietary *Lacticaseibacillus casei* lowered *il-1β* and *tnf-α* mRNA levels at days 15 and 30 relative to the unchallenged control fed the basal diet. After *Aeromonas hydrophila* challenge both genes rose above the control in the basal-diet groups at both bacterial concentrations. In the probiotic-fed fish the increase was smaller at the higher concentration, and at the lower concentration *tnf-α* did not differ from the unchallenged control and *il-1β* fell below it. The authors attribute the increased resistance to *A. hydrophila* to modulation of *tnf-α* and *il-1β* expression together with the increase in villus length and in the number of intestinal immune cells.

Jiang et al. (2024) fed zebrafish *Lactobacillus plantarum* SHY21-2 and then exposed them to *Aeromonas hydrophila*. Infection raised *tnf-α*, *il-6* and *il-1β* expression in muscle and *tnf-α*, *il-6* and *lamp2* expression in the intestine. In infected fish the probiotic lowered all six. Muscle *il-1β* and intestinal *tnf-α* and *il-6* returned to the control level, and the other three remained above it. In uninfected fish the strain lowered muscle *il-6* and *il-1β* and intestinal *il-6* below the control.

Ehsannia et al. (2022) fed zebrafish *Lactobacillus delbrueckii* (G2), *Lactobacillus acidophilus* (G3) or both (G4), and all groups were challenged with *Aeromonas hydrophila*. Intestinal *il-1β* and *tnf-α* expression was highest in the untreated control at every sampling point. Before challenge, *tnf-α* was lower than the control in G2 and G4. After challenge, no probiotic group differed from the control. Survival was lowest in the untreated control and highest in the group receiving both strains.

Wang et al. (2020) induced intestinal inflammation in zebrafish larvae with trinitrobenzenesulfonic acid and treated them with three probiotic strains by immersion.

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Lactobacillus rhamnosus HN001 lowered the number of intestinal neutrophils at the middle and high concentrations, and *Bifidobacterium animalis* subsp. *lactis* Bi-07 lowered it at all three concentrations. *Lactobacillus acidophilus* NCFM did not differ from the model group at any concentration. *il-6* expression fell under HN001 at the middle and high concentrations and did not change under Bi-07. *tgfβ-1* expression rose under HN001 at the middle and high concentrations and under Bi-07 at all three.

In Liang et al. (2023) intestinal *il-1β* expression was lower than in the control after 4 weeks of feeding a stabilised culture of *Lactobacillus rhamnosus* GCC-3, and *il-6* and *il-10* did not differ. In germ-free zebrafish larvae challenged with *Escherichia coli* O157:H7, Dey & Kang (2020) reported that *Weissella confusa* DD_A7 pre-treatment lowered transcript levels of *nf-κb-p65*, *tnf-α*, *il-1β* and *inos*. COX-2 protein fell at all three probiotic concentrations, and nitric oxide fell at the two higher concentrations. The probiotic powder was suspended in the water for 12 h before bath-immersion challenge, which the authors describe as oral administration.

Permethrin exposure in male zebrafish increased testicular *il-1β*, *il-6* and *tnf-α* transcripts and IL-1β, IL-6 and TNF-α protein (Huo et al., 2025). Dietary supplementation with viable *Lactobacillus casei* LC89 lowered these parameters in a dose-dependent manner. The six parameters no longer differed from the control at the highest dose. At the lowest dose, the decrease in IL-1β and IL-6 protein was not significant. Heat-killed cells of the same strain had no significant effect on any of the six.

Zhang et al. (2026) fed zebrafish a *Bacillus subtilis* strain secreting piscidin-1 and hepcidin in tandem, using an empty-vector strain as the reference group. After *Aeromonas hydrophila* challenge, intestinal *tlr4* and *nlrb1* expression fell significantly, as did the downstream signalling genes *myd88*, *traf6*, *ikbkb*, *fos* and *jun*. Expression of *trif*, *mapk* and *ripk2* did not change. The two strains secreting a single peptide did not alter *tlr4* or *nlrb1*. Expression of *il-10* rose and *tnf-α* declined.

Li et al. (2023) added SWFC, a cultured supernatant mixture of *Cetobacterium somerae* and *Lactococcus lactis*, to a high-fat diet at 0.3 or 0.4 g/kg. In the liver, both doses lowered *nf-κb* expression and raised *tgf-β* expression relative to the high-fat control, whereas *il-6* decreased and *il-10* increased only at 0.3 g/kg. Hepatic *tnf-α* and *il-1β* expression did not change significantly. In the intestine, both doses lowered *nf-κb*, *tnf-α* and *il-1β* expression and raised *il-10* expression. Intestinal lysozyme, *c3a*, *defbl1* and *defbl2* expression increased at 0.3 g/kg, and *defbl2* also increased at 0.4 g/kg.

García-Márquez et al. (2026) fed zebrafish a diet containing 1% lyophilised, ethanol-inactivated *Vibrio proteolyticus* DCF12.2. Gene set enrichment analysis showed downregulation of the cytokine–cytokine receptor interaction pathway and upregulation of metabolic pathways, including lipid metabolism and oxidative phosphorylation. The authors describe the combination of reduced immune sensitivity and heightened lipid turnover as an altered immunometabolic state. In their interpretation, the reduced cytokine signalling may reflect a controlled inflammatory tone, and they also raise the possibility of immunosuppression.

Gao et al. (2024) compared a yeast prebiotic, a yeast probiotic, black soldier fly larvae meal and sodium butyrate in zebrafish, then challenged them with *Pseudomonas aeruginosa* LPS or live *Flavobacterium psychrophilum*. Twenty-four hours after the pathogen challenge, butyrate lowered hepatic expression of *tnf-α*, *il-1β*, *hepcidin*, *saa*, *nf-κb/p65*, *pgrp* and *caspase-b*. Black soldier fly larvae meal lowered *nf-κb/p65*. The two yeast diets produced no significant change against the control. In unchallenged fish, *nf-κb/p65* expression did not differ significantly between the black soldier fly larvae meal group and the control. No diet affected weight gain, and no mortality followed either challenge.

These studies do not converge on a single direction of effect. Pro-inflammatory genes fall in some and rise in others, and no single feature of the preparations accounts for the difference. In Rawling et al. (2023) the live and heat-inactivated forms of one strain gave different *tnf-α*

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responses, so the effect cannot be attributed to the strain alone. The outcome of heat inactivation also differed between studies. Heat-inactivated *L. plantarum* altered cytokine expression in Rawling et al. (2023), whereas heat-killed *L. casei* did not lower permethrin-induced cytokine levels in Huo et al. (2025). Cytokine responses also differed according to whether expression was measured before or after pathogen challenge. In Gao et al. (2024) black soldier fly larvae meal lowered hepatic *nf-κb/p65* expression after challenge, whereas in unchallenged fish the difference from the control was not significant. In Liang et al. (2023) intestinal *il-1β* expression fell in unchallenged fish, and *il-6* and *il-10* did not differ. In Alavinejad et al. (2023) *L. casei* lowered *il-1β* and *tnf-α* expression before challenge, and after infection both genes increased in the unsupplemented fish at both bacterial concentrations. In the supplemented fish the increase was smaller at the higher concentration, and at the lower concentration the genes did not rise above the control. Changes in cytokine expression did not identify which groups survived pathogen challenge better. In Ehsannia et al. (2022) the group with the highest survival showed no difference from the control in post-challenge *il-1β* and *tnf-α* expression. In Nikapitiya et al. (2026) gut *tnf-α* and kidney *il-1β* expression increased under all three strains, but survival improved significantly under only two of them. In García-Márquez et al. (2026) the cytokine–cytokine receptor interaction pathway was downregulated in fish that were not challenged, and the authors raise the possibility of immunosuppression.

Oxidative stress and antioxidant defence

Oxidative stress occurs when reactive oxygen species accumulate beyond the capacity of cellular antioxidant systems. In farmed aquatic animals it contributes to organ damage, immunosuppression and metabolic disruption (Ma et al., 2025). Assessment relies on the activities of superoxide dismutase, catalase and glutathione peroxidase (Weydert and Cullen, 2010), on total antioxidant capacity, and on malondialdehyde as a marker of lipid peroxidation. These parameters have been measured in zebrafish across different tissues, doses and stressor conditions.

Esfahani et al. (2025) fed zebrafish *Lactobacillus plantarum*, *Lactobacillus reuteri* or both for 8 weeks and measured antioxidant parameters in mucus. Glutathione peroxidase activity and total antioxidant capacity increased in fish receiving *L. reuteri* or the combination, and catalase activity increased only with the combination. Superoxide dismutase activity and malondialdehyde content did not differ between groups. Expression of *gpx* and *sod* increased in all supplemented groups, and *cat* increased with *L. reuteri* and with the combination. Expression of *gpx* was highest in fish receiving both strains.

Nikapitiya et al. (2026) measured *cat* expression in gut and kidney tissue after 21 days of feeding postbiotics from three heat-inactivated *Lactobacillus* strains. Kidney *cat* expression decreased under all three treatments. *Lactiplantibacillus plantarum* HA-119 increased *cat* expression in the gut.

In Liang et al. (2023) intestinal *gpx1a*, *cu/zn sod*, *mn sod* and *gst* expression increased after 4 weeks of feeding a stabilised culture of *Lactobacillus rhamnosus* GCC-3, and *cat* did not differ from the control. Total antioxidant capacity and superoxide dismutase increased and malondialdehyde content decreased.

Qiu et al. (2025) added *Lysinibacillus sphaericus* HY3 to the culture water at three concentrations. Hepatic superoxide dismutase and catalase activities showed a significant quadratic rising trend with concentration. Values were lowest at the lowest concentration and highest at the highest. Neither enzyme differed from the control in pairwise comparison. Malondialdehyde content decreased linearly with concentration and was significantly lower than the control at the two higher doses.

In Jiang et al. (2024) *Aeromonas hydrophila* infection reduced hepatic superoxide dismutase, peroxidase, catalase and glutathione peroxidase activities and raised malondialdehyde content.

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In infected fish, supplementation with *Lactobacillus plantarum* SHY21-2 raised superoxide dismutase above the infected group. The value remained below the control. Peroxidase, catalase, glutathione peroxidase and malondialdehyde did not differ from the infected group. In uninfected fish the same strain raised superoxide dismutase and peroxidase activities above the control.

Huo et al. (2025) exposed male zebrafish to permethrin for 30 days and measured oxidative stress parameters in testicular tissue. Exposure raised reactive oxygen species and malondialdehyde and lowered total antioxidant capacity and superoxide dismutase, catalase and glutathione peroxidase activities. Dietary *Lactobacillus casei* attenuated these changes dose-dependently. Only reactive oxygen species and malondialdehyde changed at the lowest dose. The dose at which a parameter returned to control values differed between parameters. Heat-killed cells of the same strain gave no protection.

Wang et al. (2025) exposed zebrafish to PFOS, PFOA or both for 21 days, and each exposure was tested with and without dietary *Lactobacillus rhamnosus*. Hepatic superoxide dismutase activity was lower than in the control in every exposure group at all three sampling points. Intestinal superoxide dismutase activity was lower than in the control from day 14, and at day 7 it was higher than the control under PFOS. *L. rhamnosus* raised hepatic superoxide dismutase activity in the PFOA and combined-exposure groups at days 14 and 21 and did not raise it in the PFOS group. Intestinal catalase activity was higher in the combined-exposure group given *L. rhamnosus* than in the combined-exposure group at all three sampling points. Intestinal malondialdehyde content fell below the control under PFOA at all three sampling points, and *L. rhamnosus* raised it above the exposed groups from day 14. Hepatic malondialdehyde content did not differ from the control under PFOA, rose above the control under PFOS and the combined exposure at day 7 and returned to control values by day 21. Glutathione content was lower than in the control in the intestine and the liver in every exposure group, and *L. rhamnosus* did not restore it.

Li et al. (2023) fed a cultured supernatant mixture of *Cetobacterium somerae* and *Lactococcus lactis* at 0.3 or 0.4 g/kg to zebrafish on a high-fat diet. Both doses reduced hepatic malondialdehyde content and raised *ho-1* expression relative to the high-fat control. Expression of *keap1b* decreased at 0.3 g/kg and that of *keap1a* at 0.4 g/kg. Expression of *nrf2*, *sod* and *gstr* did not change significantly.

Jiang et al. (2024) and Huo et al. (2025) applied an infectious or a chemical stressor. In both, enzyme activity fell and malondialdehyde rose. Supplementation returned these parameters towards control values in Huo et al. (2025), whereas in Jiang et al. (2024) only superoxide dismutase improved. Li et al. (2023) used a high-fat diet rather than a toxic or infectious stressor and did not include a group fed a standard diet, but supplementation lowered hepatic malondialdehyde content. Wang et al. (2025) also applied a chemical stressor. Superoxide dismutase activity and glutathione content were lower than in the control in the intestine and the liver, but malondialdehyde was at or below control values at the end of exposure. Supplementation raised hepatic superoxide dismutase activity in two of the three exposure groups and did not restore glutathione. Results without a stressor were more variable. Esfahani et al. (2025) found increased expression of *gpx* and *sod* in all supplemented groups and of *cat* with *L. reuteri* and with the combination, but superoxide dismutase activity did not change in any group. In Liang et al. (2023) intestinal *gpx1a*, *cu/zn sod*, *mn sod* and *gst* expression increased and *cat* did not differ from the control. Total antioxidant capacity increased and malondialdehyde content decreased. In Nikapitiya et al. (2026) *L. plantarum* HA-119 increased *cat* expression in the gut and decreased it in the kidney. Dose responses differed among the studies that tested more than one concentration. Qiu et al. (2025) recorded a significant quadratic rising trend in superoxide dismutase and catalase activity. Neither enzyme differed

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from the control at any concentration. In Huo et al. (2025) some parameters returned to control values at the intermediate dose and others only at the highest.

Intestinal barrier integrity and histomorphology

Assessment of intestinal barrier function combines structural and molecular measurements. Villus height, crypt depth, goblet cell number, mucin chemotype and the abundance of intraepithelial leukocytes describe the structure; the expression of tight junction genes describes the molecular state. Studies in zebrafish differ in which of these parameters they measured, in the intestinal segment examined, and in whether the fish were exposed to a pathogen.

Rawling et al. (2023) compared live and heat-inactivated *Lactobacillus helveticus* HA-122 with heat-inactivated *Lactiplantibacillus plantarum* HA-119 in healthy zebrafish. In the posterior intestine, villi were longer than in the control with live *L. helveticus* and with heat-inactivated *L. plantarum*, and lamina propria width was lower than in the control with heat-inactivated *L. helveticus*. Heat-inactivated *L. plantarum* raised goblet cell density and goblet cell coverage above the control, whereas live *L. helveticus* lowered goblet cell coverage below the control. The proportion of acidic mucins increased in both heat-inactivated groups. Intraepithelial leukocyte abundance increased in all three groups, and the largest increase occurred with heat-inactivated *L. helveticus*. Expression of *zo-2a* increased in all three groups.

Ehsannia et al. (2022) measured villus length in the proximal, middle and distal regions separately and found the longest villi proximally and the shortest distally. At 28 and 56 days the group receiving both *Lactobacillus delbrueckii* and *Lactobacillus acidophilus* had longer villi than either single-strain group. Goblet cell number was higher in the middle segment than in the proximal and distal segments. Three days after *Aeromonas hydrophila* challenge villus length dropped in the untreated control. The probiotic-fed groups retained more length, and the combined treatment retained the most. Goblet cell number at that point was lower than in the untreated control in the group fed *L. acidophilus* alone and in the group fed both strains.

Alavinejad et al. (2023) measured villus height at days 0, 15 and 30. Both probiotic-fed groups had longer villi than the control at days 15 and 30. On day 35 the basal-diet group challenged at the higher bacterial concentration showed severe necrosis, submucosal oedema, inflammatory cell infiltration and epithelial shedding. The probiotic-fed group at the same concentration showed variable epithelial necrosis and had more goblet cells than the probiotic-fed unchallenged group and the basal-diet challenged group. At the lower concentration the basal-diet fish showed necrosis at the villus tips, whereas the probiotic-fed fish retained intact villus structure.

In Wang et al. (2025) zebrafish were exposed to PFOA, PFOS or both. Control fish had intact villi and undamaged epithelial cells. The pollutant-exposed groups showed fewer goblet cells, epithelial shedding and vacuolisation. Damage was least severe under PFOA and most severe under the combined exposure. *Lactobacillus rhamnosus* supplementation partially reduced these changes.

Wang et al. (2020) measured the intestinal cavity area of zebrafish larvae with trinitrobenzenesulfonic acid-induced inflammation as an index of mucosal damage. The area fell under *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* Bi-07 at the highest concentration and did not change under *Lactobacillus acidophilus* NCFM. In histological sections the intestinal dilatation and the mucosa recovered under HN001 and Bi-07, and sections from fish given NCFM resembled those of the inflammation model.

Jiang et al. (2024) reported structural breaks in the intestine and villi after *Aeromonas hydrophila* exposure. Expression of *claudin-2* and *zo-1* fell, and *occludin* did not differ from the control. *Lactobacillus plantarum* SHY21-2 attenuated the histological damage. In infected fish *zo-1* returned to the control level, and *claudin-2* rose above the infected group but remained

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below the control. In uninfected fish the same strain raised *claudin-2* and *occludin* above the control, and the intestinal villi appeared denser and more intact.
Liang et al. (2023) fed zebrafish a stabilised culture of *Lactobacillus rhamnosus* GCC-3. Villus atrophy and lamina propria thickness were reduced and histopathological scores were lower than in the control. Intestinal expression of both *zo-1* subunits increased and *occludin1* did not differ from the control. *hif-1α* transcript and HIF-1α protein both increased. Serum lipopolysaccharide content decreased.
Zhang et al. (2024) immersed zebrafish in *Lactobacillus rhamnosus* GG and found intestinal histopathological damage and higher serum lipopolysaccharide than in the control. A pilus-deficient mutant of the same strain caused less damage. Among the three recombinant pilin subunits, only SpaC raised serum lipopolysaccharide. A diet containing SpaC raised the intestinal injury score at both doses, and at 1.0 mg/g the score did not differ from that under a diet containing lipopolysaccharide. Caspase-3, GSDMEa, Caspy2 and GSDMEb were cleaved in the intestine of SpaC-fed fish, and only Caspy2 and GSDMEb in fish fed lipopolysaccharide. The authors attribute the damage to pyroptosis of the intestinal epithelium.
Qiu et al. (2025) added *Lysinibacillus sphaericus* HY3 to the culture water at three concentrations. Villus height, crypt depth and goblet cell number per villus increased linearly with concentration. The ratio of villus height to crypt depth did not change. Control fish had short, narrow and sparsely distributed villi with a thin muscle layer. At the highest concentration the villi were evenly distributed and the muscle and submucosal layers better developed.
Nikapitiya et al. (2026) assessed only goblet cell density and villus height in the intestine, and neither differed between the control and the groups fed three heat-inactivated lactobacilli. Kidney histology showed no structural alteration, inflammatory change or tissue damage in any group.
Dede & Çek (2025) fed juvenile zebrafish *Bacillus subtilis* HBB 493® at four dietary levels for 100 days. Control fish showed ruptured villi and an enlarged lumen. Villi and goblet cells remained intact at every supplementation level. No hypertrophy, villus fusion or degeneration of the mucosal lining was observed. Villus length tended to be higher than in the control, but was not quantified.
Zhang et al. (2026) measured the muscularis thickness of the anterior intestine after four *Aeromonas hydrophila* challenges. The strain secreting piscidin-1 and hepcidin in tandem gave a greater thickness than the empty-vector strain.
Four of these studies applied a pathogen. In three of them, infected fish showed histological damage, and the damage was less severe in the probiotic-fed groups. In Zhang et al. (2026) the muscularis of the anterior intestine was thicker under the strain secreting both peptides than under the empty-vector strain. Wang et al. (2025) and Wang et al. (2020) applied a chemical stressor. Supplementation reduced the mucosal damage in Wang et al. (2025), and in Wang et al. (2020) two of the three strains did so at the highest concentration. Results without a pathogen challenge were mixed. Villus height, crypt depth and goblet cell number increased with concentration in Qiu et al. (2025), whereas villus height and goblet cell density did not change in Nikapitiya et al. (2026). In Zhang et al. (2024) the supplement itself damaged the intestinal epithelium. Rawling et al. (2023) was the only study of intestinal histology to compare live and inactivated forms of one strain, and the two forms differed in villus length and lamina propria width. In Nikapitiya et al. (2026) each strain upregulated at least half of the 16 immune-related genes in the gut, but goblet cell density and villus height did not change.

Gut microbiota composition

Changes in the gut microbiota are reported at several levels of analysis, and the studies considered here differ in which levels they include. Alpha diversity indices measure the diversity within a sample, including richness and evenness, whereas beta diversity compares

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community composition between samples. Taxonomic profiles report composition at ranks such as phylum and genus. Amplicon sequence variants resolve composition at the level of individual sequences (Callahan et al., 2017), although they do not necessarily correspond to distinct species or strains (Johnson et al., 2019). Predicted functional profiles, reported in several of the studies, are inferred from marker-gene sequences and reference genomes and do not measure metabolic activity directly (Douglas et al., 2020).

García-Márquez et al. (2026) fed zebrafish an ethanol-inactivated, freeze-dried *Vibrio proteolyticus* DCF12.2 preparation. Alpha and beta diversity did not differ from the control, nor did composition at phylum or genus level. *Cetobacterium* was the most abundant genus in both dietary groups. Significant differences appeared only at the amplicon sequence variant level. Variants affiliated with *Cetobacterium* increased in both dietary groups, and variants affiliated with *Aeromonas*, *Crenobacter*, *Delftia*, *Plesiomonas*, *Ralstonia*, *Shewanella*, *Stenotrophomonas* and ZOR0006 increased in the supplemented group. Predicted microbial function did not differ.

In Liang et al. (2023) Proteobacteria decreased, and Firmicutes and Actinobacteriota increased. At genus level *Lactobacillus*, *Ralstonia* and *Mycobacterium* increased and *Herbaspirillum* decreased.

In zebrafish on a high-fat diet, Li et al. (2023) supplemented the diet with a dried cultured supernatant mixture of *Cetobacterium somerae* and *Lactococcus lactis*. At 0.3 g/kg Proteobacteria and Cyanobacteria decreased and Firmicutes, Acidobacteria and Chloroflexi increased. At genus level *Novosphingobium*, *Shewanella*, *Gemmobacter*, *Aeromonas* and an unclassified *Rhodobacteraceae* taxon decreased. Shannon and Simpson indices were higher than in the control, ACE and Chao1 did not differ, and principal coordinate analysis separated this group from both the control and the 0.4 g/kg group. At 0.4 g/kg, Firmicutes was the only one of the phyla tested that changed, and the same *Rhodobacteraceae* taxon increased.

Qiu et al. (2025) applied *Lysinibacillus sphaericus* HY3 through the culture water rather than the diet, at three concentrations. The ACE, Chao1 and Shannon indices did not differ between groups. Principal component analysis separated the supplemented groups from the control, and PERMANOVA gave a significant difference in beta diversity ($p = 0.002$). At phylum level Fusobacteriota increased and Acidobacteriota decreased at the intermediate concentration only. Planctomycetota decreased at the lowest concentration and increased at the intermediate one. At genus level *Cetobacterium* and *Aeromonas* increased at the intermediate concentration, *Paracoccus* increased at the two higher concentrations, *Bacillus* decreased at all three, and *Rhodobacteraceae* decreased at the two higher concentrations. *Gemmobacter* decreased below the control at the lowest concentration.

In Jiang et al. (2024) the Chao1 and Sob indices were higher than the control after *Aeromonas hydrophila* infection and in both groups receiving *Lactobacillus plantarum* SHY21-2, and the Shannon index did not differ. At phylum level the two probiotic groups had lower Proteobacteria and Bacteroidota and higher Firmicutes than the control, and Verrucomicrobiota was higher in the infected group than in the other three. At genus level infection enriched unclassified Parachlamydiaceae, *Rhodococcus*, unclassified Microbacteriaceae and *Bosea*, and fish receiving the probiotic did not show this enrichment. *Lactiplantibacillus*, *Leucobacter* and *Gemmobacter* increased and *Achromobacter* decreased in both probiotic groups.

Wang et al. (2025) exposed zebrafish to PFOA, PFOS or both, and each exposure was tested with and without dietary *Lactobacillus rhamnosus*. Relative to the pollutant-exposed groups, the probiotic increased Firmicutes and Actinobacteria and decreased Proteobacteria. At genus level *Leucobacter* increased and *Rhodobacter*, *Gordonia* and *Rhodococcus* decreased. *Microbacterium* increased under PFOA and PFOS and decreased under the combined exposure. The Chao1 index was higher in the supplemented groups than in the control, and principal coordinate analysis separated the supplemented from the unsupplemented groups. The

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contribution of *Rhodococcus* to the PWY-7315 pathway rose under PFOA exposure and returned towards control values in the supplemented group.

In Zhang et al. (2024) zebrafish were given the SpaC pilin subunit of *Lactobacillus rhamnosus* GG in the diet, and whole *L. rhamnosus* GG was also applied by immersion. Serum lipopolysaccharide increased in SpaC-fed fish. At the higher dietary dose Proteobacteria and Enterobacteriaceae increased, as did operational taxonomic units with predicted lipopolysaccharide-producing capacity. Immersion in whole *L. rhamnosus* GG shifted the microbiota in the same direction, and the shift was smaller with a pilus-deficient mutant. Transfer of the gut microbiota from SpaC-fed fish to germ-free fish activated the same pyroptosis pathway. The authors take this as evidence that the microbial shift contributes to the epithelial injury.

The taxa that changed differed among the studies, and some changed in opposite directions. Proteobacteria decreased in Liang et al. (2023), in Li et al. (2023) and in Jiang et al. (2024), and in Wang et al. (2025) relative to unsupplemented pollutant-exposed fish, but increased in Zhang et al. (2024) at the higher dose of purified SpaC pilin. In Li et al. (2023) the decreases in Proteobacteria, *Gemmobacter* and *Aeromonas* occurred at 0.3 g/kg. *Gemmobacter* increased in Jiang et al. (2024), and in Qiu et al. (2025) it fell below the control at the lowest concentration. *Aeromonas* increased at the intermediate concentration in Qiu et al. (2025); both Qiu et al. (2025) and Li et al. (2023) used an *Aeromonas* species as the challenge organism. *Cetobacterium*, one of the two genera used to prepare the supernatant in Li et al. (2023), increased at the intermediate concentration in Qiu et al. (2025) and was the most abundant genus in both dietary groups in García-Márquez et al. (2026). García-Márquez et al. (2026) was the only study to analyse amplicon sequence variants and found no differences at genus level, so the increase in variants affiliated with *Aeromonas* in that study is not directly comparable with the genus-level decrease in Li et al. (2023).

Pathogen resistance

Most studies report survival or cumulative mortality after a challenge. Some quantify pathogen load in host tissue, and studies in larvae track the applied organism and the pathogen simultaneously by fluorescent tagging. Species of *Aeromonas* are the most frequently used challenge organisms. The genus is a major cause of disease in aquaculture and carries zoonotic potential (Tao et al., 2026). This concentration on one genus limits what can be concluded about other agents.

Esfahani et al. (2025) challenged zebrafish with *Aeromonas hydrophila* after feeding *Lactobacillus plantarum*, *Lactobacillus reuteri* or both. Cumulative mortality over 14 days was lower in all supplemented groups than in the control and lowest with the combined treatment.

Jiang et al. (2024) exposed zebrafish to *Aeromonas hydrophila* by immersion after feeding *Lactobacillus plantarum* SHY21-2. Mortality was 4.4% in the control, 24.4% after infection and 13.3% when the probiotic was given before infection. The probiotic alone did not affect survival.

Qiu et al. (2025) challenged zebrafish with *Aeromonas hydrophila* after applying *Lysinibacillus sphaericus* HY3 through the culture water at three concentrations. Mortality in the control group started on day 3 and reached 100% by day 6. Mortality started later as the applied concentration increased, and survival at the highest concentration was higher than in the control. All groups reached 100% mortality by day 7.

Zhang et al. (2026) challenged zebrafish with *Aeromonas hydrophila* after feeding engineered *Bacillus subtilis* strains secreting piscidin-1, hepcidin, or both peptides in tandem. Survival was higher with the tandem strain than with either single-peptide strain or the empty-vector control. Intestinal pathogen load decreased with the piscidin strain, and the largest decrease occurred

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with the tandem strain. The control group received the empty-vector strain rather than an unsupplemented diet.

Li et al. (2023) challenged zebrafish with Aeromonas veronii after feeding a high-fat diet supplemented with a dried cultured supernatant mixture of Cetobacterium somerae and Lactococcus lactis. Survival at the lower dose was higher than in the control. At the higher dose the increase was not significant.

Liang et al. (2023) challenged zebrafish with spring viremia of carp virus after feeding a stabilised culture of *Lactobacillus rhamnosus* GCC-3. Survival was higher than in the control. Splenic *ifnφ1* and *ifnφ2* expression was higher 2 days after challenge. Four days after challenge *mxc* expression was higher and viral replication was lower.

Nikapitiya et al. (2026) challenged zebrafish with viral haemorrhagic septicaemia virus after 21 days of feeding postbiotics from three heat-inactivated *Lactobacillus* strains. Survival at 10 days post-challenge was numerically higher in all three postbiotic groups than in the challenged control, and survival curves differed significantly from the control for *Lactiplantibacillus plantarum* HA-119 and *Lactobacillus helveticus* HA-122. Viral copy number in the kidney of fish that survived to 10 days post-challenge did not differ among the challenged groups.

Nayak et al. (2023) co-exposed gnotobiotic zebrafish larvae to RFP-tagged *Bacillus subtilis* isolated from tilapia and to GFP-tagged *Aeromonas hydrophila* or *Edwardsiella tarda*. The isolate colonised the larval gut. Green fluorescence from the pathogens fell between 4 and 6 dpf and red fluorescence from the isolate rose. No mortality was recorded in the co-exposed larvae. The authors describe this as semi-quantitative evidence of competitive exclusion.

Nikapitiya et al. (2026) found higher survival without a difference in kidney viral load. Viral load was measured only in fish that survived to the end of the challenge, so these data do not show whether protection involved reduced viral replication. The strength of the evidence differed. Esfahani et al. (2025) gave no statistical comparison of the survival curves, Nayak et al. (2023) measured fluorescence rather than survival, and in Zhang et al. (2026) the reference group received an empty-vector strain rather than an unsupplemented diet.

Growth and reproduction

Growth is commonly assessed using weight gain and specific growth rate, and feed utilisation using feed conversion ratio. Survival is recorded as an additional parameter. Probiotics may influence growth and feed utilisation by enhancing digestive enzyme activity (Adorian et al., 2019; Rahayu et al., 2024) and modulating the intestinal microbiota (Gupta et al., 2019; Rahayu et al., 2024). Among the zebrafish studies considered here, some report improvements in growth or feed utilisation, whereas others find no detectable change.

Reproductive assessments in these studies include gonadal histology (Dede & Çek, 2025; Huo et al., 2025) and the gonadal index (Huo et al., 2025). These parameters describe the state of the gonads rather than the number of offspring produced. Among the studies listed in Table 1, only Liu et al. (2024) measured spawning, fertilisation and hatching alongside ovarian histology. Carnevali et al. (2013) reviewed zebrafish studies in which dietary *Lactobacillus rhamnosus* advanced gonadal differentiation when given from early development and improved follicle development, ovulation and embryo quality in broodstock. Aydın & Çek-Yalnız (2019) reported that work on probiotics in fish reproduction has concentrated on females and that few studies have examined sperm and semen quality.

Dede & Çek (2025) fed juvenile zebrafish *Bacillus subtilis* HBB 493® at four dietary levels for 100 days. Weight gain and daily growth ratio were higher than in the control at the two highest levels. At the highest level final weight and specific growth rate were higher and feed conversion ratio was lower than in every other group. Survival did not differ. Control testes were dominated by early spermatogenic stages, and control ovaries contained stage 1 and 2 oocytes with no exogenous yolk formation. Testicular developmental stages advanced as the

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supplementation level increased, and free spermatozoa appeared in the lumen at the highest level. Exogenous yolk formation was detected in three of the four supplemented groups. Oogenesis stages 4 to 7 were clearly present at the two highest levels.

Esfahani et al. (2025) fed zebrafish live *Lactobacillus plantarum*, *Lactobacillus reuteri* or both for 8 weeks. Weight gain increased only with the combined treatment, and feed conversion ratio decreased with *L. reuteri* alone and with the combination. Specific growth rate and survival did not differ between groups.

Jiang et al. (2024) fed zebrafish *Lactobacillus plantarum* SHY21-2 for 1 week. Body weight did not differ between groups.

Liang et al. (2023) fed zebrafish a stabilised culture of *Lactobacillus rhamnosus* GCC-3 for 4 weeks. Final weight and weight gain were higher than in the control and feed conversion ratio was lower. Survival was unaffected.

Huo et al. (2025) exposed male zebrafish to permethrin for 30 days. Growth was suppressed relative to unexposed fish, and dietary *Lactobacillus casei* reversed the suppression dose-dependently. Weight gain rate and specific growth rate remained below the control at the lowest dose. Weight gain rate in fish receiving heat-killed cells remained at the permethrin-exposed level. In the same fish permethrin lowered the testicular organ coefficient and disorganised the spermatogenic tubules. Spermatozoa decreased, the tubule epithelium partly dissolved and the interstitial space widened. *Lactobacillus casei* raised the organ coefficient above the permethrin-exposed level but not to the control value, and increased sperm concentration.

Liu et al. (2024) exposed adult female zebrafish to two concentrations of the cyanobacterial toxin microcystin-LR for 28 days, with or without *Lactobacillus rhamnosus* GG. At the higher toxin concentration cumulative spawning declined from day 13 of counting, and fertilisation and hatching rates fell. The proportion of primary oocytes rose and cortical alveolar and late/mature oocytes decreased. The probiotic raised cumulative egg production above the toxin-only group, returned fertilisation and hatching rates to control values and restored the oocyte stage distribution. Toxin accumulated in the ovaries of all exposed fish, and accumulation was lower in fish that also received the probiotic. Hepatic *vtg1* and *vtg2* expression was lower than in the control in every treated group and was lowest under the combined exposure, whereas ovarian vitellogenin protein fell at the higher concentration and partly recovered under the probiotic. Gonadosomatic index, egg weight and egg protein and lipid content did not differ from the control in any group.

Qiu et al. (2025) applied *Lysinibacillus sphaericus* HY3 through the culture water at three concentrations for 33 days. Survival, weight gain rate and specific growth rate rose numerically with concentration but did not differ significantly between groups.

Gao et al. (2024) compared a yeast prebiotic, a yeast probiotic, black soldier fly larvae meal and sodium butyrate over 63 days. Survival and weight gain did not differ between diets. Males and females were reared together, and the authors consider the difference in body weight between them a possible reason for the lack of significant differences.

Li et al. (2023) added a dried cultured supernatant mixture of *Cetobacterium somerae* and *Lactococcus lactis* to a high-fat diet at two levels for 6 weeks. Weight gain rate, feed conversion ratio and survival did not differ from the high-fat control at either level.

García-Márquez et al. (2026) fed zebrafish an ethanol-inactivated, freeze-dried *Vibrio proteolyticus* DCF12.2 preparation for 21 days. Final body weight in the supplemented group did not differ from the control, and no mortality occurred in either group.

Improvements in growth appeared in Dede & Çek (2025), Esfahani et al. (2025) and Liang et al. (2023). Qiu et al. (2025), Gao et al. (2024), Li et al. (2023), García-Márquez et al. (2026) and Jiang et al. (2024) found no change. Huo et al. (2025) measured growth under chemical exposure, so the comparison there is recovery from suppression rather than improvement over normal performance. Viable cells reversed the suppression caused by permethrin and heat-

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killed cells of the same strain did not. The same distinction applies to the reproductive data. Gonad development advanced with dose under normal conditions in Dede & Çek (2025), whereas Huo et al. (2025) and Liu et al. (2024) measured recovery from testicular and ovarian damage.

Limitations and transferability

Zebrafish have been proposed for the preliminary evaluation of diets and feed ingredients, because such trials are costly and time-consuming in some farmed species (Ulloa et al., 2014). Diets based on soybean meal reduced growth in zebrafish as well as in salmon, rainbow trout, carp, tilapia and sea bream, which was taken to indicate that the mechanisms underlying the growth response to nutrients overlap among fish, regardless of evolutionary distance or environmental conditions. In salmon, rainbow trout and zebrafish, soybean meal caused intestinal inflammation attributed to saponin, and pro-inflammatory cytokine levels increased in each of these species. The coordination of signalling cascades and its effects on growth and inflammation may be resolved in this species, and work on zebrafish nutrition could contribute to aquaculture nutrition research. In Zhang et al. (2026) the strain secreting both peptides did not differ from the single-peptide strains in an agar diffusion assay, but gave higher survival than either of them in the challenge trial. Ulloa et al. (2014) stated, however, that studies in zebrafish cannot replace studies in farmed fish and that their results cannot be extrapolated directly to other species. Ribas & Piferrer (2014) advised caution in extrapolating the results of some growth studies, and reported that breeding protocols were not controlled in most zebrafish lines, that laboratories followed their own husbandry protocols, and that growth rates differed between laboratories. Nikapitiya et al. (2026) used the zebrafish model to obtain data for the development of postbiotics for companion animals rather than for aquaculture.

Lactobacillus rhamnosus GG is well characterised as a human probiotic, and in Zhang et al. (2024) it damaged the intestinal epithelium in zebrafish. The damage was traced to the SpaC pilin subunit. Recombinant SpaC raised the pyroptotic rate in zebrafish ZF4 cells and did not raise it in human Caco-2 cells, and SpaC bound zebrafish TLR4ba and human TLR2 but not human TLR4. The species difference therefore lies in receptor recognition. Wang et al. (2025) fed the same strain to zebrafish and reported that it reduced the oxidative damage caused by PFOS and PFOA. The two studies differ in preparation, route of administration, fish age and the presence of a chemical stressor, and further work is needed before the two outcomes can be reconciled. In Wang et al. (2020) two of the three strains tested killed most larvae at concentrations above those used in the efficacy trials, and the tolerated concentration differed between strains.

Vibrio proteolyticus DCF12.2 and a culture supernatant of *Cetobacterium somerae* and *Lactococcus lactis* (SWFC) have been tested in both zebrafish and a farmed species. In two studies with shared authors, DCF12.2 was given to juvenile gilthead seabream, Sparus aurata, as cell-free extracellular products obtained from a medium containing aquafeed and agar for 62 days (Pérez-Gómez et al., 2025), and to zebrafish as ethanol-inactivated, freeze-dried cells for 21 days (García-Márquez et al., 2026). SWFC was prepared in the laboratory and added to a high-fat diet at 0.3 or 0.4 g/kg for 6 weeks in one-month-old zebrafish (Li et al., 2023). SWFC supplied by a company was added to a high-fat diet at 0.2 or 0.3 g/kg for 98 days in common carp, Cyprinus carpio, with an initial weight of about 3 g (Yu et al., 2023). The DCF12.2 studies differed in culture medium, preparation and duration. The SWFC studies differed in source, dose, duration, fish size and diet formulation. These differences limit the extent to which either pair of studies can show whether responses in zebrafish predict those in farmed species. Muthoka et al. (2026) reported that functional microbial products, including probiotics and

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postbiotics, often perform well under controlled laboratory conditions but give inconsistent results on farms. They attributed this to differences in water quality, temperature fluctuations, feed composition and stocking density, and to species-specific responses. Pérez-Gómez et al. (2025) called for the efficacy of postbiotics to be validated under commercial farming conditions and under pathogen exposure.

Conclusions

Zebrafish have been used to examine immune and antioxidant responses, intestinal structure, gut microbiota, pathogen resistance, growth and reproduction in fish given probiotics and postbiotics. In this model, strains, preparation forms and doses were compared under controlled conditions, and responses were measured in several tissues. Some studies measured gene expression, intestinal histology and survival after challenge in the same experiment, and in gnotobiotic larvae the applied bacterium and the pathogen were followed at the same time.

Across the reviewed studies, the effects of supplementation were most consistent in fish exposed to a stressor. After pathogen challenge or toxicant exposure, supplemented fish showed less intestinal damage, and in most of these studies their antioxidant enzyme activities and malondialdehyde content remained closer to control values. In fish not exposed to a stressor, the results varied between studies. The studies were designed for different purposes and differed in fish age, route of administration, strain, preparation, dose, duration and the tissues examined, so their results are not directly comparable. Within individual studies, the same gene changed in opposite directions in different tissues or before and after challenge, and a higher dose did not always produce a stronger effect. Changes in cytokine expression did not identify the groups with higher survival.

Results obtained in zebrafish may not be directly transferable to farmed species. Two of the reviewed preparations were also tested in a farmed species, but the preparation, dose or duration differed between hosts, so the responses cannot be compared directly. In addition, the performance of microbial products under farm conditions has been reported to be less consistent than under laboratory conditions. Supplementation was not uniformly beneficial. In Zhang et al. (2024) the preparation itself damaged the intestinal epithelium, and in Wang et al. (2020) two of the three strains killed most larvae at higher concentrations.

Future studies in zebrafish should define dose–response relationships, since the responses observed were not always proportional to the dose applied. The concentration that fish tolerate without harm should be established for each strain before efficacy is tested. The comparison of live and inactivated forms of the same strain should be extended to other strains and preparations. Pathogens other than *Aeromonas* species have been used in only a few of these studies, and their range should be widened. Preparations identified in zebrafish can then be evaluated for efficacy in the target farmed species under production conditions.

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Author Contribution

FA conceived the idea, FA and ŞÇ wrote the paper. Both authors read the final version of the review and approved it for publication.

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Conflict of Interest

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Salted Hilsa Fishery Products: Supply Chain And Quality Issues in Bangladesh

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Abstract

In the present study, salted Hilsa (*Tenualosa ilisha*) was collected, preserved, processed, transported and its quality evaluated priority on the packaging system. Sample was collected from the Mohipur Fish Landing Center in Patuakhali, Bangladesh. Wet and dry salting techniques were used for preserving lower-quality Hilsa fish, and packaging improvements were investigated through a seven-month period for storage. Proximate analysis showed similar values for both without-pack and normal-pack samples: moisture (35.51%), crude fat (8.01%), crude protein (32.46%), ash (23.41%), and energy content (210.024 Kcal/100g), with a consistent salt concentration of 21.024%. Over time, normal packaging showed better stability in quality. Moisture content increased up to 45.01% in without-pack and 42.90% in normal-pack samples. The pH declined to 6.22 (without pack) and 6.76 (normal pack), while free fatty acid (FFA) content rose to 0.42% and 0.32%, respectively. TBARS values indicated greater lipid oxidation in unpacked samples (14.36 mg MDA/kg) than in packed ones (12.81 mg MDA/kg). The aerobic plate count peaked at 7.45 log CFU/g in without-pack and 6.39 log CFU/g in normal-pack samples, indicating lower contamination levels in packed fish. GC-MS study has validated the stability of fatty acids and highlighted the role of packing in safeguarding polyunsaturated fatty acids. According to the findings, regular packing significantly extends the salted Hilsa's shelf life, nutritional content, and microbiological safety, making it a more viable preservation method for distribution and long-term storage.

Keywords: Hilsa fishery products, with pack, without pack

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Dire conservation need for three co-occurring freshwater megafauna in a human-dominated river of Bangladesh

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Abstract

Freshwater megafauna are among the most threatened components of global biodiversity, yet conservation in tropical river systems remains constrained by limited ecological data and poor understanding of human–wildlife interactions. This study assessed long-term stakeholder-reported encounters, threats, changes in habitat, knowledge and conservation perceptions and conservation initiatives relating to three freshwater threatened megafauna in the Lower Ganges River of Bangladesh through long-term participatory monitoring from 2001 to 2025, multi-temporal satellite imagery, structured questionnaire surveys involving key stakeholders and field surveys and analyzing literature, respectively. Results confirmed the continuous occurrence of three threatened freshwater megafauna species: the Ganges River dolphin (*Platanista gangetica*; Mammalia), narrow-headed softshell turtle (*Chitra indica*; Reptilia) and gharial (*Gavialis gangeticus*; Reptilia). Ganges River dolphin was the most frequently recorded species (51.4% of total megafauna individuals), followed by narrow-headed softshell turtle (31.5%) and gharial (17.1%). Adult individuals dominated dolphin and turtle records, whereas gharial records consisted primarily of yearlings. Substantial habitat transformation in river morphology was detected in last 25 years, with water area declined by 59.1%, while char areas, agricultural land and human settlements increased by 40.3%, 906% and 319%, respectively which might negatively affect the feeding, nesting or breeding of megafauna species. Stakeholders' conservation knowledge was extremely limited; only one riverside resident recognized these species as threatened, and no respondents recognized their ecological importance. Existing conservation initiatives were limited, only a few awareness-based, legal or species-specific, with no confirmed dedicated in-situ initiative for any of the megafauna species. The findings emphasize the importance of tropical habitats in the Global South like the Lower Ganges and highlight the dire need for the conservation of charismatic threatened co-occurring megafauna.

Keywords: Lower Ganges, Ganges River dolphin, narrow-headed softshell turtle, gharial, conservation

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Growth Enhancement of Pangasius (*Pangasius hypophthalmus*) through Effective Treatment for NH3 Raised in Bio Flock System

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Abstract

This study had shown the synergized use of organic carbon and Bacillus-based probiotic which considerably improves the growth performance and water quality in culture of *Pangasius hypophthalmus*. In comparison with all treatments, fish cultured under carbon-probiotic system (T3) had significantly better final body weight, weight gain, total biomass and survival and had also the lowest feed ratio and ammonia levels, which were much higher than the improvement that were recorded in the carbon only, probiotic only and control treatments, suggesting that the effect was synergistic and not just additive. The probiotic supplementation may have increased the efficiency of the digestive system and nutrient absorption. The negative correlation between unionized ammonia and final body weight is also very high and the positive association between ammonia and FCR further confirm the nitrogen management as a contributor to growth responses. One-way ANOVA was used to compare final weight, weight gain, survival, total ammonia nitrogen (TAN), un-ionized ammonia (NH_3) and FCR among treatments. Tukey's HSD test was applied for significant differences ($p < 0.05$). The high survival rates of all biofloc-based treatments show that microbial manipulation did not negatively affect the health of fish but rather resulted in a more stable and suitable culture condition.

Keywords: Growth, Pangasius, Bioflock, NH3, FCR

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Environmental Assessment of the Vjosa River Based on Heavy Metal and Microbiological Contamination in *Barbus prespensis* and *Squalius platyceps*

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Abstract

Rivers are experiencing substantial alterations to their ecosystems as a result of pollution originating from industrial and agricultural activities. Wastewater discharges, together with the intensive use of pesticides and chemical fertilizers, are disrupting microbial community balance and increasing the concentration of heavy metals in aquatic environments. Fish are recognized as valuable bioindicators of environmental quality due to their ability to reflect the ecological conditions of the habitats in which they live. In this study, two fish species, *Barbus prespensis* and *Squalius platyceps*, were assessed for heavy metal accumulation, specifically manganese (Mn) cadmium (Cd) and lead (Pb), during two different seasons and at two sampling sites. In addition, microbiological contamination, including total coliforms, Escherichia coli, and enterococci, was evaluated during two different seasons. Sampling was conducted at the Petran and Këlcyrë stations, with heavy metal analyses performed in 2024 and microbiological assessments carried out in 2025. The results indicated that both the microbiological contamination levels and the accumulated concentrations of heavy metals were below the maximum permissible limits established by European Union Directives. Higher concentrations of heavy metals and greater microbial contamination were observed in *Squalius platyceps* compared to *Barbus prespensis* at both sampling stations in both spring and summer. These findings indicate that the studied section of the Vjosa River is in good environmental condition, with no significant heavy metal or microbiological contamination risk.

Keywords: Freshwater pollution, heavy metals, microbial contamination, *Squalius platyceps*, *Barbus prespensis*

Introduction

The Vjosa River is one of Europe's last free-flowing rivers, originating in the Pindus Mountains of Greece and flowing into the Adriatic Sea in southern Albania. With a catchment area of approximately 6,700 km² and a mean annual discharge of 204 $m^3 s^{-1}$, the river supports a unique and highly diverse freshwater ecosystem. The river basin is home to 34 fish species, of which 28 are endemic and six non-native fish species, including *Pseuedorasbora parva, Oncorhynchus mykiss, Carassius spp., and Gambusia holbrooki*, have been introduced into the basin the last century. The lower reaches of the river are inhabited by migratory and estuarine

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species such as the European eel (*Anguilla anguilla*), mullets (*Mugil cephalus, Chelon ramada, Chelon saliens, and Chelon labrosus*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), European flounder (*Platichthys flesus*), soles (*Solea spp.*), and several marine-associated species. Among these, *Atherina boyeri* and *Aphanius fasciatus* are numerically dominant and play important ecological roles within the lower Vjosa River ecosystem (Shumka et al., 2018, 2023). Heavy metals enter river ecosystems primarily through industrial, agricultural, and urban wastewater discharges, contributing to the contamination of aquatic environments and the organisms inhabiting them (Maitera et al., 2011). Consequently, aquatic organisms may accumulate substantial concentrations of heavy metals even when their concentrations in the surrounding water remain relatively low, posing potential ecological risks and significant threats to human health through the consumption of contaminated fish. Fish are widely recognized as effective bioindicators of aquatic ecosystem health because they occupy relatively high trophic levels and can accumulate heavy metals through both dietary intake and direct uptake from water and sediments (Yılmaz et al., 2007). Previous research conducted in the Vjosa River demonstrated that fish collected during the dry season exhibited higher concentrations of all six investigated heavy metals (Fe, Zn, Cu, Mn, Cd, and Pb) in both muscle and liver tissues than fish collected during the wet season (Muçollari et al., 2025). Microbiological contamination of fish is also closely associated with environmental conditions and habitat quality. Fish may become contaminated through continuous exposure to polluted water and sediments, contaminated food sources, feeding behavior, and the overall microbial quality of their habitat. Consequently, microbial contamination in fish reflects the ecological condition of aquatic ecosystems and serves as an important indicator of environmental quality and food safety (Eizenberga et al., 2015; Olafsen, 2001; Geldreich & Clarke, 1966). Seasonality also plays a significant role in determining the levels of fecal microbial contamination in river ecosystems. During the dry season, reduced river discharge and elevated water temperatures create favorable conditions for bacterial survival and proliferation while reducing the natural dilution and dispersion of contaminants. As a result, concentrations of fecal indicator microorganisms generally increase during periods of low flow (Azzam & Omran, 2025). The present study aimed to evaluate the environmental status of the Vjosa River by determining the concentrations of toxic heavy metals (Mn, Cd, and Pb) in the muscle and liver tissues of two fish species, *Squalius platyceps* and *Barbus prespensis*, and by assessing fecal microbial contamination using total coliforms, *Escherichia coli*, and enterococci as indicator microorganisms. These fish species were selected because they are widely distributed throughout the Vjosa River and exhibit different feeding habits and ecological lifestyles. In addition, the study investigated the effects of season, tissue type, and fish species on heavy metal accumulation and patterns of fecal microbial contamination.

Materials and Methods

Fish sampling

A total of 24 fish specimens belonging to two fish species, *Barbus prespensis* and *Squalius platyceps*, were collected from two sampling stations (Petran and Këlcyrë) in the Vjosa River during autumn 2024 and spring 2025. Fish were sampled for the determination of heavy metal concentrations in muscle and liver tissues. For microbiological analyses, two individuals of each species (four specimens) were collected from each sampling station during both sampling seasons (autumn 2024 and spring 2025) for the determination of fecal indicator bacteria, including total coliforms, *Escherichia coli*, and enterococci

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Heavy metal analysis

Fish were dissected immediately after collection, and liver and muscle tissues were excised for heavy metal analysis. Samples were transported to the laboratory in sterile hermetically sealed bags under refrigerated conditions (-20 °C). Approximately 5 g of each tissue sample was digested using a mixture of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (3:1, v/v). Samples were heated at 150 °C for 20 min, cooled to room temperature, diluted to a final volume of 50 mL with ultrapure water, and filtered prior to analysis. Concentrations of Mn, Cd, and Pb were determined using Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Microbiological analysis

Fish samples were transported under refrigerated conditions (4 ± 2 °C) in accordance with ISO 19458 and analyzed within three days of collection. Total coliforms and *Escherichia coli* were enumerated using the membrane filtration method according to ISO 9308-2. Briefly, 100 mL of each sample was filtered through a membrane filter, which was subsequently placed onto Chromogenic Coliform Agar (CCA) and incubated at 36 ± 2 °C for 21 ± 3 h. Total coliforms were identified as pink-to-red colonies, whereas *E. coli* colonies were identified by their dark blue to violet fluorescence under UV light (365 nm). Enterococci were determined according to ISO 7899-2 using membrane filtration. After filtration of 100 mL of sample, membranes were incubated on Slanetz and Bartley agar at 37 ± 1 °C for 4 h followed by 44 ± 0.5 °C for 40 ± 4 h. Presumptive colonies were confirmed on Bile Aesculin Azide Agar following the ISO protocol.

Statistical analysis

Statistical analyses were performed using R software (R version 4.6.1(2026-06-24). Descriptive statistics were calculated for heavy metal concentrations and microbiological parameters. Because of the limited sample size and non-normal distribution of the data, comparisons between groups were performed using the Wilcoxon rank-sum test. Statistical significance was accepted at $p < 0.05$. Graphical visualizations were generated using the ggplot2 package.

Results

The concentrations of heavy metals (Mn, Cd, and Pb) determined in the two fish species, *Barbus prespensis* and *Squalius platyceps*, are presented in Table 1. As shown in the table, the highest concentration of heavy metals was recorded for cadmium (Cd) in the liver tissue of *Barbus prespensis* during the autumn season, reaching 9.7±4.6 µg g^{-1}. In contrast, the lowest concentration was observed for lead (Pb) in the liver tissue of *Squalius platyceps* 0.06±0.1 during the spring season. Overall, liver tissue exhibited higher concentrations than muscle tissue in both fish species. In contrast, Pb accumulation in *Barbus prespensis* was higher in muscle than in liver tissue, with mean concentrations of 0.20 ± 0.10 mg/kg in spring and 0.07 ± 0.10 mg/kg in autumn (Figure 1).

Table 1. Heavy metal concentrations in muscle and liver samples collected during the spring and autumn of 2024

Species	HM	N spring	N autumn	Muscle Spring	Muscle Autumn	Liver Spring	Liver Autumn
Barbus prespensis	Mn	12	12	0.6±0.1	0.5±0.3	1.4±0.2	1.3±0.5
Barbus prespensis	Cd	12	12	4.5±0.8	4.8±1.4	9.6±3.9	9.7±4.6
Barbus prespensis	Pb	12	12	0.2±0.1	0.2±0.1	0.07±0.1	0.09±0.1

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Squalius platyceps	Mn	12	12	0.7±0.1	0.6±0.2	1.6±0.5	1.8±0.6
Squalius platyceps	Cd	12	12	0.8±0.2	0.9±0.4	2.6±0.8	2.9±0.5
Squalius platyceps	Pb	12	12	0.1±0.2	0.2±0.4	0.06±0.1	0.12±0.1

The order of heavy metal accumulation, from the highest to the lowest mean concentration, was Cd > Mn > Pb, with mean concentrations of 4.47 µg g^{-1}, 1.06 µg g^{-1}, and 0.97 µg g^{-1}, respectively. Descriptive analysis revealed that the mean concentrations of the three heavy metals were highest in the liver of *Barbus prespensis*, reaching 3.7 ± 5.2 µg g^{-1} in autumn and 3.69 ± 5.16 µg g^{-1} in spring (Table 2). In muscle tissue, the corresponding mean concentrations were 1.8 ± 2.57 µg g^{-1} in autumn and 1.76 ± 2.37 µg g^{-1} in spring. In *Squalius platyceps*, the highest mean concentration was also recorded in the liver during autumn (2.53 ± 0.63 µg g^{-1}), whereas the lowest mean concentration was observed in muscle tissue during spring (2.26 ± 0.57 µg g^{-1}). Overall, the concentrations of Mn, Cd, and Pb measured in the muscle tissues of both fish species were below the maximum permissible limits established by the relevant food safety legislation (EU Regulation 2023/915).

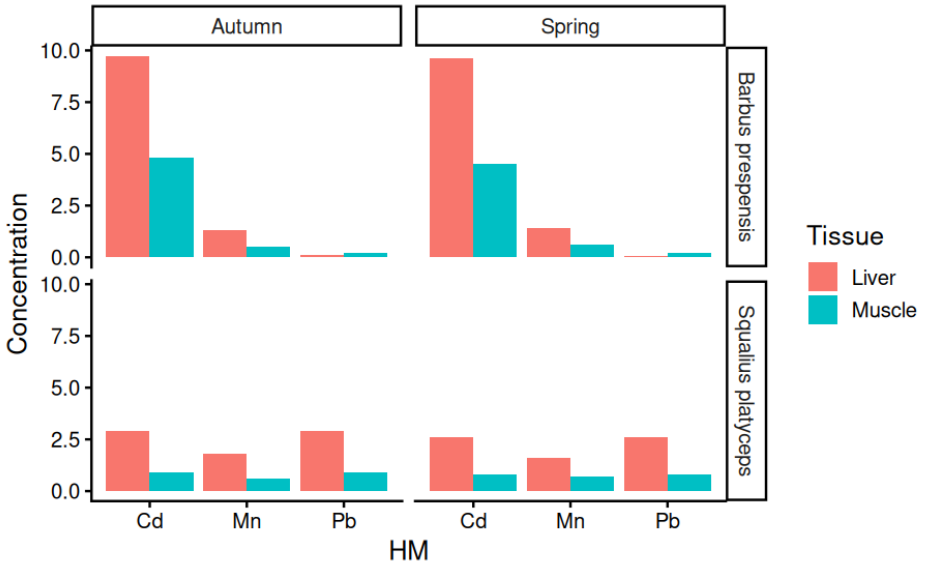


Figure 1. Mean concentration of heavy metals (Cd, Mn and Pb) in muscle and liver tissues of *S. platyceps* and *B. prespensis* during spring and autumn 2024

Table 2. Description analyses of heavy metals concentration in the muscle and liver of barbell and chub collected from Këlcyrë and Petran during spring–autumn 2024

Species	Tissue	Season	N	Mean	SD	Median	Min	Max
Barbus prespensis	Liver	Autumn	3	3.6966667	5.23412202	1.3	0.09	9.7
Barbus prespensis	Liver	Spring	3	3.69	5.16123047	1.4	0.07	9.6
Barbus prespensis	Muscle	Autumn	3	1.8333333	2.57358375	0.5	0.2	4.8
Barbus prespensis	Muscle	Spring	3	1.7666667	2.37557011	0.6	0.2	4.5
Squalius platyceps	Liver	Autumn	3	2.5333333	0.6350853	2.9	1.8	2.9
Squalius platyceps	Liver	Spring	3	2.2666667	0.57735027	2.6	1.6	2.6
Squalius platyceps	Muscle	Autumn	3	0.8	0.17320508	0.9	0.6	0.9
Squalius platyceps	Muscle	Spring	3	0.7666667	0.05773503	0.8	0.7	0.8

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In addition to heavy metal analysis, fecal microbiological contamination for total coliforms, *Escherichia coli*, and enterococci was evaluated in *Squalius platyceps* and *Barbus prespensis* collected from the Petran and Këlcyrë sampling stations during spring and summer 2025. The results showed that all microbiological values were below the maximum limits established by Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Among the three microbiological parameters, total coliforms exhibited the highest concentrations, whereas *E. coli* and enterococci were not detected in any of the fish samples. The highest total coliform concentrations were recorded in *S. platyceps* collected from the Petran station, reaching 17 CFU g^{-1} during summer 2025 and 16 CFU g^{-1} during spring 2025. In contrast, the lowest concentrations were observed in *B. prespensis* collected during spring 2025, with 2 CFU g^{-1} at the Petran station and 5 CFU g^{-1} at the Këlcyrë station. The Petran station showed a higher overall mean total coliform concentration (11 CFU g^{-1}) than the Këlcyrë station (7 CFU g^{-1}). Similarly, the mean microbial load was higher during summer 2025 (10.5 CFU g^{-1}) than during spring 2025 (7.5 CFU g^{-1}). Overall, both fish species collected from the Petran station exhibited higher total coliform concentrations than those collected from Këlcyrë. The only exception was *B. prespensis* sampled during spring 2025, for which the specimen collected at the Këlcyrë station showed a higher total coliform concentration than the specimen collected at Petran. An independent-samples t-test was performed to compare total coliform concentrations between the Petran and Këlcyrë sampling stations. No statistically significant difference was observed between the two locations ($t = -1.124$, $df = 3.25$, $p = 0.337$).

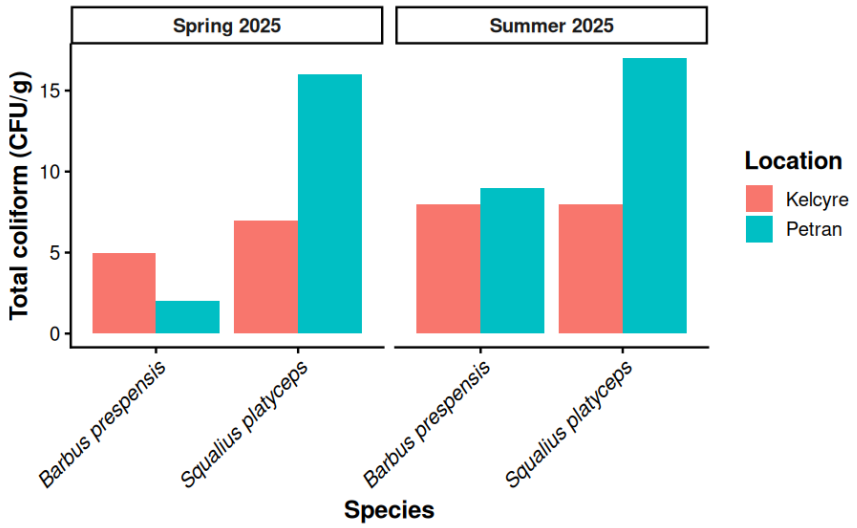


Figure 2. Mean concentrations of total coliforms, *Escherichia coli*, and enterococci in the muscle of *S. platyceps* and *B. prespensis* during spring and autumn 2025

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Table 3. Description analyses of microbiological analyses at barbell and chub collected from Këlcyrë and Petran during spring–autumn 2025

Location	Season	Species	Coliform_mean	Ecoli_mean	Enterococci_mean
Këlcyrë	Spring 2025	*Barbus prespensis*	5	0	0
Këlcyrë	Spring 2025	*Squalius platyceps*	7	0	0
Këlcyrë	Summer 2025	*Barbus prespensis*	8	0	0
Këlcyrë	Summer 2025	*Squalius platyceps*	8	0	0
Petran	Spring 2025	*Barbus prespensis*	2	0	0
Petran	Spring 2025	*Squalius platyceps*	16	0	0
Petran	Summer 2025	*Barbus prespensis*	9	0	0
Petran	Summer 2025	*Squalius platyceps*	17	0	0

Conclusion

The present study demonstrated that the concentrations of heavy metals (Mn, Cd, and Pb) and the levels of faecal microbiological contamination in the two analysed fish species, *Squalius platyceps and Barbus prespensis*, collected from the Petran and Këlcyrë stations of the Vjosa River were below the maximum permissible limits established by international and European Union standards. These findings indicate a generally good environmental status of the investigated river section and suggest that fish from these locations do not currently pose a significant risk to consumers with respect to the investigated contaminants. Seasonal variation influenced both heavy metal accumulation and microbiological contamination. Higher concentrations of heavy metals were recorded during the dry season than during the wet season, most likely due to lower river discharge and reduced dilution capacity, which increase the concentration and bioavailability of contaminants. Continued long-term monitoring of chemical and microbiological contaminants is recommended to support the conservation of this unique river ecosystem and to ensure the safety of fish intended for human consumption.

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Phylogeography and integrative taxonomy of water mites (Hydrachnidia) in Great Britain: implications for postglacial colonization processes

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Abstract

Water mites (Hydrachnidia) are among the most diverse groups of freshwater invertebrates, yet their evolutionary history and postglacial colonization of Great Britain remain poorly understood. This project aims to reconstruct postglacial colonization routes and investigate the genetic and taxonomic diversity of British water mites using an integrative approach that combines molecular and morphological data.

Specimens will be collected from a wide range of freshwater habitats across Great Britain, including rivers, streams, lakes, springs, and peat bogs. Species identification will be supported by DNA barcoding based on the mitochondrial cytochrome c oxidase subunit I (COI) gene, complemented by the nuclear markers 18S rDNA and 28S rDNA. Molecular Operational Taxonomic Units (MOTUs) will be delimited using BIN, ASAP, and bPTP approaches, while phylogenetic relationships will be reconstructed using Maximum Likelihood and Bayesian inference. Morphological analyses will be integrated with molecular data to detect cryptic diversity and facilitate the description of potential new species.

The project will test hypotheses concerning the influence of geographical barriers, habitat preferences, and host specificity on dispersal patterns in water mites. Furthermore, it will provide insights into the postglacial colonization history of Great Britain following the retreat of the Devensian Ice Sheet and evaluate the biogeographical relationships between British and continental European populations. The results are expected to improve our understanding of freshwater biodiversity, reveal previously unrecognized diversity within Hydrachnidia, and provide a modern integrative taxonomic framework for the British water mite fauna.

Keywords: Hydrachnidia; Great Britain; biogeography; integrative taxonomy; postglacial colonization

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Discovery of a new aggregation site of Sandbar Sharks (*Carcharhinus plumbeus*) in Alayurt Cove, Eastern Mediterranean

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Abstract

The sandbar shark (*Carcharhinus plumbeus*) is an endangered coastal species whose population dynamics depend on the integrity of nursery and aggregation sites. In the Mediterranean, nurseries are documented, but aggregation sites remain poorly understood: known areas occur off Israel and Florida, while the Eastern Mediterranean has few records.

We report a new aggregation site in Alayurt Cove (Dalaman, Muğla, Türkiye), based on systematic surveys conducted from 2017 to 2026. Observations were standardized between 06:00–09:00 h, and rebreathers were used from 2018 onward to reduce disturbance. The highest counts consistently occurred on the first survey day. In 2017, five adult males were observed in early June. In 2019, numbers declined over three consecutive days (3–5 June). No individuals were detected in 2020 (22–27 June) or 2022 (27–28 May), highlighting temporal variability. Aggregation peaked in 2025, but surveys conducted on the same dates in 2026 yielded no sightings, possibly due to delayed seasonal warming. All observed individuals were male, indicating sex-segregated behavior. Sharks were most frequently encountered at 3 m depth and 18–21 °C, suggesting a narrow thermal preference.

These findings expand the known spatial ecology of *C. plumbeus* in the Eastern Mediterranean and identify Alayurt Cove as a critical hotspot. From a conservation perspective, immediate measures—including habitat monitoring, fishing-ban enforcement, and awareness programs—are recommended to safeguard this site.

Keywords: *Carcharhinus plumbeus*, sandbar shark, aggregation site, Alayurt Cove, biodiversity, sustainable fisheries, conservation

Introduction

The sandbar shark (*Carcharhinus plumbeus*) is widely distributed in temperate and subtropical waters (Compagno, 1984). It is currently listed as *Endangered* in the Mediterranean by the International Union for Conservation of Nature (Rigby et al., 2021), primarily due to overfishing, habitat degradation, and slow life-history traits such as late maturity and low fecundity (McAuley et al., 2007; Jabado et al., 2017).

Although *C. plumbeus* is a globally recognized species, verified nursery habitats within the Mediterranean Sea are still scarce (Ferretti et al., 2008). At present, confirmed or repeatedly reported nursery and breeding grounds of *C. plumbeus* are limited to Boncuk Cove in Gökova Bay (southwestern Turkey) (Filiz, 2019), the Gulf of Gabès in southern Tunisia (Bradai et al.,

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2005), and the Yumurtalık Bight in the Gulf of İskenderun (northeastern Turkey) (Basusta et al., 2021). In addition, river-influenced systems such as the Po Delta in the northern Adriatic (Barbato et al., 2023) and the Bojana River Delta in the southern Adriatic (Cetkovic et al., 2022) have been suggested as potential nursery habitats based on recurrent observations of juveniles and neonates. Aggregation sites, however, remain poorly studied. Seasonal male aggregations have been reported off Israel (Bigal et al., 2024) and Lampione Island, Sicily (Cattano et al., 2021).

This study reports the discovery of a new aggregation site in Alayurt Cove, Eastern Mediterranean, based on long-term surveys (2017–2026). The findings broaden the ecological knowledge of *C. plumbeus* and emphasize conservation priorities.

Materials and Methods

Annual underwater surveys were conducted between 2017 and 2026, targeting late spring and early summer when shark presence was anticipated in Alayurt Cove (Fig. 1). All dives were standardized between 06:00 and 09:00 h along the same transect, observations were conducted along a transect extending 100 m eastward and 150 m westward from the point (36° 40’ 3.22 N, 28° 48’ 10.53 E), covering a total length of 205 m (see Fig. 1, right side, yellow lines) to minimize diel variability. Closed-circuit rebreather systems were employed from 2018 onwards to minimize diver-induced disturbance (Ward-Paige et al., 2010). Each year comprised 2–5 consecutive survey days. Depth, water temperature, and visibility were recorded systematically. Interannual variability in shark presence was assessed by comparing annual survey results.

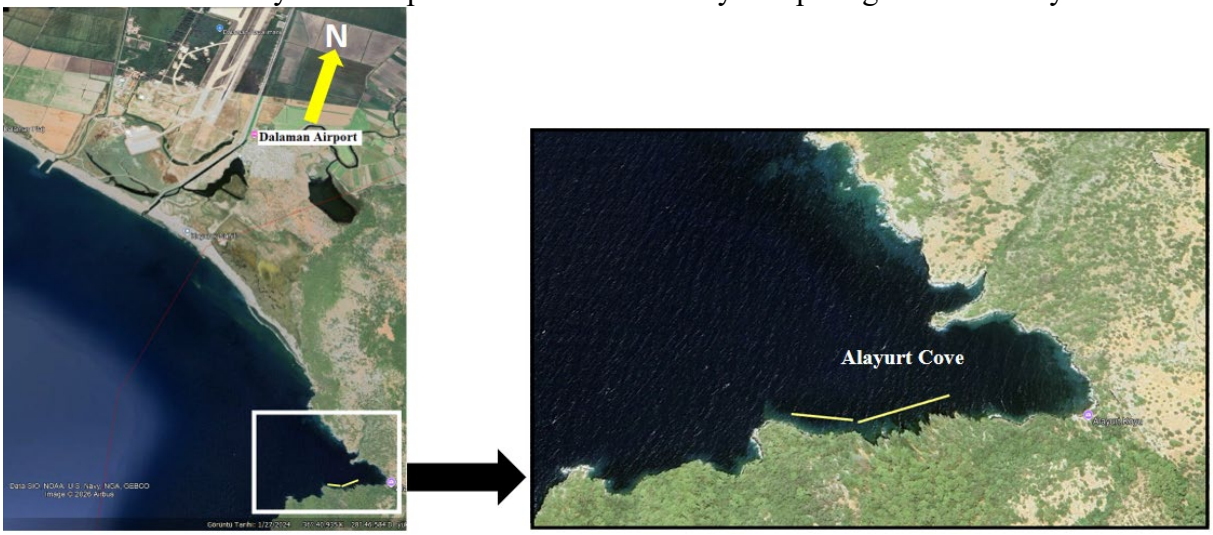


Figure 1. Location of Alayurt Cove on the Dalaman coast, Eastern Mediterranean, yellow lines indicating the sandbar shark observation transects (Maps produced by Google Earth).

Results and Discussion

Observations revealed pronounced interannual variability in sandbar shark occurrence at Alayurt Cove. In early June 2017, five adult males were recorded. During the consecutive survey days of 3-5 June 2019, a declining trend in individual counts was observed. Despite intensive survey efforts between 2020 and 2022, no specimens were detected, suggesting possible shifts in temporal visitation patterns or environmental suitability. The highest aggregation density was documented in 2025, whereas surveys conducted during the corresponding period in 2026 yielded no sightings, potentially attributable to delayed seasonal warming and associated oceanographic anomalies.

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All encountered individuals were exclusively adult males, with the highest frequency of observations recorded at approximately 3 m depth and sea-surface temperatures ranging between 18 °C and 21 °C. This narrow thermal affinity, coupled with the absence of females, points toward sex-segregated aggregation behavior, a pattern consistent with previous reports from coastal waters off Israel (Bigal et al., 2024) and Lampione Island in the central Mediterranean (Cattano et al., 2021).

Comparisons with Boncuk Cove, where year-round aggregations including females have been documented (Filiz, 2019), highlight ecological differences between nursery and aggregation habitats. Temporal variability underscores the influence of environmental fluctuations—particularly sea-surface temperature anomalies—on shark occurrence (Piercy et al., 2016).

The discovery of Alayurt Cove as an aggregation site broadens the known distribution of *C. plumbeus* in the Eastern Mediterranean (Figure 2). The exclusively male presence suggests sex-segregated behavior, consistent with reports from Israel and Lampione Island (Sicily) (Cattano et al., 2021).

From a conservation perspective, Alayurt Cove represents a critical hotspot. Immediate protective measures are required, including strict enforcement of fishing bans, habitat monitoring, and awareness programs (WWF, 2025).

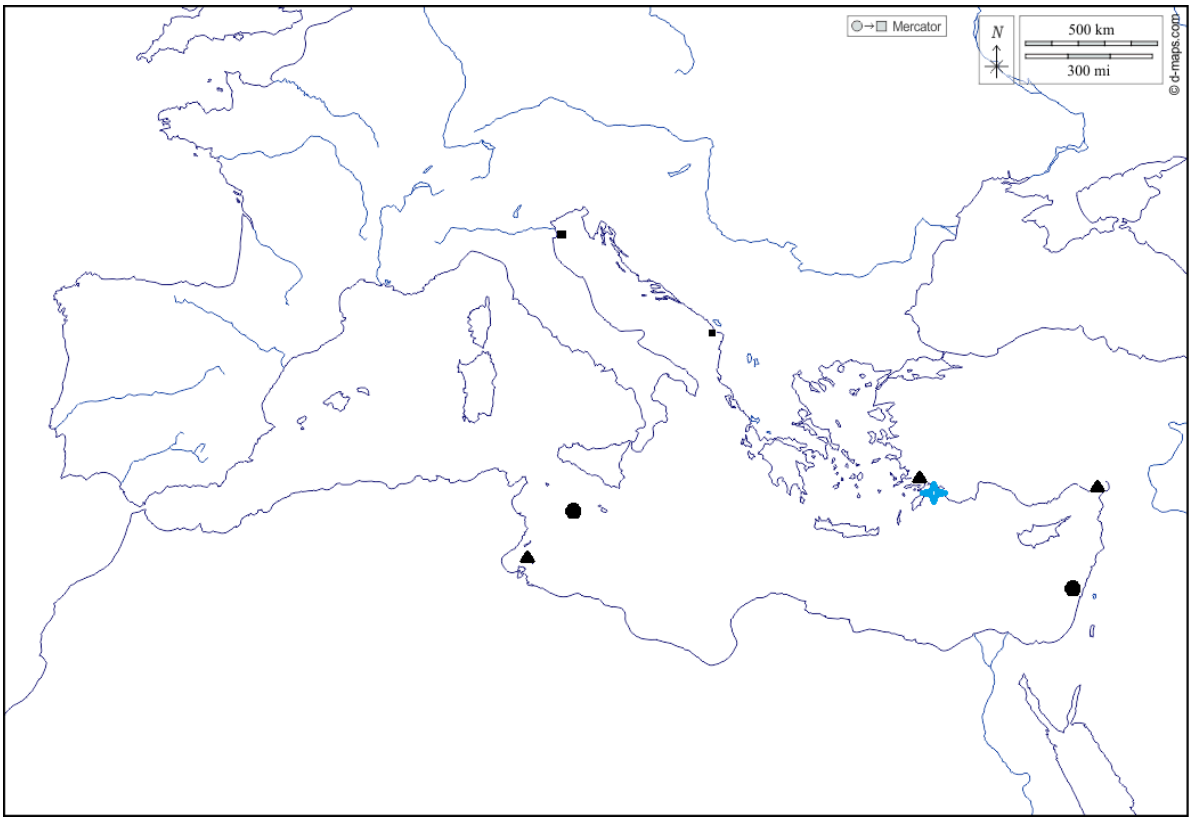


Figure 2. Confirmed nursery and breeding grounds [marked with black triangles; Boncuk Cove in Gökova Bay (southwestern Turkiye), the Gulf of Gabès in southern Tunisia and the Yumurtalık Bight in the Gulf of İskenderun (northeastern Türkiye)], potential nursery habitats [marked with black squares; Po Delta in the northern Adriatic and the Bojana River Delta in the southern Adriatic] and aggregation sites [marked with black circles; off Israel and Lampione Island, Sicily, Italy] of *C. plumbeus* in the Mediterranean Sea. The blue star indicates a new potential aggregation site, Alayurt Cove. (maps reproduced from https://www.d-maps.com/carte.php?num_car=3125&lang=en).

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Conclusion

This study documents the first male-dominated aggregation site of *C. plumbeus* in the Eastern Mediterranean. The findings emphasize:

- The ecological significance of aggregation sites.
- The necessity of long-term monitoring to capture variability.
- The urgent need for protective measures to ensure population resilience.

By integrating these findings with existing datasets from Boncuk Cove and other Mediterranean localities, this research contributes to a more comprehensive understanding of *C. plumbeus* spatial ecology and conservation requirements.

Funding
No external funding was reported for this study.

Ethical statement

This study was conducted in full compliance with national and international ethical standards for marine research. All underwater surveys were strictly observational and non-invasive, involving no capture, handling, or disturbance of sandbar sharks (*Carcharhinus plumbeus*) or other marine organisms. No experimental procedures were performed on animals, and no permits for animal experimentation were required. Fieldwork was carried out under the regulations of the Turkish Ministry of Environment, Urbanization and Climate Change, and the Ministry of Agriculture and Forestry. The authors affirm that the research respected local conservation rules, including fishing bans and protected area guidelines, and that no conflicts of interest exist regarding the ethical conduct of this study.

Conflict of interest

The authors declare that there is no conflict of interest.

Data Availability

All data generated and analyzed during this study are included in the published article. Raw field observations (2017–2026) from Alayurt Cove, including survey dates, counts, and environmental parameters, are archived at Muğla Sıtkı Koçman University, Faculty of Fisheries. These datasets are available from the corresponding author upon reasonable request. No proprietary or restricted data were used in this research.

Author contributions

Halit Filiz, conceived and designed the study, coordinated field surveys, analyzed ecological data, and prepared the manuscript for submission. Sait Özgür Gedikoğlu participated in survey planning and logistics, contributed to field observations, and assisted with data documentation. Ertan Taşkavak provided taxonomic expertise, contributed to the interpretation of ecological findings, and critically revised the manuscript.

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Food Safety Evaluation of Edible Bivalves from the Marmara Sea: Potentially Toxic Elements, Dietary Exposure, and Human Health Risk Assessment

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Abstract

In this study, the concentrations of ten potentially toxic elements (PTEs; Zn, Fe, Mn, Cu, Al, Cd, Co, Cr, Pb, and Ni) were determined in the edible soft tissues of wedge clam (*Donax trunculus*), Mediterranean mussel (*Mytilus galloprovincialis*), and grooved carpet shell (*Ruditapes decussatus*) collected from the Marmara Sea. Samples were digested using a microwave-assisted wet digestion procedure and analyzed by inductively coupled plasma–mass spectrometry (ICP-MS). Based on the analytical results, consumer health risk assessments were performed using estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR). According to the mean concentrations (mg/kg), the highest levels of Zn (39.09 ± 7.20) and Cu (3.67 ± 0.50) were detected in *M. galloprovincialis*, Mn (1.97 ± 0.78) in *D. trunculus*, and Fe (110.49 ± 15.49), Al (20.27 ± 5.00), Cd (0.45 ± 0.03), Co (0.38 ± 0.12), and Cr (0.97 ± 0.20) in *R. decussatus*. The highest Pb concentration (1.06 ± 0.20 mg/kg) was observed in both *D. trunculus* and *R. decussatus*. Based on the health risk assessment results for all ten PTEs, the consumption of these bivalve species was not associated with significant non-carcinogenic or carcinogenic health risks for consumers.

Keywords: Potentially toxic elements, edible bivalves, food safety, dieraty exposure, Marmara Sea

*** This study is based on a master's thesis.**

Introduction

Bivalve molluscs play an important role in human nutrition because they are valuable sources of high-quality protein, essential amino acids, vitamin B_{12}, macro-minerals such as Ca, K, and Na, essential trace elements including Fe, Mn, Zn, Cu, Co, and Se, and long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while generally containing low levels of total and saturated fat. (Orban et al., 2007, Biandolino et al., 2019, Mutić et al., 2021).

Bivalve molluscs consumed in Türkiye are obtained from both capture fisheries and aquaculture. According to fisheries statistics published by the Ministry of Agriculture and Forestry, a total of 28,499 tonnes of bivalve molluscs were produced from marine waters in 2025, of which 64.9% originated from capture fisheries and 35.1% from aquaculture (Ministry of Agriculture and Forestry, 2026). According to the Food and Agriculture Organization of the

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United Nations, global bivalve production reached approximately 18.4 million tonnes in 2023 (FAO, 2024).

The Sea of Marmara is an inland sea exposed to PTEs pollution originating from industrial, urban, agricultural, and maritime transport activities. The occurrence of PTEs in its sediments has been documented by studies conducted at different times and locations (Tan and Aslan, 2020; Bat et al., 2025; Balcıoğlu İlhan et al., 2026; Kükrer et al., 2026). Contaminant levels in sediments are important indicators of the temporal accumulation of pollution in the marine environment. Bivalve molluscs feed by filtering phytoplankton, organic particles, and microorganisms from the water column through their gills. During filter feeding, they may take up not only food particles but also PTEs. Therefore, monitoring PTEs in bivalves intended for human consumption is essential for protecting consumer health.

The aim of this study was to determine the concentrations of PTEs in the edible soft tissues of wedge clam (*Donax trunculus*), Mediterranean mussel (*Mytilus galloprovincialis*), and grooved carpet shell (*Ruditapes decussatus*) collected from different locations in the Sea of Marmara. The study also aimed to estimate dietary exposure to these elements and assess the potential carcinogenic and non-carcinogenic health risks associated with the consumption of these bivalves, thereby providing information relevant to seafood safety and consumer health.

Materials and Methods

Bivalve specimens were randomly selected from individuals harvested from authorized capture fisheries and aquaculture areas in the Sea of Marmara, with *R. decussatus* and *M. galloprovincialis* specimens sampled following depuration and *D. trunculus* specimens sampled following relaying. The samples comprised *Donax trunculus* (n = 25), *Mytilus galloprovincialis* (n = 20), and *Ruditapes decussatus* (n = 20), resulting in a total sample size of 65 individuals. The sampling locations are shown in Figure 1. The production area at Locality 1 was classified as Class C, whereas those at Localities 2 and 3 were classified as Class B.

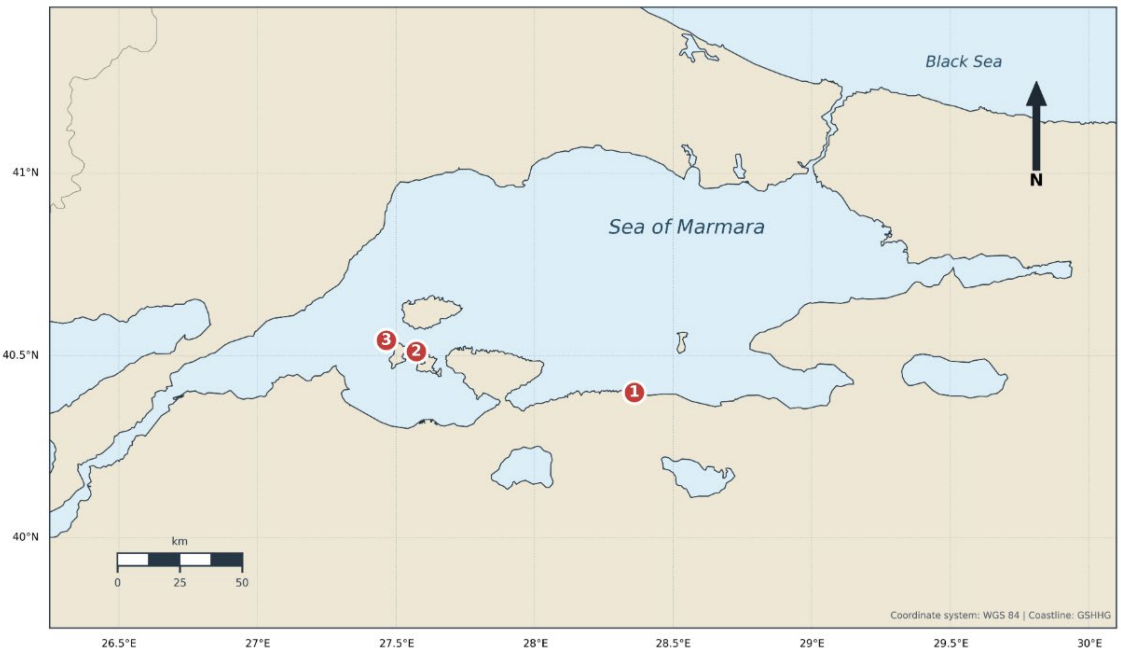


Figure 1. Sampling locations of the bivalve specimens examined in the Sea of Marmara: (1) *Donax trunculus*, Karacabey; (2) *Mytilus galloprovincialis*, Koyun Island; and (3) *Ruditapes decussatus*, Kalınburun. The map was generated using the sampling coordinates in the WGS 84 coordinate system and coastline data from the Global Self-consistent, Hierarchical, High-resolution Geography Database (GSHHG).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Sample Digestion

The samples were digested using a modified closed-vessel microwave-assisted acid digestion procedure with HNO_3 and H_2O_2. Prior to dissection, the bivalve specimens were thoroughly rinsed with deionized water to remove any potential surface contaminants. After the shell lengths of randomly selected specimens had been measured, their soft tissues were carefully dissected and homogenized using a laboratory blender equipped with stainless-steel blades. Approximately 0.3 g of each homogenized sample (wet weight) was weighed and transferred into a polytetrafluoroethylene (PTFE) digestion vessel containing 65% HNO_3 and 30% H_2O_2 at a ratio of 4:2 (v/v). The samples were subsequently digested in a microwave digestion system (CEM Discover SP-D 80) at 200 °C for 5 min, following the procedure proposed by Licata et al. (2014). After digestion, the resulting solutions were transferred into tubes and diluted to a final volume of 25 mL with ultrapure water.

Determination of PTEs Concentrations

The concentrations of PTEs in the samples were determined using inductively coupled plasma–atomic emission spectrometry (ICP–AES; PerkinElmer Series 2000). A calibration curve was generated for each analytical run using a Labsert multielement standard solution. The limits of quantification (LOQs, mg kg^{-1}) were **10.70 for Zn, 11.50 for Fe, 7.50 for Mn, 12.60 for Cu, 0.01 for Al, 0.01 for Cd, 7.50 for Co, 0.01 for Cr, 0.02 for Pb, and 0.01 for Ni.**

The recovery study was performed using 100 and 60 µg L^{-1} standard solutions prepared from a 1000 mg L^{-1} Labsert custom-made multielement stock standard. Element concentrations were determined by ICP–AES, and the recovery results are presented in Table 1. All recoveries were within the generally acceptable range of **80–120%**, indicating satisfactory accuracy of the ICP-AES method.

Table 1. Recovery results for the ICP-AES method

Element	Spiked concentration (µg L^{-1})	Measured concentration (µg L^{-1}, mean ± SD)	Recovery (%)
Zn	100	111.90 ± 4.70	111.90
Fe	100	97.24 ± 0.55	97.24
Mn	100	100.90 ± 0.40	100.90
Cu	100	95.54 ± 1.76	95.54
Al	60	61.05 ± 1.03	101.75
Cd	60	63.80 ± 1.31	106.33
Co	60	56.31 ± 1.19	93.85
Cr	60	59.04 ± 0.73	98.40
Pb	60	59.09 ± 0.68	98.48
Ni	100	109.10 ± 5.41	109.10

Recovery was calculated as: Recovery (%) = (measured concentration/spiked concentration) × 100. Values are expressed as mean ± standard deviation (SD).

Consumer Health Risk Assessment

In this study, the potential health risks associated with oral exposure to Zn, Fe, Mn, Cu, Al, Cd, Co, Cr, Pb, and Ni through the consumption of the examined bivalve molluscs were evaluated by calculating the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR).

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Estimated Daily Intake (EDI)

The estimated daily intake (EDI) represents the estimated amount of a contaminant—in this study, Zn, Fe, Mn, Cu, Al, Cd, Co, Cr, Pb, and Ni—ingested daily by an individual through dietary exposure. EDI was calculated using the following equation proposed by Islam et al. (2014):

$$EDI = \frac{C\ x\ IRd}{BW}$$

In the equation, C represents the mean concentration of the element determined in the bivalve molluscs; IRd represents the ingestion rate (annual per capita fish consumption in 2023: 7.2 kg, according to the 2024 fisheries statistics published by the Turkish Statistical Institute); and BW represents body weight [70 kg for adults (Brodberg & Klasing, 2003)].

Target Hazard Quotient (THQ)

The target hazard quotient (THQ) is used in health risk assessments to evaluate potential non-carcinogenic health risks. A THQ value of ≤1 indicates that adverse health effects are unlikely, whereas a THQ value of >1 suggests the possibility of potential adverse health effects. THQ was calculated using the following equation (Chien et al., 2002):

$$\text{THQ} = \frac{EF\ x\ ED\ x\ IRd\ x\ C}{Rfd\ x\ BW\ x\ ATn} x10^{-3}$$

In the equation:

- EF is the exposure frequency (365 days year^{-1});
- ED is the exposure duration (70 years);
- RfD is the oral reference dose (Al = 1.0, Cd = 0.001, Mn = 0.0003, Co = 0.0003, Cu = 0.04, Zn = 0.3, Fe = 0.7, Cr = 1.5, Ni = 0.02, and Pb = 0.0035 mg kg^{-1} day^{-1}); and
- ATn is the averaging time for non-carcinogenic effects (365 days year^{-1} × ED).

Hazard Index (HI)

The hazard index (HI) represents the overall non-carcinogenic health risk resulting from an individual's simultaneous exposure to multiple potentially toxic substances. According to USEPA (1989), the HI was calculated using the equation below:

$$\text{HI} = \sum_{i=1}^{n} THQi$$

Where THQi is the target hazard quotient of the ith element and n is the total number of PTEs investigated. An HI value of ≤1 indicates that the combined exposure to the evaluated elements is unlikely to result in appreciable non-carcinogenic health effects. In contrast, an HI value of >1 indicates that the possibility of adverse non-carcinogenic health effects cannot be excluded and that further assessment may be warranted. However, an HI value exceeding 1 does not necessarily indicate that adverse effects will occur; rather, it signifies a potential health concern (USEPA, 1989, Jović and Stanković, 2014).

Carcinogenic Risk (CR)

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Carcinogenic risk (CR) represents the estimated probability of an individual developing cancer over a lifetime as a result of dietary exposure to a carcinogenic substance. It was calculated using the following equation recommended by the USEPA (2019):

$$CR = \frac{EF\ x\ ED\ x\ IRd\ x\ C\ x\ CSFo}{BW\ x\ ATx} x10^{-3}$$

In the equation, CSFo represents the oral cancer slope factor. The CSFo values used were 0.38 for Cd, 0.0085 for Pb, 1.7 for Ni, and 0.50 for Cr $(mgkg^{-1}day^{-1})^{-1}$, based on the U.S. EPA Integrated Risk Information System (IRIS) and the relevant literature (U.S. EPA, 1989, 2010, 2019; Onuoha et al., 2016). Because no CSFo values were available for the other elements, CR was calculated only for Cd, Cr, Ni, and Pb.
CR values of $<10^{-6}$ indicate a negligible carcinogenic risk, values ranging from 10^{-6} to 10^{-4} are generally considered acceptable or tolerable, and values of $>10^{-4}$ indicate an unacceptable carcinogenic risk (Kazami et al., 2022).

Statistical Analyses

Statistical analyses were performed using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). The data were summarized using descriptive statistics, including the arithmetic mean and standard deviation.

Results and Discussion

The biometric characteristics of the examined specimens are presented in Table 2.

Table 2. Mean length and weight of the bivalve species examined in this study.

Biometric parameter	*Donax trunculus*	*Mytilus galloprovincialis*	*Ruditapes decussatus*
Length (cm) ± SD	1.84 ± 0.30	7.26 ± 0.45	4.52 ± 0.90
Weight (g) ± SD	0.66 ± 0.73	2.62 ± 0.35	6.68 ± 2.90

SD: Standart deviation

The mean PTE concentrations determined in the soft tissues of the species are presented in Figure 2.

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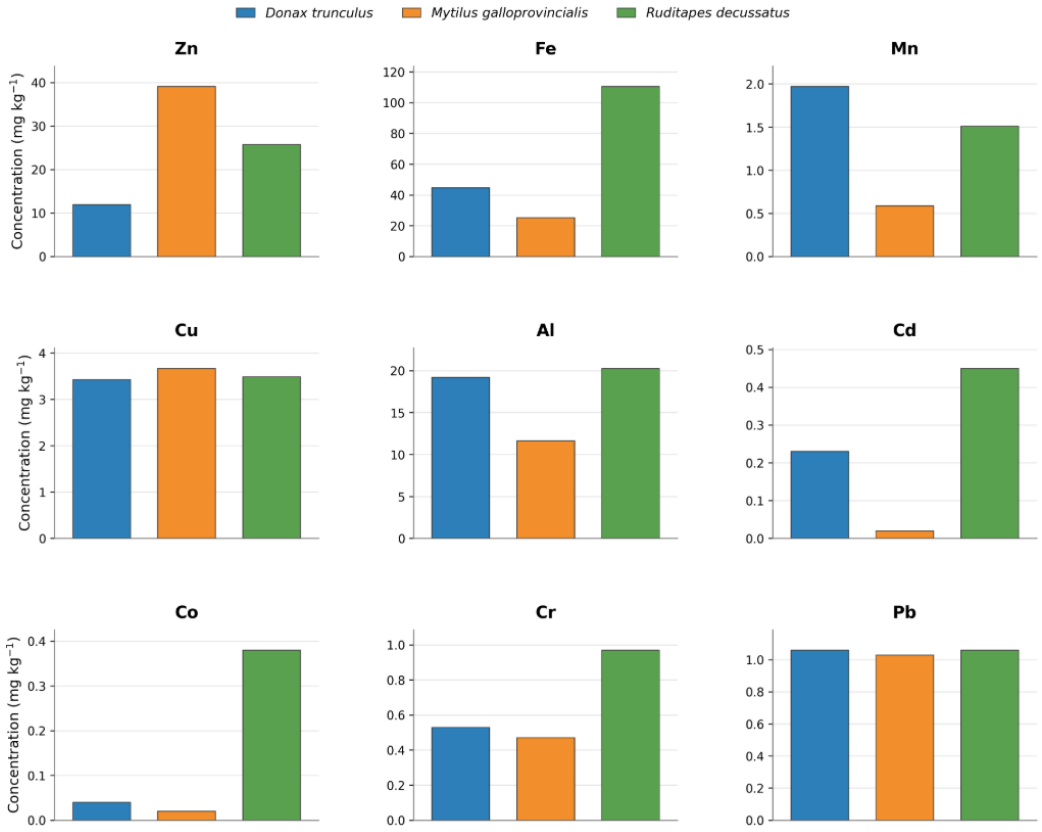


Figure 2. Mean concentrations of potentially toxic elements (PTEs) in the soft tissues of *Donax trunculus*, *Mytilus galloprovincialis*, and *Ruditapes decussatus*.

According to the mean concentrations (mg kg^{-1}), the highest levels of Zn and Cu were detected in *M. galloprovincialis*, Mn in *D. trunculus*, and Fe, Al, Cd, Co, and Cr in *R. decussatus*. The highest Pb concentration was observed in both *D. trunculus* and *R. decussatus*.
The estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR) values calculated for PTEs are presented in Table 3.

Table 3. Estimated daily intake and human health risk assessment results for PTEs in the investigated bivalve species groups.

Species		Zn	Fe	Mn	Cu	Al	Cd	Co	Cr	Pb	Ni	HI
DT	EDI	3.37E -03	1.26E -02	5.55E -04	9.67E -04	5.41E -03	6.48E -05	1.13E -05	1.49E -04	2.99E -04	1.83E -04	1.14E -02
	THQ	1.12E -05	1.80E -05	1.11E -02	2.42E -05	5.41E -06	6.48E -05	3.76E -05	4.98E -05	7.47E -05	9.16E -06	
	CR	---	---	---	---	---	2.46E -08	---	7.47E -08	2.54E -09	3.11E -07	
MG	EDI	1.10E -02	7.11E -03	1.66E -04	1.03E -03	3.28E -03	5.64E -06	5.64E -06	1.32E -04	2.90E -04	1.89E -04	3.56E -03
	THQ	3.67E -05	1.02E -05	3.33E -03	2.59E -05	3.28E -06	5.64E -06	1.88E -05	4.42E -05	7.26E -05	9.44E -06	
	CR	---	---	---	---	---	2.14E -09	---	6.62E -08	2.47E -09	3.21E -07	
RD	EDI	7.27E -03	3.11E -02	4.26E -04	9.84E -04	5.71E -03	1.27E -04	1.07E -04	2.73E -04	2.99E -04	1.16E -03	9.32E -03
	THQ	2.42E -05	4.45E -05	8.51E -03	2.46E -05	5.71E -06	1.27E -04	3.57E -04	9.11E -05	7.47E -05	5.79E -05	
	CR	---	---	---	---	---	4.82E -08	---	1.37E -07	2.54E -09	1.9 E -06	

DT: *Donax trunculus*; MG: *Mytilus galloprovincialis*; RD: *Ruditapes decussatus*.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

The EDI values, representing dietary exposure to PTEs through the consumption of the investigated bivalve molluscs, were evaluated against the available tolerable daily and weekly intake values. The relevant health-based guidance values for the investigated PTEs are presented in Table 4. The highest EDI values calculated for the PTEs did not exceed the corresponding guidance values.

Table 4. Health-based guidance values and adult Tolerable Upper Intake Levels used to evaluate dietary exposure to the investigated elements

Guidance values	Zn	Fe	Mn	Cu	Al	Cd	Co	Cr	Pb	Ni
TDI/PMTDI (mg kg^{-1} bw day^{-1})	0.3–1.0	0.8	NE	0.5	-	-	NE	0.3*	NE	0.013
TWI/PTWI (mg kg^{-1} bw week^{-1})	—	—	—	—	1.0	0.0025	—	—	WD	—
Adult UL (mg person^{-1} day^{-1})	40	45	11	10	NR	NR	NR	NE	NR	1.0**
Adult UL normalized for a 70-kg individual[1] (mg kg^{-1} bw day^{-1})***	0.571	0.643	0.157	0.143	—	—	—	—	—	0.0143
Referances	JECFA, 1982 IOM, 2001	JECFA, 1984 IOM, 2001	IOM, 2001	JECFA, 1982 IOM, 2001	EFSA, 2008	EFSA, 2009	—	EFSA, 2014 IOM, 2001	EFSA, 2010	EFSA, 2020 IOM, 2001

* The TDI value applies to Cr(III).
** The IOM UL applies to soluble nickel salts.
*** Body-weight-adjusted ULs were calculated by dividing the adult UL by the assumed adult body weight of 70 kg; these values were not directly reported by IOM (2001).

TDI: tolerable daily intake; PMTDI: provisional maximum tolerable daily intake; TWI: tolerable weekly intake; PTWI: provisional tolerable weekly intake; UL: tolerable upper intake level; NE: not established; NR: not reviewed in IOM (2001); WD: withdrawn.

The **THQ** values obtained were substantially below 1, indicating that individual exposure to PTEs through the consumption of the investigated bivalves is unlikely to cause appreciable non-carcinogenic health effects. The calculated **HI** values were also substantially below 1, indicating that combined exposure to the investigated elements is unlikely to pose an appreciable non-carcinogenic health risk. Most element-specific **CR** values were below 10^{-6}, indicating a negligible carcinogenic risk. The highest CR value was calculated for Ni in *R. decussatus* (1.90×10^{-6}), which was within the generally acceptable risk range of 10^{-6}–10^{-4}.

The concentrations of PTEs (Cd, Cu, Fe, Pb, and Zn) detected in *M. galloprovincialis* specimens collected from the Çanakkale Strait were reported to be at levels considered safe for human health, consistent with the findings of the present study (Demir & Akkuş, 2018). Similarly, Cd and Pb exposure associated with the consumption of *Ruditapes philippinarum* and *D. trunculus* collected from the Sea of Marmara was found to be below the corresponding TDI values (Künili et al., 2021). Furthermore, EDI, THQ, and HI assessments of Cd, Cu, Pb, and Zn in *M. galloprovincialis* harvested from the Sea of Marmara indicated no appreciable health risk (Hacısalihoğlu, 2025). Likewise, EDI- and THQ-based assessments of Fe, Zn, Mn, Cu, Pb, and Cd in *M. galloprovincialis* collected from the Turkish coast of the Black Sea

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indicated no appreciable health risk (Bat et al., 2018). In contrast, Al concentrations detected in the soft tissues of *R. decussatus* collected from the Homa Lagoon in İzmir Bay exceeded the relevant critical threshold values (Bilgin & Uluturhan, 2015).

Mussels collected from natural habitats may exhibit elevated PTE concentrations attributable to exposure to environmental contaminants. In contrast, mussels cultivated under controlled conditions in relatively uncontaminated waters that meet the criteria established by the Turkish Ministry of Agriculture and Forestry may generally show lower PTE accumulation in their soft tissues. Moreover, depuration of farmed mussels by holding them in clean, aerated seawater may contribute to reducing contaminant levels (Akdemir, 2025).

Conclusion

Based on the EDI, THQ, HI, and CR results, consumption of the three bivalve species harvested from Class B and Class C production areas in the Sea of Marmara and sampled following depuration or relaying was not associated with an appreciable health risk from the investigated PTEs (Zn, Fe, Mn, Cu, Al, Cd, Co, Cr, Pb, and Ni) under the evaluated consumption scenario. However, aquatic ecosystems are dynamic and are subject to pressures from both point and diffuse pollution sources. Therefore, continuous and systematic monitoring of PTE concentrations in marine environments and seafood is essential. Because comparisons with regulatory maximum levels alone may not adequately characterize potential adverse health effects, assessments based on dietary exposure and both carcinogenic and non-carcinogenic health risks should also be conducted to ensure consumer safety.

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Development of a Hemocyanin-Based Wound Care Gel Prototype from Blue Crab (*Callinectes sapidus*)

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Abstract

The blue crab (*Callinectes sapidus*) is an invasive species along the Marmara, Çanakkale Strait, Aegean, and Mediterranean coasts of Türkiye, where its rising numbers pressure local organisms and fisheries. At the same time, its protein content and bioactive compounds make it a potential raw material for biotechnological products. This study aims to prepare a wound care gel prototype from hemocyanin obtained from blue crab hemolymph and to examine its basic properties. Crabs will be obtained from commercial fishers and transported under cold-chain conditions. Hemolymph will be drawn from live individuals through the arthrodial membrane, and hemocyanin will be purified by low-speed centrifugation, sequential filtration, and chromatography, with purity checked by SDS-PAGE. The purified protein will be incorporated into a biocompatible polymer gel to form a stable formulation. The gel's activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* will be tested by disk diffusion and MIC methods, and its stability will be monitored at 4 °C, 25 °C, and 37 °C. Since hemocyanins have been reported to show antimicrobial activity, this study offers an initial evaluation of the protein in gel form and provides insight into the economic use of an invasive species.

Keywords: *Callinectes sapidus*, hemocyanin, hemolymph, wound care, invasive species

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Exploring Fish Otoliths in Orontes River Research: A Bibliometric Approach

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Abstract

Otoliths, commonly known as 'ear stones', are believed to help determine the age of fish and the frequency of fish blooms, as well as the rate of species reproduction. They also help to prevent fractures and structural deterioration. Data on the otoliths of fish species in the Orontes River are crucial for ensuring the long-term sustainability of inland fisheries, as they provide valuable insights into environmental conditions. This study uses bibliometric methods to analyse the academic literature on the discovery of fish otoliths in the River. By analysing a comprehensive dataset of academic articles from the WOS database, the study identifies the most cited articles, leading authors and key institutions that have contributed to research on the discovery of fish otoliths in the River. The Web of Science database was used to identify all original articles, reviews, books, and book chapters on this subject published between 1982 and 2025. After collecting relevant bibliographic and citation data, keywords, citation networks and co-citations, the analysis was performed using VOSviewer software. A total of 84 articles were retrieved from the WOS database. These articles were cited a total of 2,469 times (2,422 times excluding self-citations). The highest number of citations occurred in 2024, with 225 citations across four publications. The h-index is 27. Of the 39 countries involved, the United States led the way with 41 published articles. Türkiye ranked 24th with two articles. The most active research area was 'Fisheries' (n = 47). The most prolific publisher was Wiley (n = 19). The Transactions of the American Fisheries Society was the journal with the most published articles (n = 8). In conclusion, this bibliometric analysis provides a historical overview of the development of the field and offers valuable insights into future research directions and policy implications for advancing the global subject area.

Keywords: Bibliometric analysis, Orontes River, Freshwater Fish, Otolith, WOS database

Introduction

Otoliths, commonly known as 'ear stones', are found in the inner ear of bony fish. These consist of three pairs of calcium carbonate-based otoliths: the sagitta, lapillus and asteriscus. These hard structures consist of calcium carbonate crystals and organic matter. While they serve similar functions, otoliths vary in shape, size, growth patterns, weight and chemical composition depending on the species. Therefore, they reflect a fish's age, size, growth and development, and can be used to identify the species (Karlou-Riga, 2000; Campana, 2004; Geldiay & Balık, 2007; Zorica et al., 2010).

The Orontes (known as 'Asi' in Turkish and Arabic) is a major river that flows into the Levantine coast of the Mediterranean Sea. Of the river's total length of 380 km, approximately 94 km lies

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within Turkey. It originates in Lebanon's Bekaa Valley and flows northward through western Syria, passing through the cities of Homs and Hama. It then enters the Hatay province in southern Turkey, where it turns sharply southwest to reach the sea approximately 30 km downstream from Antakya, emptying into the Mediterranean Sea at Samandağ (Bridgland et al., 2012; Özcan et al., 2013).

A total of 390 species of freshwater fish across 37 families have been identified in Turkey's inland waters. Of these, 367 are native species and 23 are naturalised non-native taxa. 202 of these species (55%) are endemic to Turkey (Kaya et al., 2025). To date, more than 50 fish species have been recorded in the Orontes River and its basin (Krupp & Schneider, 1991; Fricke et al., 2007; Yalçın-Özdilek, 2007; Özcan, 2013).

Bibliometric analysis is a powerful tool for examining scientific literature through quantitative measurement. However, of the three bibliometric analysis tools (Bibliometrix, VOSviewer and CiteSpace 5.5) (Xie et al., 2021), only the Web of Science (WoS) database offers valuable insights into how research on the discovery of fish otoliths in the Orontes River has evolved. Such analysis offers a comprehensive understanding of this evolution, highlighting trends, themes and influential studies while bringing new areas of interest to light that could shape future research agendas. This study aims to conduct such an analysis and present a systematic review of the academic literature from a global perspective. Through this analysis, we aim to identify key research areas, highlight trends and shifts in focus, and recognise significant contributions to the field. The increase in unstructured data today has rendered traditional data processing methods inadequate. Consequently, specialised data and information processing techniques, such as natural language processing (NLP) and machine learning (ML), have become important. These methods enable meaningful information to be extracted from unstructured data (Zhang et al., 2021).

This study will analyse scientific research on fish otoliths in the Orontes River using bibliometric methods to identify key trends, influential publications and emerging research areas.

Materials and Methods

Research Design and Search Methodology

A bibliographic (retrospective) review was conducted using the bibliometric analysis method (Zhang et al., 2021). The Web of Science Core Collection (WoSCC) electronic database, one of the bibliometric analysis methods widely used by scientists and providing comprehensive and consistent exportable data, was used to create the literature dataset. WoSCC was chosen as the main data source for the study because of its long history as a reliable source for biomedical research and its ability to provide a strong and reliable foundation for research. Its ability to provide comprehensive citation data was emphasized.

Data Collection

The data search terms were "Fish Otolith" AND "Orontes River" (Discovery of Fish Otoliths in Orontes River Research: A Bibliometric Approach). To eliminate bias and account for frequent updates to the WoSCC database, the literature search was performed on a single day (July 27, 2026). The research was conducted between 1982 and 2025.

Standards for Study Selection

A comprehensive search in WoSCC using the terms "Fish Otolith" AND "Orontes River" (Discovery of Fish Otoliths in Orontes River Research: A Bibliometric Approach) resulted in the identification of a total of 97 studies. Subsequently, by selecting original articles, from the document type tab, the search yielded 84 records.

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Data collection

The primary data points were publication titles, publication years, names of journals in which the research was published, author names, keywords, names of the institutions to which the authors were affiliated and countries of origin and were extracted in plain text format. The analysis considered the total number of articles and total citations retrieved from the WoSCC database. WoS publications were saved as .txt files and subsequently exported to Microsoft Office Excel 2019 (Los Angeles, CA, USA) for further analysis.

Network analytics

In this bibliometric study, VOSviewer (version 1.6.10, Leiden University, Netherlands) was used to import data and identify trends, collaborations, and key findings. The software was also used to collect and analyze data related to authorship, links, keywords, citations, and thematic words. The program aided in the analysis of keywords, concurrent uses, citations, co-authorship, and co-citations.

Results

During the study period from 1982 to 2025, publications were produced in four languages. The most common language used was English (Table 1).

Table 1. The languages in which articles were published.

Languages	Record Count	% of 84
English	80	95,238%
French	2	23,81%
Chinese	1	11,90%
Japanese	1	11,90%

A total of 26 countries/regions have been published in the period from1982 to 2025. The ten countries with the most publications are shown in Figure 1.

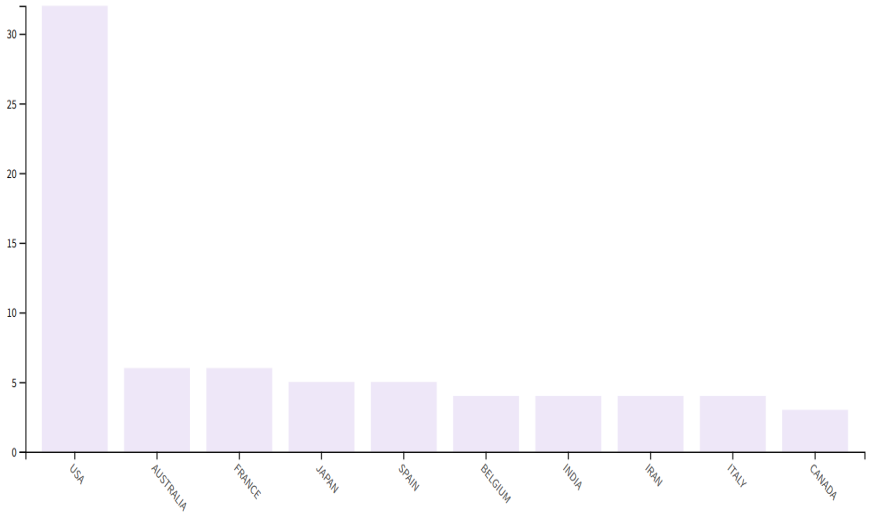


Figure 1. Most productive countries

Over the course of the study, which ranged from 1982 to 2025, 17 independent research areas were discovered. Table 2 shows the top 10 most extensively researched areas.

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Table 2. Most active research areas

Research Areas	Record Count	% of 84
Fisheries	37	44,048%
Marine Freshwater Biology	32	38,095%
Environmental Sciences Ecology	18	21,429%
Zoology	13	15,476%
Oceanography	10	11,905%
Biodiversity Conservation	6	7,143%
Science Technology Other Topics	6	7,143%
Anatomy Morphology	2	2,381%
Water Resources	2	2,381%
Audiology Speech Language Pathology	1	1,190%

Showing first 10 out entries

In the years between 1982 and 2025, a total of 199 affiliations have been published. Figure 2 presents the affiliations of the ten journals with the highest number of publications.



Figure 2. Most prolific affiliations

Between 1982 and 2025, ten entries were detected, representing 42 different publishers. Table 3 lists the most prolific publishers.

Table 3. Most prolific publishers

Publishers	Record Count	% of 84
Wiley	16	19,048%
Elsevier	8	9,524%
Mdpi	6	7,143%
Amer Fisheries Soc	4	4,762%
Taylor & Francis	4	4,762%
Springer Nature	3	3,571%
Biodiversity Research Center, Acad Sinica	2	2,381%
Canadian Science Publishing	2	2,381%
Csiro Publishing	2	2,381%
KLUWER ACADEMIC PUBL	2	2,381%

Showing first 10 outentries

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A total of 58 journal articles have been published on this topic. Table 4 includes the top 10 journals with the most publications.

Table 4. Journals publishing the most articles

Journals	Record Count	% of 84
TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY	7	8,333%
CANADIAN JOURNAL OF FISHERIES AND AQUATIC SCIENCES	3	3,571%
ENVIRONMENTAL BIOLOGY OF FISHES	3	3,571%
FISHERIES RESEARCH	3	3,571%
FISHES	3	3,571%
JOURNAL OF FISH BIOLOGY	3	3,571%
NORTH AMERICAN JOURNAL OF FISHERIES MANAGEMENT	3	3,571%
AUSTRALIAN JOURNAL OF MARINE AND FRESHWATER RESEARCH	2	2,381%
COPEIA	2	2,381%
ECOLOGY OF FRESHWATER FISH	2	2,381%

Showing 10 out of entries

The evaluated articles generated a total of 2,058 citations (2,031 of which were self-citations), with an average h-index of 27. The highest number of citations (225) and publications (4) were found in 2024 (Figure 3).

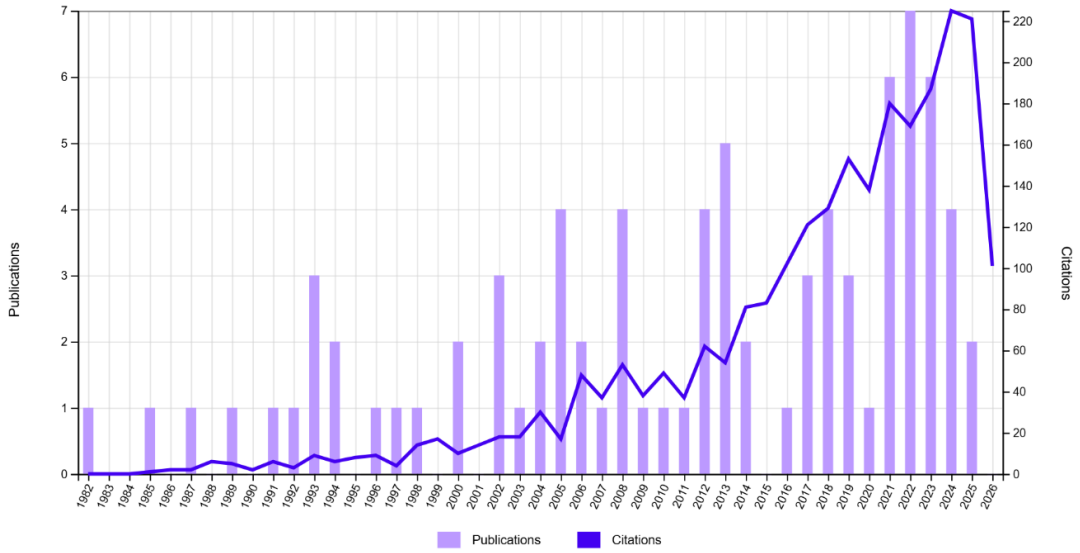


Figure 3. Publication and citation frequency

As shown in Figure 4, our bibliometric study has revealed the most frequently used keywords. It was found that the font size of the keywords was closely proportional to the frequency with which they were repeated in the articles.

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Figure 4. Key Word visualization map of articles

The size of the bubbles was proportional to the amount of research done in each country in the network representation of bibliographic coupling between nations. The width of the lines connecting the countries indicates the degree to which they are linked, while the colours of the lines indicate the cluster to which each country is assigned. For this study, we have included at least one document and one citation from each country (Figure 5).

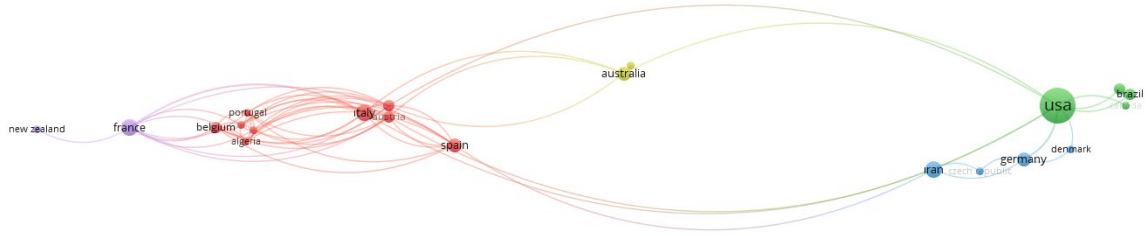


Figure 5. Network visualisation of bibliographic coupling betweencountries

The relationship between authors with at least five publications and at least five citations is shown in Figure 6.

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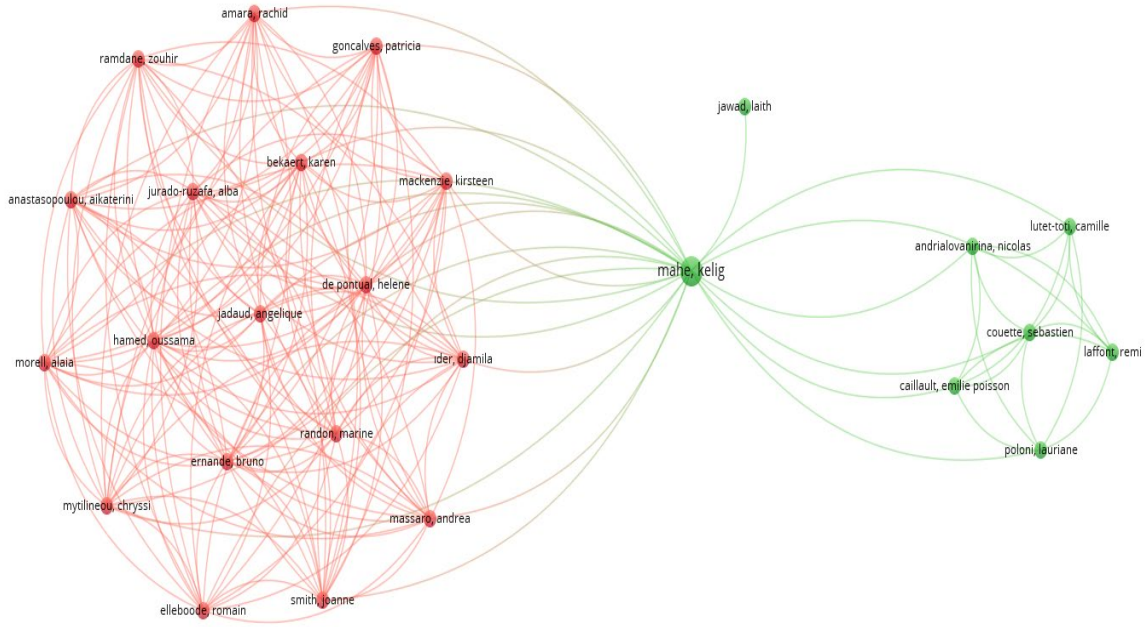


Figure 6. The relationships between authors

Discussion

Otolith analysis is of great importance in determining fish population dynamics and stock structure (Zampatti et al., 2021). In freshwater systems, the geometric morphometry of otoliths can distinguish between very similar cryptic species and stocks, particularly those that are morphologically similar (Dörtbudak et al., 2022; Özpiçak, 2020). For example, it has been proven that *Cyprinion kais* and *Cyprinion macrostomus*, previously considered the same species in the Tigris River, can be successfully distinguished by differences in otolith shape (Dörtbudak et al., 2022). *Barbus tauricus* stocks in the Black Sea basin (Akçay, Engiz, Terme, Karadere and Değirmenağzı) were classified with 75.3% accuracy using discriminant analysis (Dörtbudak et al., 2022; Özpiçak, 2020). Similarly, otolith shape analyses are expected to provide high population discrimination power in distinguishing fish stocks in the Asi (Orontes) River.

In freshwater ecosystems, environmental parameters such as water temperature and pH can influence the growth and shape of otoliths. In carp, the degree of asymmetry in asteriscus otoliths has been found to vary depending on geographical region and specific environmental conditions (Jawad & Mahé, 2022). Although high otolith mass asymmetry can negatively impact acoustic sensitivity and sound orientation, it is generally not a functional problem for most fish species (Lychakov & Rebane, 2005).

In migratory river fish, otolith microchemistry is a valuable method for determining habitat use, migration routes and breeding time and location (Pease et al., 2023; Zampatti et al., 2021). Sr:Ca analyses performed on the *Centropomus undecimalis* species in the Usumacinta River (Mexico) revealed insights into habitat use and ecological relationships (Pease et al., 2023). In contrast, strontium isotopes in the Murray River in Australia provided information on the reproductive cycle and migratory movements of the *Macquaria ambigua* species (Zampatti et al., 2021).

Otolith shape indices (e.g. form factor, circularity, aspect ratio and ellipticity) and chemical marking methods are effective for monitoring the population and stock structure of river fish (Durham & Wilde, 2008; Rai & Rani, 2022). Otolith morphometry studies of fish in the Asi (Orontes) River are expected to reveal the species-specific effects of regional environmental conditions.

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This bibliometric study evaluated research on fish otoliths in the Orontes River basin. According to the Web of Science (WoS) database, findings covering the period 1982–2025 show that scientific output has increased, particularly in recent years. Studies are primarily focused on Fisheries and Marine and Freshwater Biology. While the United States leads the way in this field of research, Türkiye is ranked 24th, indicating the need for more regional-scale studies targeting the dynamics of the basin.

Findings obtained through otolith morphometry and microchemistry in large river systems such as the Tigris, Usumacinta and Murray constitute an important reference for aquatic systems with high endemism under intense anthropogenic pressure, such as the Orontes River. Future studies should focus on elucidating the stock infrastructure of endemic and commercially valuable Asi (Orontes) River fish species, determining their species and stock structures, and identifying their natal areas and migration routes. Integrating otolith analyses with classical biological methods would significantly contribute to sustainable fishing, habitat restoration, and aquatic conservation policies.

Funding

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Ethics Approval

No need to ethical approval for this study.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data supporting the findings of this study were derived from the Web of Science Core Collection database. The bibliometric dataset generated and analysed during the current study is available from the corresponding author upon reasonable request due to privacy reasons.

Limitations

One limitation of this study is its reliance on the Web of Science database. This database may have excluded some relevant articles from other databases or grey literature, thus overlooking important perspectives on the Discovery of Fish Otoliths in the Orontes River research. The exclusion of case reports and other document types may have led to underrepresentation of certain research trends or reviews that could complement the primary research findings. Furthermore, since the study only covers publications in English and a few other languages, significant contributions from non-English speaking regions may have been excluded. The use of citation counts and h-indexes as baseline measures may not fully reflect the qualitative impact of the research. These measures tend to favor older and more widely cited studies compared to newer and more innovative studies.

Author Contribution

The authors conceived and designed the study, collected and analyzed the data, prepared the figures, wrote the manuscript, and approved the final version of the manuscript.

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Genetic erosion and demographic signatures in Mediterranean white sharks (*Carcharodon carcharias*): insights from Tunisian waters

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Abstract

The white shark (*Carcharodon carcharias*) is a globally distributed apex predator of high conservation concern, with Mediterranean populations considered rare and poorly characterized genetically. In this study we assessed mitochondrial genetic diversity and demographic history of individuals sampled from Tunisian waters using two markers: the cytochrome c oxidase subunit I (COI) gene and the control region (D-loop). A total of 15 COI sequences (550 bp) and 9 D-loop sequences (856 bp) were analyzed. COI data revealed low genetic diversity, with only 9 polymorphic sites, 3 haplotypes, a haplotype diversity (h) of 0.257 and a nucleotide diversity (π) of 0.00239. Neutrality tests showed significant negative values (Tajima's D = -1.97, $P < 0.05$; Fu and Li's F = -2.59, $P < 0.05$) suggesting an excess of rare variants consistent with recent population expansion or purifying selection. However, mismatch distribution and demographic analyses showed a relatively high raggedness index (r = 0.613) and a positive Fu's Fs value (Fs = 1.56), not fully supporting a classic expansion model. In contrast, the D-loop exhibited extremely low variability, with only one segregating site, two haplotypes (h= 0.222), and very low nucleotide diversity (π = 0.00026). Neutrality tests were negative but not statistically significant, and demographic inference remained inconclusive due to the limited polymorphism. Overall, both markers consistently indicate very low mitochondrial diversity in Tunisian white sharks. The COI signal of recent demographic expansion contrasts with the near invariance of the D-loop, likely reflecting reduced effective population size, historical bottlenecks, or strong maternal lineage structuring in the Mediterranean. These findings align with previous reports of low genetic diversity in Mediterranean *C. carcharias* and reinforce concerns about its conservation status.

Keywords: *Carcharodon carcharias*, COI, D-loop, genetic diversity, population genetics, Mediterranean Sea.

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Crustacean Fauna of Iskenderun Bay: A Review of Nearly a Century of Studies

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Abstract

Iskenderun Bay, located in the northeastern Levantine Sea, is a biologically diverse coastal system exposed to urbanisation, industrial activities, fisheries, port infrastructure, and the introduction of alien species. This review compiles and synthesises published information on the crustacean fauna of Iskenderun Bay based on studies conducted since the 1930s. A total of 56 scientific publications, including taxonomic studies, species inventories, benthic community investigations, ecological assessments, fisheries studies, and reports of alien species, were evaluated. Records were included when they directly referred to Iskenderun Bay or when the study area explicitly encompassed the bay. A total of 256 crustacean species larger than 0.5 mm, representing ten taxonomic orders, were recorded. Amphipoda was the most species-rich order (104 species), followed closely by Decapoda (101 species). The remaining species were distributed among Isopoda (24 species), Cumacea (8 species), Balanomorpha and Mysida (5 species each), Stomatopoda (4 species), Tanaidacea (3 species), and Scalpellomorpha and Leptostraca (1 species each). Of these, 34 were reported as alien species, accounting for approximately 13% of the total crustacean fauna. Decapoda constituted the largest component of the alien species records, with 30 species. The results are a species richness based on the literature and not a direct estimate of the current abundance or distribution, due to differences in sampling effort, habitat coverage, geographical extent and taxonomic resolution of the reviewed studies. This synthesis provides a regional baseline for future taxonomic, ecological and biodiversity studies of crustaceans in Iskenderun Bay and emphasizes the need for standardised, long-term monitoring programmes.

Keywords: Iskenderun Bay; crustacean fauna; biodiversity; alien species.

Introduction

Iskenderun Bay is a semi-enclosed coastal system located in the northeastern Levantine Basin of the eastern Mediterranean. Its geographical position, hydrographic characteristics and proximity to the Suez Canal make the bay an important area for studying marine biodiversity, species dispersal and biogeographical change. At the same time, the bay is exposed to various anthropogenic pressures, including industrial activities, port operations, fisheries, coastal settlements and land-based pollution. Industrial contamination has been detected in several coastal areas of the bay, with some stations showing distinct pollution signatures associated

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with factory-related inputs (Yücel & Çam, 2021). Land-based nutrient inputs have also been reported to influence the physicochemical conditions and phytoplankton dynamics of coastal waters, particularly in nearshore areas (Polat, 2002). In addition, historical changes in river discharge and sedimentation indicate that human activities have influenced the environmental development of the bay over long time scales (Spezzaferri et al., 2000).

The biological structure of the eastern Mediterranean has been substantially modified since the opening of the Suez Canal. The canal created a pathway for the movement of Red Sea and Indo-Pacific organisms into the Mediterranean, resulting in the establishment of numerous alien species, especially in the eastern Mediterranean subregion. This process is generally referred to as Lessepsian migration or Erythraean migration and is regarded as a major biogeographical process in the Mediterranean Sea (Zakaria, 2015). The eastern Mediterranean contains a particularly high proportion of thermophilic non-indigenous species, and crustaceans, molluscs and fishes are among the taxonomic groups in which alien species are especially well represented (Zenetos et al., 2010). In addition to migration through the Suez Canal, maritime transport, including the movement of organisms through ballast water and ship-associated vectors, may contribute to the introduction and spread of alien species (Streftaris et al., 2005).

Crustaceans also have ecological and economic importance as components of marine food webs and fisheries. They may act as consumers, detritivores, predators and prey, and they constitute important food items for commercially and ecologically significant fish species. In Iskenderun Bay, fishes such as lionfish have been reported to consume crustaceans, including shrimps, indicating that crustaceans contribute to predator–prey interactions in the bay's benthic and demersal food webs (Demirci & Demirhan, 2022). Penaeid and other commercially important shrimp species are also associated with regional fisheries and may be affected by environmental contamination. Microplastics have been detected in commercially important shrimp species from the northeastern Mediterranean, including species collected from Iskenderun Bay, demonstrating both the exposure of crustaceans to marine pollutants and the potential for human exposure through seafood consumption (Yücel & Kılıç, 2025). Recent research has additionally identified localized metal accumulation in green tiger shrimp from Iskenderun Bay, emphasizing the need to consider the bay as a potential contamination hotspot in assessments of crustacean health and seafood safety (Göçer et al., 2026).

Despite their ecological, biogeographical and economic importance, information on the crustacean fauna of Iskenderun Bay remains fragmented across taxonomic, faunistic, ecological, fisheries and biogeographical studies. Earlier investigations generally focused on particular taxonomic groups or habitats, whereas more recent studies have increasingly addressed community-level distribution patterns, biological invasions and environmental pressures. Direct comparisons among these studies are often difficult because of differences in sampling design, gear type, station distribution, depth range, taxonomic resolution and habitat classification. A comprehensive synthesis is therefore needed to integrate the available records and establish a reliable baseline for future research.

In this study, data from 56 scientific publications covering approximately a century of research are synthesized to evaluate the crustacean fauna of Iskenderun Bay. The recorded species are assessed in terms of taxonomic composition, habitat preference, depth distribution and biogeographical origin, with particular emphasis on alien. This synthesis provides a reference framework for future taxonomic revisions, biodiversity assessments, ecological monitoring and the detection of long-term changes in benthic communities of the eastern Mediterranean.

Materials and Methods

Iskenderun Bay is surrounded by densely populated coastal areas, industrial facilities, port infrastructure, agricultural land and fishing grounds. Its coastal morphology includes a variety of natural and artificial habitats such as soft-bottom areas (Mutlu 2002; 2015; Özcan et al.,

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2008; 2010b), rocky shores (Bakır et al., 2007; Özcan et al., 2010a; İnandı et al., 2014; Ergüden et al., 2021; Özcan & Özcan, 2025), bivalve beds (Doğan et al., 2008), vegetated habitats (Çevik et al., 2012), port structures (Şen et al., 2010) and shallow coastal zones (Sezgin et al., 2007; Türeli & Leba, 2023; Alkan & Özcan, 2025).
The area shown in Figure 1 was used in this study. Only records from sampling stations located in this designated area of Iskenderun Bay were considered. Some studies covered a larger geographical area than Iskenderun Bay, but only the Crustacea species reported from stations within the boundaries specified were included in the list in Table 1 (Kocataş & Katağan, 1978; Çınar et al., 2008).

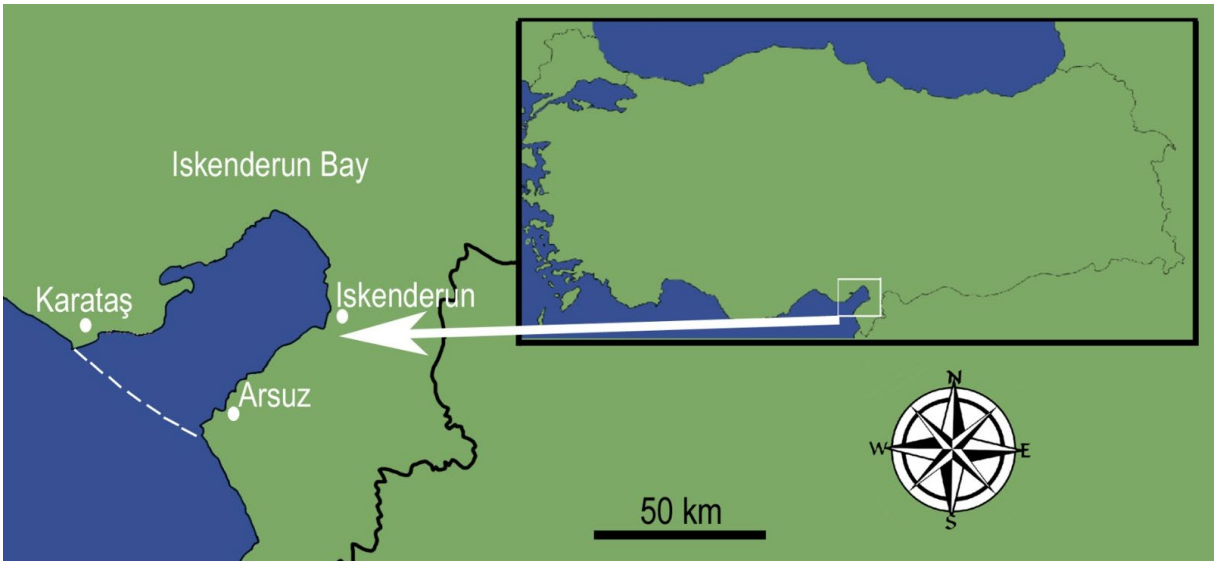


Figure 1. Map of the study area. The dashed line between Karataş and Arsuz in the right panel delineates the geographical boundary applied for the inclusion of species records in this review.

A comprehensive literature search was conducted across open-access scientific publications, conference proceedings, and research project reports documenting Crustacea species in Iskenderun Bay. This review covers taxonomic studies, species inventories, benthic community studies, ecological assessment, fisheries research and reports on alien species, but does not include data from academic theses.
The present review was restricted to crustacean taxa larger than 0.5 mm. This 0.5-mm threshold was established as an operational inclusion criterion to increase comparability of data between studies using different sampling gears and targeting different habitats. Thus records of predominantly microcrustacean taxa such as Copepoda and Cladocera were excluded.

Results and Discussion

The literature review found 56 studies that were conducted within the defined regional limits and during the years 1932 to 2026, reporting a total of 256 species of Crustacea. Based on these compiled data records, it was observed that the identified species in the region are distributed along a broad depth gradient from shallow coastal waters (≤ 50 m) to deep marine environments (> 50 m) and include a highly diverse range of benthic and pelagic habitats, both soft and hard substrates (Table 1).
The early studies of Iskenderun Bay by Monod (1932) and Gruvel (1936) documented both Red Sea/Indo-Pacific and native Mediterranean crustacean species, such as *Penaeus* (*Eopenaeus*) *semisulcatus*, *Penaeus* (*Marsupenaeus*) *pulchricaudatus*, *Portunus pelagicus*, and *Myra subgranulata*, as well as the native Mediterranean species such as *Penaeus* (*Melicertus*) *kerathurus* and *Parapenaeus longirostris* These historical records indicate that Iskenderun Bay

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has long been an important area for the northward expansion and establishment of Lessepsian species introduced through the Suez Canal. However, because the early studies did not provide station-specific sampling information, they cannot be used to make reliable assessments of habitat preferences, depth distributions or relative abundances. Accordingly, these publications should be considered primarily as historical and biogeographical records of crustacean research in Iskenderun Bay rather than as standardized ecological surveys.

Among the studies conducted in Iskenderun Bay, the investigation by Çınar et al. (2008), which examined the structural characteristics of zoobenthic communities along the Levantine coast, recorded the highest number of crustacean species. This study also represents the most comprehensive survey conducted in the region to date. Samples were collected between 0 and 50 m depth from a range of biotopes, including rocky and sandy bottoms, mussel beds formed by *Brachidontes pharaonis* and *Modiolus barbatus*, and algal assemblages dominated by *Jania rubens*, *Cystoseira elegans*, *Padina pavonica*, *Ulva* sp. and *Dictyota dichotoma*. A total of 109 crustacean species were identified.

During the literature review, *Penaeus japonicus* was also encountered in several historical records. However, this species was excluded from the checklist because recent genetic evidence indicates that specimens reported from the Turkish coast correspond to *P. pulchricaudatus* rather than *P. japonicus* (Deval et al., 2023). Accordingly, the records of *P. japonicus* reported by Monod (1932), Enzenross and Enzenross (1987) and Can et al. (2004) were reassigned to *P. pulchricaudatus*. Similarly, the record of *Myra fugax* reported by Monod (1932) was regarded as a misidentification and omitted from the checklist. Recent taxonomic studies have shown that specimens from the Turkish coast identified as *M. fugax* actually belong to *Myra subgranulata* (Bakır et al., 2024).

Table 1. Reported marine crustacean taxa from Iskenderun Bay, including source references, depth range, habitat type [Alien species are marked with an asterisk (*). Depth categories are defined as S: shallow water (≤50 m) and D: deep water (>50 m). Habitat codes are as follows: Hs, hard substratum, including algae, sponges, and mussels; Ss, soft substratum, including sediments and seagrass-associated habitats; P, pelagic habitat; Ps, parasitic association; and W, submerged wood. E: economic importance, BC: Barcelona Convention and BN: Bern Convention.].

Species	Sources	Depth range	Habitat
Order: Balanomorpha			
Amphibalanus amphitrite (Darwin, 1854)	22, 28	S	Hs
**Amphibalanus improvisus* (Darwin, 1854)	12	S	Hs
Chthamalus montagui Southward, 1976	22, 35	S	Hs
Chthamalus stellatus (Poli, 1791)	22, 35	S	Hs
Microeuraphia depressa (Poli, 1791)	22	S	Hs
Order: Scalpellomorpha			
Scalpellum scalpellum (Linnaeus, 1767)	42	D	Hs
Order: Leptostraca			
Nebalia bipes (Fabricius, 1780)	12	S	Ss
Order: Stomatopoda			
**Clorida albolitura* Ahyong & Naiyanetr, 2000	42, 43, 55	S	Ss
**Erugosquilla massavensis* (Kossmann, 1880)	5, 42, 43, 50, 52, 55	S-D	Ss
Rissoides desmaresti (Risso, 1816)	55	S	Ss
Squilla mantis (Linnaeus, 1758)	42	S-D	Ss
Order: Mysida			

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Gastrosaccus sanctus (Van Beneden, 1861)	22	S	Ss
Gastrosaccus spinifer (Goës, 1864)	12, 22	S	Ss
Mesopodopsis slabberi (Van Beneden, 1861)	45	S	Ss-P
Paramysis (*Longidentia*) *helleri* (G.O. Sars, 1877)	22	S	Ss
Siriella clausii G.O. Sars, 1877	22	S	Ss-P
Order: Amphipoda			
Ampelisca brevicornis (A. Costa, 1853)	12, 31, 34	S	Ss
Ampelisca diadema (A. Costa, 1853)	31, 37	S	Ss
Ampelisca gibba G.O. Sars, 1883	34	S	Ss
Ampelisca massiliensis Bellan-Santini & Kaim-Malka, 1977	37	D	Ss
Ampelisca multispinosa Bellan-Santini & Kaim-Malka, 1977	34	S-D	Ss
Ampelisca provincialis Bellan-Santini & Kaim-Malka, 1977	34	D	Ss
Ampelisca pseudosarsi Bellan-Santini & Kaim-Malka, 1977	22	S-D	Ss
Ampelisca ruffoi Bellan-Santini & Kaim-Malka, 1977	37	S-D	Ss
Ampelisca sarsi Chevreux, 1888	34	S-D	Ss
Ampelisca tenuicornis Liljeborg, 1856	34	S	Ss
Ampelisca truncata Bellan-Santini & Kaim-Malka, 1977	34	S-D	Hs-Ss
Ampelisca typica (Spence Bate, 1857)	34	S-D	Ss
Ampithoe ferox (Chevreux, 1902)	4	S	Hs-Ss
Ampithoe ramondi Audouin, 1826	4, 22, 31, 51	S	Hs-Ss
Ampithoe riedli Krapp-Schickel, 1968	4, 22, 31	S	Hs-Ss
Aora spinicornis Afonso, 1976	4	S-D	Hs-Ss
Apherusa chiereghinii Giordani Soika, 1950	22	S	Ss
Apohyale crassipes (Heller, 1866)	4, 22	S	Hs-Ss
Apohyale perieri (Lucas, 1846)	4, 22	S	Hs
Apolochus neapolitanus (Della Valle, 1893)	4	S	Ss
Apolochus picadurus (J.L. Barnard, 1962)	22, 31	S	Hs-Ss
Apocorophium acutum (Chevreux, 1908)	22	S	Hs-Ss
Bathyporeia lindstromi Stebbing, 1906	12	S-D	Ss
Bathyporeia guilliamsoniana (Spence Bate, 1857)	4, 12, 22, 34	S-D	Ss
Biancolina algicola Della Valle, 1893	4	S	Hs-Ss
Caprella acanthifera Leach, 1814	22	S	Hs-Ss
Caprella grandimana Mayer, 1882	22	S	Hs
Caprella hirsuta Mayer, 1890	22	S	Hs
Centraloecetes dellavallei (Stebbing, 1899)	22, 34	S	Ss
Cheirocratus sp.	37	S-D	Hs
Cheiriphotis mediterranea Myers, 1983	34	S	Ss
Chelura terebrans Philippi, 1839	22	S	Hs-Ss-W
Colomastix pusilla Grube, 1861	34	S	Hs
Deflexilodes acutipes (Ledoyer, 1983)	34	S	Ss
Dexamine spiniventris (A. Costa, 1853)	22, 31	S	Hs-Ss
Dexamine spinosa (Montagu, 1813)	4, 12, 22, 34	S	Hs-Ss
Echinogammarus sp.	31	S	Hs
Elasmopus pectenicrus (Spence Bate, 1863)	21, 22	S	Hs-Ss
Elasmopus pocillimanus (Spence Bate, 1863)	4, 22, 31, 44	S	Hs-Ss
Ericthonius brasiliensis (Dana, 1853)	4, 12, 22, 31	S	Hs
Ericthonius difformis H. Milne Edwards, 1830	31	S	Hs

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Ericthonius punctatus (Spence Bate, 1857)	22, 31	S	Hs
Eriopisa elongata (Bruzelius, 1859)	34, 37	D	Ss
Gammarella fucicola (Leach, 1814)	4, 22, 31	S	Hs-Ss
Gammaropsis sophiae (Boeck, 1861)	31	D	Hs-Ss
Haploops nirae Kaïm-Malka, 1976	34	S-D	Ss
Harpinia antennaria Meinert, 1890	37	S-D	Ss
Harpinia crenulata (Boeck, 1871)	37	S-D	Ss
Harpinia dellavallei Chevreux, 1911	34, 37	S-D	Ss
Harpinia pectinata G.O. Sars, 1891	34, 37	S-D	Ss
Harpinia truncata Sars, 1891	34	S-D	Ss
Hippomedon massiliensis Bellan-Santini, 1965	34	S-D	Ss
Jassa falcata (Montagu, 1808)	4	S	Ss
Jassa marmorata Holmes, 1905	22, 31	S	Ss
Kroyera carinata Spence Bate, 1857	12	S-D	Ss
**Latigammaropsis togoensis* (Schellenberg, 1925)	19, 22	S	Hs-Ss
Lembos websteri Spence Bate, 1856	22, 34	S	Hs-Ss
Leptocheirus bispinosus Norman, 1908	37	S	Hs-Ss
Leptocheirus pectinatus (Norman, 1869)	34	S-D	Ss
Leptocheirus pilosus Zaddach, 1844	22	S-D	Hs-Ss
Leucothoe lilljeborgi Boeck, 1861	4, 34	S-D	Ss
Leucothoe occulta Krapp-Schickel, 1975	34	S	Ss
Leucothoe richiardii Lessona, 1865	12, 37	S-D	Ss
Leucothoe spinicarpa (Abildgaard, 1789)	22, 44	S	Ss
Liropus elongatus Mayer, 1890	34	S	Ss
Lysianassa caesarea Ruffo, 1987	22	S	Hs-Ss
Lysianassa costae H. Milne Edwards, 1830	22, 34	S-D	Ss
Lysianassa pilicornis Heller, 1866	4	S-D	Ss
Lysianassina longicornis (Lucas, 1846)	22	S-D	Ss
Maera grossimana (Montagu, 1808)	22	S	Ss
Maera sodalis Karaman & Ruffo, 1971	34	D	Ss
Medicorophium rotundirostre (Stephensen, 1915)	37	S	Ss
Megaluropus massiliensis Ledoyer, 1976	22	S	Ss
Melita hergensis Reid, 1939	22	S	Ss
Metaphoxus gruneri Karaman, 1986	34	S	Ss
Metaphoxus simplex (Spence Bate, 1858)	12	S-D	Ss
Microdeutopus anomalus (Rathke, 1843)	22	S	Ss
Microdeutopus bifidus Myers, 1977	34	S	Hs-Ss
Microdeutopus similis Myers, 1977	22	S	Ss
Microdeutopus stationis Della Valle, 1893	4	S	Ss
Neoischyrocerus inexpectatus (Ruffo, 1959)	22	S	Hs
Nototropis massiliensis (Bellan-Santini, 1975)	22	S-D	Ss
Othomaera schmidtii (Stephensen, 1915)	22, 34, 37	S-D	Ss
Paraphoxus oculatus (G. O. Sars, 1879)	37	S-D	Ss
Parhyale aquilina (Costa, 1857)	4	S	Hs
Pereionotus testudo (Montagu, 1808)	22	S	Ss
Perioculodes aequimanus (Kossman, 1880)	22, 34	S-D	Ss
Perioculodes longimanus (Spence Bate & Westwood, 1868)	12, 22	S-D	Ss

Phtisica marina Slabber, 1769	4, 31, 34	S-D	Ss
Platorchestia platensis (Krøyer, 1845)	4	S	Ss
Plumulojassa ocia (Spence Bate & Westwood, 1862)	31	S	Ss
Podocerus variegatus Leach, 1814	22	S	Hs
Pontocrates arenarius (Spence Bate, 1858)	22, 34	S	Ss
Protohyale (*Boreohyale*) *camptonyx* (Heller, 1866)	4, 22	S	Hs
Protohyale (*Protohyale*) *schmidtii* (Heller, 1866)	4, 22	S	Hs
Pseudoprotella phasma (Montagu, 1804)	22	S	Ss
Ptilohyale littoralis (Stimpson, 1853)	22	S	Hs
Quadrimaera inaequipes (A. Costa in Hope, 1851)	22, 44	S	Ss
Stenothoe eduardi Krapp-Schickel, 1976	22	S-D	Hs-Ss
Stenothoe gallensis Walker, 1904	22	S	Hs
Tritaeta gibbosa (Spence Bate & Westwood, 1862)	22, 34	S	Hs-Ss
Tryphosa nana (Krøyer, 1846)	12, 37	S	Ss
Urothoe grimaldii Chevreux, 1895	12, 22, 34	S	Ss
Urothoe poseidonis Reibish, 1905	12	S	Ss
Order: Isopoda			
Anilocra physodes (Linnaeus, 1758)	12	S-D	Ps
Anthura gracilis (Montagu, 1808)	37	S	Hs
Apanthura corsica Amar, 1953	22	S	Ss
Cleantis prismatica (Risso, 1827)	22	S	Hs
Compecopea sp.	22	S	Hs
Cyathura carinata (Krøyer, 1847)	22	S-D	Ss
Cymodoce spinosa (Risso, 1816)	22	S	Hs-Ss
Cymodoce truncata Leach, 1814	22	S	Hs-Ss
Cymodoce tuberculata A. Costa in Hope, 1851	22	S	Hs-Ss
Dynamene bicolor (Rathke, 1836)	22, 44	S	Hs-Ss
Dynamene edwardsi (Lucas, 1849)	22	S	Hs-Ss
Eurydice affinis Hansen, 1905	22	S	Hs-Ss
Eurydice pulchra Leach, 1816	12, 37	S-D	Ss
Eurydice spinigera Hansen, 1890	22	S	Ss-P
Eurydice truncata (Norman, 1868)	37	S-D	Ss
Gnathia vorax (Lucas, 1849)	22, 44	S-D	Ss
Idotea balthica (Pallas, 1772)	51	S	Hs-Ss-P
Jaera sp.	22	S	Hs
Janira maculosa Leach, 1814	22, 44	S-D	Hs-Ss
Lekanesphaera monodi (Arcangeli, 1934)	22	S	Ss
Ligia italica Fabricius, 1798	22	S	Hs
Limnoria tripunctata Menzies, 1951	28	S	Hs-W
Paradella sp.	22	S	Hs
Paranthura costana Bate & Westwood, 1866	22	S	Ss
Order: Tanaidacea			
Apseudopsis latreillii (Milne Edwards, 1828)	12, 22, 31, 37, 44	S	Hs-Ss
Chondrochelia savignyi (Kroyer, 1842)	22, 44, 51	S-D	Hs-Ss
*Tanais dulongii (*Audouin, 1826)	22	S	Hs-Ss
Order: Cumacea			
Bodotria scorpioides (Montagu, 1804)	12, 22	S-D	Hs-Ss

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Campylaspis glabra Sars, 1878	37	D	Ss
Cumella (*Cumella*) *limicola* Sars, 1879	22	S	Hs-Ss-P
Cumella (*Cumella*) *pygmaea* G.O. Sars, 1865	37	S	Ss-P
Eudorella truncatula (Bate, 1856)	37	S-D	Ss
Iphinoe elisae Băcescu, 1950	12	S-D	Ss
Eucuma sp.	22	S	Hs
Pseudocuma (*Pseudocuma*) *longicorne* (Bate, 1858)	12	S-D	Hs-Ss-P
Order: Decapoda			
Acanthonyx lunulatus (Risso, 1816)	22, 29	S	Hs-Ss
Achaeus gracilis (O.G. Costa, 1839)	22	S	Hs-Ss
Aegaeon cataphractus (Olivi, 1792)	42	S	Hs
Aegaeon lacazei (Gourret, 1887)	42	D	Ss
Albunea carabus (Linnaeus, 1758)	14	S	Ss
Alpheus dentipes Guérin, 1832	29	S-D	Hs
Alpheus glaber (Olivi, 1792)	12, 22	S-D	Hs
**Alpheus inopinatus* Holthuis & Gottlieb, 1958	31	S-D	Hs
**Alpheus migrans* Lewinsohn & Holthuis, 1978	22, 31, 37	S	Hs
**Alpheus rapacida* De Man, 1908	22, 31	S-D	Hs
Anapagurus laevis (Bell, 1845)	37	S-D	Ss
Ashtoret lunaris (Forskål, 1775)	38	S	Ss
**Atergatis roseus* (Rüppell, 1830)	5, 6, 7, 15, 42	S	Hs-Ss
Athanas nitescens (Leach, 1814)	22, 29	S-D	Hs-Ss
Brachynotus foresti Zariquiey Álvarez, 1968	22	S	Hs
Calcinus tubularis (Linnaeus, 1767)	22	S	Hs
Callianassa subterranea (Montagu, 1808)	12, 37	S	Ss
*, [E]*Callinectes sapidus* Rathbun, 1896	5, 10, 15, 17, 32, 43, 55	S	Ss
[E]*Carcinus aestuarii* Nardo, 1847	55	S	Hs-Ss
**Carupa tenuipes* Dana, 1852	42	S	Hs
Cestopagurus timidus (Roux, 1830)	22	S-D	Hs
**Charybdis* (*Charybdis) hellerii* (A. Milne-Edwards, 1867)	5, 6, 15, 20, 22, 27	S	Hs-Ss
**Charybdis* (*Archias*) *longicollis* Leene, 1938	6, 15, 24, 41, 42, 43, 55	S	Ss
Clibanarius erythropus (Latreille, 1818)	22	S	Hs-Ss
**Coleusia signata* (Paulson, 1875)	5, 6	S	Hs-Ss
Crangon crangon (Linnaeus, 1758)	12	S-D	Ss
Dardanus calidus (Risso, 1827)	14	S	Ss
Diogenes pugilator (Roux, 1829)	12, 22	S	Hs-Ss
Dromia personata (Linnaeus, 1758)	15, 22	S-D	Hs-Ss
[E]*Eriphia verrucosa* (Forskål, 1775)	15	S	Hs-Ss
Ethusa mascarone (Herbst, 1785)	15, 42	S-D	Hs-Ss
**Eucrate crenata* (De Haan, 1835)	6, 7	S	Ss
**Eurycarcinus integrifrons* De Man, 1879	26, 30, 31	S	Ss
Gilvossius tyrrhenus (Petagna, 1792)	12	S	Hs-Ss
Goneplax rhomboides (Linnaeus, 1758)	12, 15, 42	S-D	Ss
Homola barbata (Fabricius, 1793)	42	S-D	Hs
Hippolyte leptocerus (Heller, 1863)	22	S	Hs-Ss
Latreillia elegans Roux, 1830	42	S-D	Ss

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

**Leptochela pugnax* De Man, 1916	12, 22, 31, 37	S-D	Ss
Liocarcinus depurator (Linnaeus, 1758)	15, 42	S-D	Ss
**Liomera rugipes* (Heller, 1861)	54	S	Hs
Inachus communissimus Rizza, 1839	42	S	Hs-Ss
**Ixa monodi* Holthuis & Gottlieb, 1956	5, 6, 15, 42, 43, 55	S-D	Ss
Macrophthalmus (*Macrophthalmus*) *graeffei* A. Milne-Edwards, 1873	8, 12, 22, 31, 37	S-D	Hs-Ss
Macropodia linaresi Forest & Zariquiey Álvarez, 1964	42	S-D	Hs-Ss
Macropodia longirostris (Fabricius, 1775)	42	S-D	Hs-Ss
Macropodia rostrata (Linnaeus, 1761)	15, 31, 42	S-D	Hs-Ss
Macropodia tenuirostris (Leach, 1814)	42	S-D	Hs-Ss
[BN, E]*Maja squinado* (Herbst, 1788)	15	S-D	Hs-Ss
**Matuta victor* (Fabricius, 1781)	46, 49	S-D	Ss
Medorippe lanata (Linnaeus, 1767)	15, 42	S	Ss
**Metapenaeopsis aegyptia* Galil & Golani, 1990	43, 55	S-D	Ss
*, [E]*Metapenaeus monoceros* (Fabricius, 1798)	9, 13, 16, 40, 42, 43, 47, 53, 55	S	Ss
*, [E]*Metapenaeus stebbingi* Nobili, 1904	9, 55	S	Ss
**Micippa thalia* (Herbst, 1803)	8	S	Hs
Monodaeus couchii (Couch, 1851)	42	S	Ss
Munida sp.	42	S-D	Hs
**Myra subgranulata* Kossmann, 1877	1, 15, 55	S-D	Hs-Ss
[BC, BN]*Ocypode cursor* (Linnaeus, 1758)	15	S-D	Ss
**Ogyrides mjoebergi* (Balss, 1921)	22, 25	S	Ss
Pachygrapsus marmoratus (Fabricius, 1787)	15, 22, 23, 35	S	Hs
Pachygrapsus maurus (Lucas, 1846)	22, 23	S	Hs
Paguristes eremita (Linnaeus, 1767)	14	S	Hs-Ss
Pagurus alatus Fabricius, 1775	14	S-D	Ss
Pagurus anachoretus Risso, 1827	22	S-D	Hs-Ss
Pagurus chevreuxi (Bouvier, 1896)	42	S-D	Hs-Ss
Pagurus prideaux Leach, 1815	42	S-D	Ss
Palaemon serratus (Pennant, 1777)	12,	S-D	Hs-Ss
Paragalene longicrura (Nardo, 1869)	36	S	Hs
[E]*Parapenaeus longirostris* (Lucas, 1846)	1, 40, 42	S-D	Ss
Pasiphaea sivado (Risso, 1816)	42	D	Ss
*, [E]*Penaeus* (*Eopenaeus*) *semisulcatus* De Haan, 1844	1, 2, 5, 9, 16, 24, 40, 42, 43, 47, 53, 55, 56	S-D	Ss
*, [E]*Penaeus* (*Farfantepenaeus*) *aztecus* Ives, 1891	33, 53	S	Ss
*[E]*Penaeus* (*Fenneropenaeus*) *merguiensis* De Man, 1888	18	S	Ss
*[E]*Penaeus* (*Marsupenaeus*) *pulchricaudatus* Stebbing, 1914	1, 2, 5, 13, 16, 42	S	Ss
[E]*Penaeus* (*Melicertus*) *kerathurus* (Forskål, 1775)	1, 13, 16, 42, 43, 55	S-D	Ss
*, [E]*Penaeus* (*Oleopenaeus*) *hathor* Burkenroad, 1959	11, 16, 20, 40	S	Ss
Pilumnus hirtellus (Linnaeus, 1761)	15, 22, 23, 29, 42	S-D	Hs
Pisa armata (Latreille, 1803)	42	S	Hs-Ss
Pisa nodipes Leach, 1815	42	S-D	Hs-Ss
Pisidia bluteli (Risso, 1816)	22	S	Hs-Ss
[E]*Plesionika edwardsii* (Brandt, 1851)	42	D	Ss

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Polycheles perarmatus Holthuis, 1952	42	D	Ss
Portumnus latipes (Pennant, 1777)	12, 15	S	Hs-Ss
Portunus pelagicus (Linnaeus, 1758)	1, 2, 3, 6, 9, 10, 15, 17	S	P
[E]Portunus segnis (Forskål, 1775)	32, 43, 55	S	P
Processa canaliculata Leach, 1815	42	S-D	Hs-Ss
Processa edulis (Risso, 1816)	37	S	Hs-Ss
Processa elegantula Nouvel & Holthuis, 1957	12	S-D	Hs-Ss
Processa nouveli Al-Adhub & Williamson, 1975	12	S-D	Hs-Ss
Processa robusta Nouvel & Holthuis, 1957	12	S	Hs-Ss
Sicyonia carinata (Brünnich, 1768)	42	S-D	Hs-Ss
**Sicyonia lancifer* (Olivier, 1811)	39, 42	D	Ss
[E]Solenocera membranacea (Risso, 1816)	42	S-D	Ss
Spinolambrus macrochelos (Herbst, 1790)	42	S-D	Ss
Synalpheus africanus Crosnier & Forest, 1965	42	S	Hs
**Thalamita poissonii* (Audouin, 1826)	12, 22, 31, 42	S	Hs-Ss
[E]Upogebia pusilla (Petagna, 1792)	12, 31	S-D	Hs-Ss
Upogebia tipica (Nardo, 1869)	22, 31	S	Ss
**Urocaridella pulchella* Yokes & Galil, 2006	48	S	Hs
Xantho pilipes A. Milne-Edwards, 1867	22	S-D	Hs-Ss

1: Monod, 1932; 2:Gruvel, 1936; 3: Holthuis & Gottlieb, 1958; 4: Kocataş & Katağan, 1978; 5: Enzenross & Enzenross, 1987; 6: Enzenross & Enzenross, 1990; 7: Enzenross et al., 1992; 8: Enzenross & Enzenross, 1995; 9: Kumlu et al., 1999; 10: Türeli et al., 2000; 11: Kumlu et al., 2002; 12: Mutlu, 2002; 13: Can et al., 2004; 14: Koçak et al., 2005; 15: Özcan et al., 2005; 16: Türkmen, 2005; 17: Özcan & Akyurt, 2006; 18: Özcan et al., 2006; 19: Bakır et al., 2007; 20: Özcan et al., 2007; 21: Sezgin et al., 2007; 22: Çınar et al., 2008; 23: Doğan et al., 2008; 24: Fırat, et al., 2008; 25: Özcan et al., 2008; 26: Özcan et al., 2010a; 27: Özcan et al., 2010b; 28: Şen et al., 2010; 29: Özcan & Katağan, 2011; 30: Turan et al., 2011; 31: Çevik et al., 2012; 32: Özcan, 2012; 33: Bilecenoğlu et al., 2013; 34: Bakır & Katağan, 2014; 35: İnandı et al., 2014; 36: Gönülal et al., 2015; 37: Mutlu, 2015; 38: Turan_et al., 2015; 39: Gönülal et al., 2016; 40: Kaymacı & Altun, 2016; 41: Özcan & Katağan, 2016; 42: Gönülal & Dalyan, 2017; 43: Yeşilyurt & Türeli, 2018; 44: Çınar et al., 2019; 45: Özcan et al., 2019; 46: Yeşilyurt et al., 2019; 47: Can et al., 2021; 48: Ergüden et al., 2021; 49: Türeli & Leba, 2023; 50: Yeşilyurt & Türeli, 2024; 51: Alkan & Özcan, 2025; 52: Başusta & Çevik, 2025; 53: Özbek & Demirci, 2025; 54: Özcan & Özcan, 2025; 55: Türeli et al., 2025; 56: Göçer et al., 2026.

The recorded fauna comprised ten crustacean orders. Amphipoda was the most species-rich order, with 104 species, followed by Decapoda with 101 species and Isopoda with 24 species. Together, these three orders accounted for 229 of the 256 reported species. The remaining orders were Cumacea (8 species), Balanomorpha and Mysida (5 species each), Stomatopoda (4 species), Tanaidacea (3 species) and Scalpellomorpha and Leptostraca (1 species each) (Figure 2).

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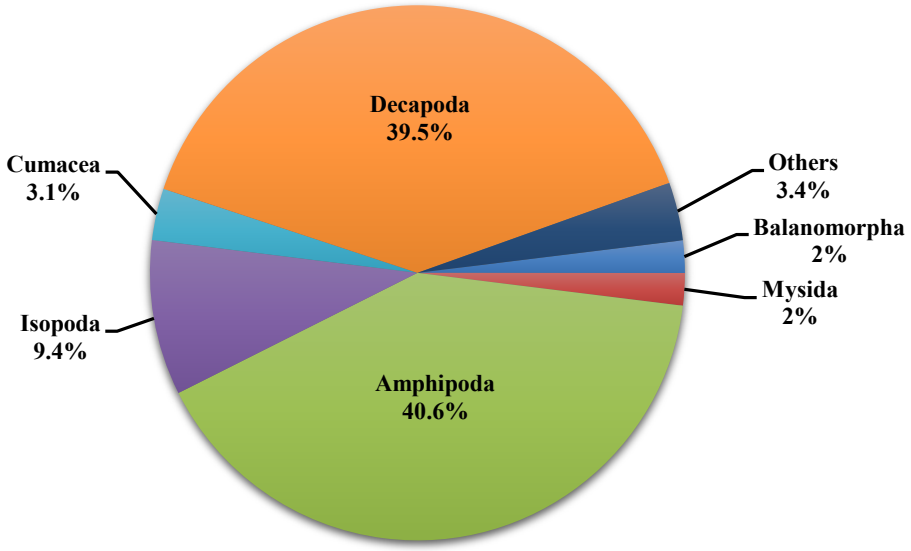


Figure 2. Relative contribution of crustacean orders to the total number of reported species in Iskenderun Bay. "Others" includes Balanomorpha, Scalpellomorpha, Leptostraca, Stomatopoda, Mysida, and Tanaidacea.

The compiled data revealed distinct habitat preferences among the recorded species: 121 species were found to exclusively inhabit soft substrates, while 49 species are restricted to hard substrates. The remaining 86 species consist of taxa with diverse ecological traits, including those occupying multiple substrate types, as well as pelagic (*P. pelagicus* and *P. segnis*), parasitic, and wood-boring (submerged wood) (*Chelura terebrans*) organisms.
In terms of depth distribution, 153 species are confined to shallow waters (≤ 50 m), whereas 91 species were identified as either exclusively inhabiting deep waters (> 50 m) or possessing a broader bathymetric tolerance, occurring in both shallow and deep zones.

Alien species
The total number of alien crustacean species recorded from Iskenderun Bay was 34 which corresponds to about 13 % of 256 species reported in the present dataset. The species comprised one Balanomorpha, two Stomatopoda, one Amphipoda and 30 Decapoda. Thus, Decapoda was the group with the highest number of reported species among the alien taxa, representing 87.1% of all alien species (Table 2). This dominance is in accordance with the important role of decapods in Lessepsian migration and their ability to exploit a wide range of habitats in the Eastern Mediterranean (Corsini-Foka et al., 2017).

Table 2. Reported alien crustacean species from Iskenderun Bay and their proposed or presumed introduction pathways (Compiled from Enzenross & Enzenross, 1990; 1995; Çınar et al., 2021; Özcan & Özcan, 2025).

Ordo	Species	Pathways
Balanomorpha	*Amphibalanus improvisus*	Shipping
Stomatopoda	*Clorida albolitura*	Suez Canal
Stomatopoda	*Erugosquilla massavensis*	Suez Canal
Amphipoda	*Latigammaropsis togoensis*	Shipping
Decapoda	*Alpheus inopinatus*	Suez Canal
Decapoda	*Alpheus migrans*	Suez Canal
Decapoda	*Alpheus rapacida*	Suez Canal
Decapoda	*Atergatis roseus*	Suez Canal

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Decapoda	*Callinectes sapidus*	Shipping
Decapoda	*Carupa tenuipes*	Suez Canal
Decapoda	*Charybdis* (*Charybdis*) *hellerii*	Suez Canal
Decapoda	*Charybdis* (*Archias*) *longicollis*	Suez Canal
Decapoda	*Coleusia signata*	Suez Canal
Decapoda	*Eucrate crenata*	Suez Canal
Decapoda	*Eurycarcinus integrifrons*	Shipping
Decapoda	*Leptochela pugnax*	Suez Canal
Decapoda	*Liomera rugipes*	Suez Canal
Decapoda	*Ixa monodi*	Suez Canal
Decapoda	*Matuta victor*	Suez Canal
Decapoda	*Metapenaeopsis aegyptia*	Suez Canal
Decapoda	*Metapenaeus monoceros*	Suez Canal
Decapoda	*Metapenaeus stebbingi*	Suez Canal
Decapoda	*Micippa thalia*	Suez Canal
Decapoda	*Myra subgranulata*	Suez Canal
Decapoda	*Ogyrides mjoebergi*	Suez Canal
Decapoda	*Penaeus* (*Eopenaeus*) *semisulcatus*	Suez Canal
Decapoda	*Penaeus* (*Farfantepenaeus*) *aztecus*	Shipping
Decapoda	*Penaeus* (*Fenneropenaeus*) *merguiensis*	Suez Canal
Decapoda	*Penaeus* (*Marsupenaeus*) *pulchricaudatus*	Suez Canal
Decapoda	*Penaeus* (*Oleopenaeus*) *hathor*	Suez Canal
Decapoda	*Portunus segnis*	Suez Canal
Decapoda	*Sicyonia lancifer*	Suez Canal
Decapoda	*Thalamita poissonii*	Suez Canal
Decapoda	*Urocaridella pulchella*	Suez Canal

Protected species

Only two species of crustacean identified from Iskenderun Bay, *Maja squinado* and *Ocypode cursor* were found to be listed under the Bern and/or Barcelona conventions (Table 1). *M. squinado* is under the Bern Convention and *O. cursor* is under both the Bern Convention and the Barcelona Convention.

The occurrence of *O. cursor* in the protected species is of particular importance, as this species is strongly linked to sandy beaches and dune habitats that are highly sensitive to coastal development, recreational activities, beach cleaning, and other anthropogenic disturbances (Marchessaux et al., 2024). Recent studies have reported declines of *O. cursor* populations in intensively used Mediterranean coastal areas. They have also shown that recreational disturbance may reduce burrow abundance and alter the activity patterns of the species (Galil et al., 2024). The results suggest that conservation of *O. cursor* in Iskenderun Bay should not be limited to the protection of individual animals but should also include the preservation of suitable sandy-shore habitats and restriction of activities that cause habitat degradation.

Economic Importance

Eighteen crustacean species of economic importance have been reported from the Iskenderun Bay. Among these species, shrimps are particularly prominent (Table 1). The economically important shrimp species reported from the region include *Metapenaeus monoceros*, *M. stebbingi*, *Parapenaeus longirostris*, *Penaeus* (*Eopenaeus*) *semisulcatus*, *P.* (*Farfantepenaeus*) *aztecus*, *P.* (*Fenneropenaeus*) *merguiensis*, *P.* (*Marsupenaeus*) *pulchricaudatus*, *P.* (*Melicertus*) *kerathurus*, *P.* (*Oleopenaeus*) *hathor*, *Plesionika edwardsii*, *P. martia* and

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Solenocera membranacea (Kumlu et al., 1999; Can et al., 2004; Türkmen, 2005; Can et al., 2006; Kaymacı & Altun, 2016; Özbek & Demirci, 2025). In addition to shrimps, economically important crab species include *Callinectes sapidus*, *Carcinus aestuarii*, *Eriphia verrucosa*, *Maja squinado* and *Portunus segnis* (Türeli et al., 2000; Özcan & Akyurt, 2006; Özcan, 2012; Harlıoğlu et al., 2018). *Upogebia pusilla* is economically important primarily as a live bait, not for direct consumption (Aydın & İlkyaz, 2021). However, the economic importance of these species can vary according to their market value, the volume of their catch, their stock status, their contribution to regional fisheries and the pattern of utilization. For the alien or lessepsian species included in the list, their economic value should be evaluated together with their potential ecological effects (Zenetos et al., 2010).

Conclusions

This review synthesizes the fragmented data on the crustacean fauna of Iskenderun Bay and provides a solid baseline for future taxonomic, ecological and biodiversity studies. Since the bay is under multiple pressures (urbanization, industrial activities, port infrastructure, fishing pressure and presence of non-native species), regular monitoring of faunal changes is necessary. In this context, the establishment of standardized long-term monitoring programs with comparable sampling methods across a range of habitats and seasons is considered a primary requirement. Such initiatives are crucial for assessing the current distribution and abundance of species, monitoring the spread of alien taxa and providing a stronger scientific basis for the conservation of regional biodiversity.

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Ethics Approval

Not applicable. This study is a literature review and did not involve any experiments on humans or animals; therefore, ethical approval was not required.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Author Contribution

Ozge OZGEN: Investigation, Data curation, Writing – original draft. Elif ACAR: Investigation, Data curation.

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Crustacean Fauna of *Zostera marina* Meadows off the Dardanos Coast, Çanakkale Strait: A Preliminary Study

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Abstract

This study examined the crustacean taxa inhabiting *Zostera marina* meadows off the Dardanos coast of the Çanakkale Strait, and assessed their distribution with respect to depth (0.5, 1, 2 and 4 m) and season. Samples were collected in winter and spring 2025 by SCUBA diving using 30×30 cm quadrats, with three replicates per depth and season. In the field, samples were sieved (0.5 mm) and fixed in 4% formaldehyde. In the laboratory, individuals were sorted under a stereomicroscope and identified to the lowest taxonomic level possible, following Ruffo (1982–1998), Naylor (1972), Jones (1976), Holdich & Jones (1983) and Bakır et al. (2014). Taxon names follow WoRMS. A total of 26 crustacean species/taxa were recorded. The most abundant were *Ampithoe* sp. (n = 71), *Dexamine* sp. (n = 60), *Ampelisca* sp. (n = 50) and *Dynamene* sp. (n = 43). Amphipoda accounted for the largest number of taxa, whereas Isopoda and Decapoda were represented by fewer individuals. *Apseudes latreillii* (Tanaidacea), *Iphinoe serrata* and *Cumella limicola* (Cumacea) were also recorded. Most individuals came from the shallow stations at 0.5 and 1 m, where the highest counts of *Ampithoe* sp. and *Dexamine* sp. were also found. More taxa were recorded in winter than in spring, while in spring the numbers of individuals were more variable among stations. This study presents the first observations on the crustacean fauna of the Dardanos Z. marina meadows and indicates that a variety of crustacean taxa are associated with them. Sampling is planned to continue so that the effects of depth and season can be assessed in more detail. This study was supported by the TÜBİTAK 2209-A University Students Research Projects Support Programme.

Keywords: *Zostera marina*, Crustacea, macrobenthic fauna, biodiversity, Çanakkale Strait

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Growth, Reproduction, and Population Structure of Silver Scabbardfish (*Lepidopus caudatus*, Euphrasen, 1788) in Gökova Bay (Southern Aegean Sea, Türkiye)

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Abstract

This study aimed to determine the population structure, growth, and reproductive characteristics of the silver scabbardfish (*Lepidopus caudatus,* Euphrasen, 1788) in Gökova Bay (Southern Aegean Sea). Sampling was conducted monthly from September 2009 to May 2010 using trawl and gill nets. A total of 115 individuals (71 males, 44 females) were examined. Total length ranged from 31.0 to 99.0 cm, and total weight ranged from 16.9 to 685.7 g. The male-to-female ratio was 1.61:1, indicating a predominance of males, likely due to the sampling method. Age determination from otolith readings identified age groups 0–III, with age group II (44.35%) being dominant. Von Bertalanffy growth parameters for the overall population were estimated as $L\infty$ = 151.64 cm, k = 0.27 yr^{-1}, and t_0 = -0.34. The length–weight relationship was $W = 0.0003 \times L^{3.20}$ (r^2 = 0.99), indicating positive allometric growth. Monthly gonadosomatic index (GSI) values and macroscopic gonad examinations showed that reproductive activity begins in February and continues through late November, with distinct peaks in October and May, suggesting a multiple-spawning strategy. Overall, the Gökova Bay population exhibited biological characteristics consistent with other Mediterranean and Atlantic populations of the species, but with lower maximum age and size. This study provides the first detailed biological data for this species in the bay, highlighting its role as an important trophic link in the regional food web and as a baseline for sustainable fisheries management.

Keywords: silver scabbardfish, *Lepidopus caudatus*, population biology, growth parameters, reproductive biology, Gökova Bay, Aegean Sea

Introduction

The silver scabbardfish *Lepidopus caudatus* Euphrasen, 1788 is a benthopelagic member of the family Trichiuridae with a broad distribution extending through the eastern Atlantic, the Mediterranean Sea, the southern Indian Ocean and the south-western Pacific (Froese and Pauly 2010; Mytilineou et al. 2005). The species typically inhabits continental-shelf and upper-slope grounds over muddy or sandy bottoms and performs nocturnal vertical movements while feeding on crustaceans, cephalopods and fishes (Parin 1986). In addition to its predatory role, it is an important prey item for several large fishes, sharks, skates and marine mammals, indicating a functionally relevant position in demersal food webs (Ebert et al. 1991, 1992; Gomes et al. 1998; Morato et al. 2003).

In Turkish waters, *L. caudatus* has been recorded from both the Aegean and Mediterranean seas, including reports from Edremit Bay, İzmir Bay and Gökova Bay (Bilecenoğlu et al. 2002; Torcu and Aka 2000; Çoker and Mater 2006; Özi and Yılmaz 2006). Despite these records, detailed biological information for the species in Türkiye has remained scarce, and no

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comprehensive assessment from Gökova Bay had been prepared before the present study. This lack of information is noteworthy because life-history traits such as age structure, growth and reproductive timing are central to evaluating population status and to supporting ecosystem-based fisheries management (Demestre et al. 1993; D'Onghia et al. 2000; Tuset et al. 2006). Previous studies from the Catalan Sea, Ionian Sea and Canary Islands have shown substantial geographic variation in growth, size composition and reproductive traits of *L. caudatus* (Demestre et al. 1993; D'Onghia et al. 2000; Tuset et al. 2006). Such differences may be associated with environmental conditions, prey availability, local hydrography, fishing pressure and sampling design. Accordingly, the present study aimed to describe the length and weight composition, sex ratio, age structure, growth parameters, length-weight relationship and reproductive characteristics of *L. caudatus* from Gökova Bay, thereby establishing a regional baseline for the species in the southern Aegean Sea.

Materials and Methods

Gökova Bay is located in the south-eastern Aegean Sea between the Bodrum and Datça peninsulas and has been designated as a Special Environmental Protection Area since 1989 (Gülahin 2008). The bay extends approximately 100 km in the east-west direction and reaches about 30 km in width in its western sector, whereas the present study focused on the inner bay where sampling depths reached approximately 50 m (Gülahin 2008).

A total of 115 specimens of *L. caudatus* were collected monthly between September 2009 and May 2010. Most individuals were obtained from commercial gillnet catches, while a smaller proportion came from bottom-trawl operations. Trawl sampling was conducted with a commercial vessel (22.6 m length, 580 HP) using a standard Mediterranean bottom trawl with a cod-end mesh size of 22 mm, towed for approximately 60 min at a speed of about 2.5 nautical miles h^{-1}.Gillnet samples were collected during retrieval of commercial fishing gear used by local fishers in the Akyaka sector, where the small-scale fleet is dominated by gillnet and combined longline-gillnet vessels (Akyol et al. 2007).

All specimens were transported to the laboratory on ice for morphometric and biological examination. Total length (TL) was measured to the nearest 1 mm or 0.1 cm, depending on the draft version, and total body weight and gonad weight were recorded to the nearest 0.01 g. Fifty randomly selected fish were also used for additional morphometric measurements, including standard length, fork length, pre-anal length, post-anal length, maximum body depth, maximum body width, snout length, head length, eye diameter, pectoral-fin height and dorsal-fin length. Descriptive statistics for these morphometric measurements are presented in Table 1**.**

Table 1. Morphometric characteristics of *Lepidopus caudatus* from Gökova Bay (n = 50). Values are mean ± standard deviation (range in parentheses).

MEASUREMENT	VALUE (CM)
Total Length (TL)	47.6 ± 22.8 (31.0–99.0)
Standard Length (SL)	47.0 ± 21.5 (30.0–94.0)
Fork Length (FL)	46.7 ± 21.9 (30.5–96.0)
Pre-anal Length (PreA-L)	21.8 ± 11.0 (14.1–46.0)
Post-anal Length (PostA-L)	25.8 ± 11.9 (16.6–53.0)
Maximum Body Height (MBH)	2.8 ± 1.6 (1.7–6.7)
Maximum Body Width (MBW)	1.3 ± 0.9 (0.7–3.8)
Snout Length (SnL)	2.6 ± 1.3 (1.6–5.5)
Head Length (HL)	6.8 ± 3.6 (4.5–14.5)
Eye Diameter (ED)	1.3 ± 0.8 (0.7–3.0)
Pectoral Height (Pect-H)	0.6 ± 0.3 (0.3–1.3)
Dorsal Fin Length (DL)	40.4 ± 19.7 (27.0–85.0)

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Sex and gonadal maturity were determined macroscopically, and female gonads were classified following Holden and Raitt (1974) into immature, developing, mature and spent stages. Because the reproductive analysis was based primarily on macroscopic staging and gonadosomatic index values, the reproductive interpretation should be regarded as provisional rather than histologically validated.

Sagittal otoliths were removed from fresh specimens, cleaned with distilled water and stored dry for age determination. Otoliths were examined under reflected light at 14X magnification, and each otolith was read independently by three readers without access to fish length, sex, date or locality information. An age estimate was accepted when at least two readers agreed, and unresolved otoliths were excluded from analysis. The relative precision between the readings was assessed using the index of mean percentage error (IAPE) (Beamish & McFarlane, 1983). Growth was modelled using the von Bertalanffy growth function (von Bertalanffy 1938):

$$Lt = L\infty(1-e^{-k}(t-t^{0})),$$

where L_t is total length at age t, L∞ is asymptotic length, k is the growth coefficient and t_0 is the theoretical age at zero length. The growth-performance index was reported as 3.79 for pooled sexes. Comparisons of von Bertalanffy parameters from different localities are shown in Table 2**.**

Table 2. Comparison of von Bertalanffy growth parameters of *Lepidopus caudatus* from different localities.

AUTHOR(S)	LOCALITY	SEX	L∞ (CM)	K (YEAR^{-1})	T₀ (YEAR)	Φ'
Fitch & Lavenberg (1968)	California	♂+♀	95.0	0.064	---	2.76
Moli et al. (1990)	NW Mediterranean	♂	185.2	0.333	0.34	4.06
Moli et al. (1990)	NW Mediterranean	♀	195.4	0.297	0.32	4.05
D'Onghia et al. (2000)	Ionian Sea	♂	174.3	0.310	-0.53	3.97
D'Onghia et al. (2000)	Ionian Sea	♀	182.5	0.300	-0.50	4.00
Tuset et al. (2006)	Canary Islands	♀	195.1	0.23	-1.23	3.94
Tuset et al. (2006)	Canary Islands	♂+♀	125.3	0.38	-0.38	3.78
Mariño-Briceño et al. (2022)	Azores	♂+♀	171.6	0.12	---	3.52
Rebac & Ferri (2025)	Eastern Adriatic	♂+♀	134.9	0.32	-1.01	---
Present study	Gökova Bay	♂+♀	151.64	0.27	-0.34	---

The length-weight relationship was described by the equation (Keys 1928):

$$W=aL^{b},$$

where W is total body weight in g, $L$is total length in cm, and a and b are regression parameters. The relationship was estimated from log-transformed data, the coefficient of determination was calculated, and departure of b from the isometric value of 3 was evaluated with a t-test; 95% confidence intervals were calculated after Zar (1984). Length-weight parameters for males, females and pooled sexes are presented in **Table 3**, and comparisons with other studies are provided in **Table 4**.

Table 3. Length–weight relationship parameters of *Lepidopus caudatus* from Gökova Bay (*A(+): positive allometric growth*).

Sex	n	Length range (cm)	Weight range (g)	a	b ± 95% CI	r²	Growth type
Males	71	32.0–94.0	18.3–685.7	0.0003	3.20 ± 0.12	0.99	A(+)
Females	44	31.0–99.0	16.9–637.0	0.0002	3.25 ± 0.10	0.99	A(+)
Combined	115	31.0–99.0	16.9–685.7	0.0003	3.20 ± 0.06	0.99	A(+)

Table 4. Comparison of length–weight relationship parameters of *Lepidopus caudatus* from different localities.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

AUTHOR(S)	LOCALITY	A	B	R^2
Fitch & Lavenberg (1968)	California	---	2.76	---
Moli et al. (1990)	NW Mediterranean	---	4.06	---
D'Onghia et al. (2000)	Ionian Sea	---	3.97–4.00	---
Tuset et al. (2006)	Canary Islands	---	3.78	---
Borges et al. (2003)	Algarve (Portugal)	0.0033	2.97	0.92
Filiz & Bilge (2004)	Sığacık Bay (N. Aegean)	0.00013	3.28	0.98
Torres et al. (2012)	Gulf of Cadiz (SW Spain)	0.0003	3.23	0.98
Present study	Gökova Bay	0.0003	3.20	0.99

The gonadosomatic index (GSI) for females was calculated as follows (King 1995):

$$GSI = \frac{gonad}{body} \times 100$$

Monthly GSI values were used to describe seasonal changes in reproductive activity during the sampling period. Because sampling ended in May 2010, the GSI profile documents only the observed period and does not provide a complete annual reproductive cycle.

Results

A total of 115 specimens were examined, comprising 71 males (61.7%) and 44 females (38.3%), which corresponds to a male-biased sex ratio of 1.61:1 (Table 5). Total length ranged from 31.0 to 99.0 cm, while total weight ranged from 16.9 to 685.7 g. Mean total length for pooled sexes was 47.6 ± 22.8 cm and mean total body weight was 124.3 ± 20.5 g. Mean total length was similar between sexes, measuring 47.7 ± 23.4 cm in males and 47.6 ± 23.3 cm in females (Table 6).

Table 5. Comparison of sex ratios of *Lepidopus caudatus* from different studies.

AUTHOR(S)	LOCALITY	SEX RATIO (M:F)
Tuset et al. (2006)	Canary Islands	0.11:1
Mariño-Briceño et al. (2022)	Azores	0.46:1
Rebac & Ferri (2025)	Adriatic Sea	3.2:1
Present study	Gökova Bay	1.61:1

Table 6. Descriptive statistics of total length and weight of *Lepidopus caudatus* from Gökova Bay by sex.

SEX	N	TOTAL LENGTH (CM)	TOTAL WEIGHT (G)
Males	71	47.7 ± 23.4 (32.0–94.0)	126.3 ± 20.5 (18.3–685.7)
Females	44	47.6 ± 23.3 (31.0–99.0)	120.9 ± 21.5 (16.9–637.0)
Combined	115	47.6 ± 22.8 (31.0–99.0)	124.3 ± 20.5 (16.9–685.7)

Morphometric measurements obtained from 50 randomly selected individuals showed that mean standard length, fork length, pre-anal length and post-anal length were 47.0 ± 21.5 cm, 46.7 ± 21.9 cm, 21.8 ± 11.0 cm and 25.8 ± 11.9 cm, respectively (Table 1). These measurements confirm the elongate body form characteristic of the species and provide descriptive information for the Gökova Bay population.

Age was determined independently by two readers, with both readers agreeing on the age of 90 fish (78%). The variability of IAPE was 14.7%. Otolith readings identified four age classes, from 0 to 3 years (Table 7). Age class 2 was dominant, representing 44.35% of the sample, followed by age class 1 (37.39%), age class 3 (17.39%) and age class 0 (0.87%). The maximum observed age was therefore 3 years.

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Table 7. Age distribution and mean total lengths of *Lepidopus caudatus* from Gökova Bay based on otolith readings.

LENGTH CLASS (CM)	AGE CLASSES				TOTAL
	0	I	II	III	
30.0–39.9	1	1			2
40.0–49.9		28	12		40
50.0–59.9		11	3	1	15
60.0–69.9		3	5		8
70.0–79.9			1	8	9
80.0–89.9			23	8	31
90.0–99.0				1	1
n	1	43	51	20	115
Mean TL (cm)	31.0	45.7	88.9	99.0	
Percentage	0.87	37.39	44.35	17.39	100.0

The von Bertalanffy growth parameters estimated for pooled sexes were $L_\infty = 151.64$ cm, $k = 0.27$ year and $t_0 = -0.34$ years (Table 2). The corresponding growth-performance index was 3.79. Together, these estimates indicate moderate growth and provide the first growth model available for this species from Gökova Bay.

Length-weight relationships indicated positive allometric growth in males, females and pooled sexes (Table 3). The pooled regression was $W = 0.0003L^{3.20}$ with $r^2 = 0.99$, while the estimated b values were 3.20 for males, 3.25 for females and 3.20 for pooled sexes. The high coefficient of determination indicates a strong association between total length and total body weight within the sampled population. A comparison of length-weight parameters from different geographical regions is presented in Table 4**.**

Monthly female GSI values varied considerably during the sampling period. GSI increased during autumn and reached a local maximum in October 2009, declined until January 2010, and rose again from February onward to attain the highest observed value in May 2010. This pattern indicates pronounced seasonal changes in ovarian development and suggests elevated reproductive activity during autumn and spring within the sampled interval.

Macroscopic observations of gonads, together with the bimodal GSI pattern, are compatible with extended reproductive activity and possibly multiple spawning events. However, because no samples were available after May 2010, the full annual spawning period cannot be defined with certainty and should not be overstated.

Discussion

The present study provides the first detailed biological assessment of *L. caudatus* from Gökova Bay and, more broadly, one of the first focused datasets for the species from Turkish waters. The maximum total length recorded in Gökova Bay (99.0 cm) exceeds published values from southern Brazil, Algarve and the northern Aegean Sea, but remains lower than maximum sizes reported from the Catalan Sea, Ionian Sea and Canary Islands (Table 8) (Demestre et al. 1993; Haimovici and Velasco 2000; Borges et al. 2003; Filiz and Bilge 2004; Tuset et al. 2006). This regional contrast is consistent with earlier suggestions that population traits in *L. caudatus* vary geographically (Demestre et al. 1993; D'Onghia et al. 2000; Tuset et al. 2006).

The maximum observed age of 3 years was lower than the 7 years reported from the Ionian Sea and Canary Islands (D'Onghia et al. 2000; Tuset et al. 2006). Likewise, the dominance of age class 2 in Gökova Bay differs from the predominance of older age classes reported elsewhere (Table 7). These differences may reflect true population-level variation, but they may also result from differences in sample size, seasonal coverage, habitat depth and fishing-gear selectivity. Because the present dataset was derived mainly from fishery-dependent sampling in a restricted time window, the observed age structure should be interpreted cautiously. Comparable studies

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revealed longer lifespans in the Azores (up to 7 years; Mariño-Briceño et al., 2022) and shorter ones in the Adriatic (maximum 4 years; Rebac & Ferri, 2025). This contrast highlights regional heterogeneity in longevity and growth dynamics of *L. caudatus* populations.

Table 8. Comparison of maximum total lengths of *Lepidopus caudatus* from different localities.

AUTHOR(S)	LOCALITY	MAX. TL (CM)
Demestre et al. (1993)	Catalan Sea (Spain)	196.0
Merella et al. (1997)	Balearic Islands (Spain)	106.0
D'Onghia et al. (2000)	Ionian Sea (Italy)	164.0
Haimovici & Velasco (2000)	Brazil	78.5
Borges et al. (2003)	Algarve (Portugal)	66.7
Filiz & Bilge (2004)	Sığacık Bay (N. Aegean)	81.5
Tuset et al. (2006)	Canary Islands (Atlantic)	195.8
Mariño-Briceño et al. (2022)	Azores	198.0
Rebac & Ferri (2025)	Eastern Adriatic	123.0
Present study	Gökova Bay (S. Aegean)	99.0

The male-biased sex ratio in Gökova Bay contrasts with the female-dominated ratio reported for the Canary Islands (Table 5) (Tuset et al. 2006). Sex-specific habitat use, seasonal movements, differential mortality and gear selectivity may all contribute to such variation. For that reason, the present sex ratio should not be considered an unbiased population estimate, but rather a description of the sampled catch composition. In contrast, the Azorean population is strongly female-dominated (M:F 0.46:1; Mariño-Briceño et al., 2022), whereas the Adriatic population shows male predominance (Rebac & Ferri, 2025). These differences suggest sex-specific ecological strategies that vary geographically.

The pooled length-weight regression coefficient of 3.20 indicates positive allometric growth and is close to estimates reported from other parts of the Mediterranean and adjacent Atlantic waters (Table 4) (Merella et al. 1997; D'Onghia et al. 2000; Borges et al. 2003; Filiz and Bilge 2004; Tuset et al. 2006). The high r^2 value further indicates that the model closely described the relationship between body size and mass in the examined material. This agreement with previous studies suggests that somatic growth form in the Gökova Bay population is broadly consistent with that reported elsewhere for the species.

Comparison of von Bertalanffy growth parameters across different localities reveals substantial geographic variation (Table 2). The estimated L∞ (151.64 cm) and k (0.27 year^{-1}) values for Gökova Bay fall within the range reported for other populations, although the maximum length and age were comparatively lower. The growth-performance index (Φ' = 3.79) is similar to values reported for the Canary Islands (Tuset et al. 2006) but lower than those from the Ionian Sea and northwestern Mediterranean (D'Onghia et al. 2000; Molí et al. 1990), suggesting regional differences in growth dynamics. The estimated parameters for Gökova Bay ($L\infty$ = 151.6 cm, k = 0.27) fall between the slower growth reported in the Azores ($L\infty$ = 171.6 cm, k = 0.12; Mariño-Briceño et al., 2022) and the faster growth observed in the Adriatic ($L\infty$ = 134.9 cm, k = 0.32; Rebac & Ferri, 2025). This intermediate position underscores the influence of local environmental and fishery conditions.

The GSI profile suggests elevated reproductive activity in autumn and spring and is broadly comparable with the long reproductive periods reported from the Adriatic and Catalan seas (Parin 1986; Demestre et al. 1993). However, the present data do not justify a definitive conclusion that spawning extended continuously from February to late November, because the sampling period did not cover the full year. A more conservative interpretation is therefore that reproductive activity was clearly detected within the sampled months and that peaks occurred in October and May.

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Several limitations should be recognised when interpreting the results. The sample size was modest, sampling ceased in May 2010, and the reliance on commercial gears likely introduced selectivity effects into the size, sex and age composition (Tables 6–7). In addition, maturity staging was based on macroscopic gonad observations, so histological validation would be necessary to confirm spawning frequency and the exact duration of the reproductive season.

Conclusions

The *Lepidopus caudatus* population sampled in Gökova Bay was characterised by positive allometric growth, a male-biased sex ratio and a dominance of age class 2 (Tables 7, 3, 5**).** Maximum observed total length and age were 99.0 cm and 3 years, respectively (Tables 6, 8), and the estimated von Bertalanffy parameters for pooled sexes were $L_\infty = 151.64$ cm, $k = 0.27$ year^{-1}, and $t_0 = -0.34$ years (Table 2). Female GSI values indicated increased reproductive activity in October and May during the sampled period, but a full annual spawning cycle could not be resolved from the available material.

These findings establish an important regional baseline for *L. caudatus* in the southern Aegean Sea. The morphometric data provided in Table 1 contribute to the descriptive biology of the species in Turkish waters. Future studies should employ standardised year-round sampling, incorporate fishery-independent surveys, quantify otolith reading precision and validate reproductive stages histologically. Additional work on trophic ecology and population connectivity would further clarify the ecological role of the species in Gökova Bay and support future management of demersal fish communities. When compared with other Mediterranean and Atlantic populations, our results emphasize that *L. caudatus* exhibits marked geographic variation in growth and sex ratios. The Gökova Bay population occupies an intermediate position between the slow-growing, female-dominated Azorean stock and the fast-growing, male-biased Adriatic stock.

In conclusion, this study presents the first detailed biological dataset for *L. caudatus* in Gökova Bay, providing valuable baseline information on length–weight relationships (Table 3), age structure (Table 7), growth parameters (Table 2), sex ratio (Table 5), morphometry (Table 1), and reproductive biology—thus filling an important geographical gap in Turkish waters. The methodology is clearly described, the discussion critically compares findings with other Mediterranean and Atlantic populations (Tables 8, 4), and the bibliography is comprehensive. However, the study has several notable limitations: the sample size (115 individuals) is modest, the sampling covers only 9 months (September 2009 – May 2010), preventing a complete annual reproductive cycle from being resolved, and the reliance on fishery-dependent gillnet catches introduces gear selectivity biases. Additionally, gonad maturity was assessed macroscopically without histological confirmation, otolith reading precision was not quantified, and von Bertalanffy parameters lack confidence intervals. While the manuscript provides a valuable regional contribution, these methodological and presentation issues should be addressed, either through revision or by reframing the work as a preliminary short communication, to strengthen its scientific rigour and suitability for peer-reviewed publication. Therefore, incorporating such regional baselines into broader Mediterranean stock assessments is essential to avoid misinterpretation and to design location-specific management strategies.

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Ethical statement

Fish specimens were obtained from commercial catches and fishing operations, and no live experimental procedures were conducted on animals during the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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Data Availability

All data generated and analyzed during this study are included in the published article. The raw datasets (length, weight, age, and gonadosomatic index values) obtained from Gökova Bay between September 2009 and May 2010 are archived at Muğla Sıtkı Koçman University, Faculty of Fisheries. These data are available from the corresponding author upon reasonable request. No proprietary or restricted datasets were used in this research.

Author contributions

Altan Kunduz: conceptualisation, field sampling, laboratory analyses, data curation and writing of the original draft. Halit Filiz: supervision, study design, interpretation of results and review and editing of the manuscript. Ertan Taşkavak**:** logistical support, reading otoliths, critical review and editing of the manuscript.

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Groundwater Biodiversity in the Taza Region (Morocco): Hydrobiidae Distribution and Conservation Priorities

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Abstract

Subterranean freshwater ecosystems are unique and fragile habitats characterized by stable environmental conditions and highly specialized fauna. Among groundwater gastropods, the family Hydrobiidae is particularly notable for its high degree of endemism and sensitivity to environmental disturbance, making these mollusks valuable indicators of groundwater ecosystem quality. Despite Morocco's remarkable subterranean biodiversity, groundwater mollusks remain insufficiently studied, particularly in the karstic systems of the Taza region, where increasing anthropogenic pressures may threaten these vulnerable ecosystems.

This study presents the results of exploratory sampling campaigns conducted in the Taza region (Morocco) to investigate the occurrence and distribution of Hydrobiidae in groundwater wells and evaluate their relationship with environmental conditions. The surveys were conducted within the framework of the WWF Mediterranean North Africa Grant. Wells exhibiting contrasting hydrological and morphometric characteristics were surveyed. Biological sampling was carried out using a phreatobiological net, and collected specimens were preserved in ethanol and identified using morphological criteria. Physico-chemical analyses included measurements of pH, electrical conductivity, dissolved oxygen, total dissolved solids, nitrates, nitrites, sulfates, orthophosphates, and chemical oxygen demand.

Hydrobiidae were recorded in several investigated wells, displaying a heterogeneous spatial distribution across the study area. Their presence was associated with relatively stable environmental conditions, greater water depth, and lower exposure to surface disturbances. In contrast, they were absent from shallow or unprotected wells characterized by higher mineralization and elevated nutrient concentrations, suggesting that groundwater quality strongly influences habitat suitability for these specialized taxa.

These findings highlight the ecological significance of groundwater Hydrobiidae as sensitive indicators of aquifer health and emphasize the conservation importance of the Taza region's subterranean freshwater ecosystems. Protecting these habitats through regular groundwater monitoring, improved well management, and integrated conservation strategies is essential for preserving their biodiversity. Further taxonomic and molecular studies are needed to uncover hidden diversity and support conservation of groundwater fauna.

Keywords: Hydrobiidae, Groundwater biodiversity conservation, Subterranean freshwater ecosystems, Groundwater quality

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Conservation of the Bouhlou Key Biodiversity Area, A hope for the Critically Endangered Freshwater Mussel *Pseudunio marocanus* (Pallary, 1918) (Mollusca: Margaritiferidae), Morocco

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Abstract

Bouhlou river, a tributary of the Sebou basin in Taza Province, is a Key Biodiversity Area supporting one of the last significant populations of *Pseudunio marocanus*, a Critically Endangered endemic freshwater mussel ranked among the world's 100 most threatened species. Between 2025 and 2026, an integrated conservation and monitoring work was carried out across this KBA within the RestoreBouhlou project, funded by WWF under the Restore NAW programme. This project allowed to re-define the spatial distribution of *P. marocanus* while estimating its density using the Capture-Per-Unit-Effort method across several stations, revealing a core population concentrated at station SB2. It allowed also the application of Capture-Mark-Recapture, using Morocco's first individual marking protocol for freshwater bivalves, first with adhesive resin tags and later refined through non-lethal shell engraving, to estimate population abundance and test the reliability of individual tracking over time. The population structure was also characterized, notably through size-based biometric data, confirming active juvenile recruitment at station SB2 and a more adult-dominated population upstream. A third mission, conducted immediately after major regional flooding, confirmed the persistence of marked individuals, demonstrating population resilience despite a marked drop in mark-detection rates. In parallel, complementary scientific work characterized the endemic fish community as potential mussel hosts and revealed a diverse, largely undocumented groundwater fauna in local wells. Within this broader assessment, the invasive bivalve *Corbicula fluminea* was also identified at high density in the watershed, constituting an emerging competitive threat to native species. Beyond fieldwork, the project engaged local communities through awareness workshops, school environmental clubs, and socio-economic surveys on water use and biodiversity perception, fostering a sense of ownership among riverside populations that is essential for the mussel's long-term protection. These combined scientific and social efforts contributed to Bouhlou's recognition as a Site d'Intérêt Biologique et Écologique, equivalent to an IUCN OECM.

Keywords: *Pseudunio marocanus*; Freshwater conservation; KBA; CMR; Morocco

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Geographical and Environmental Distribution of Water Mites (Acari: Hydrachnidia) in Pakistan: An Integrative Morphological and DNA Barcoding Study

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Abstract

Water mites (Acari: Hydrachnidia) are one of the most species-rich groups of freshwater arachnids and play essential ecological roles as predators, parasites, and bioindicators of freshwater ecosystem integrity. Despite Pakistan supporting diverse of freshwater habitats, including glacier-fed rivers, mountain streams, springs, lakes, wetlands, reservoirs, and the Indus River system, its water mite fauna remains unexplored. No comprehensive investigation has combined field surveys, morphological taxonomy, ecological assessment, and molecular identification to document the diversity and distribution of Hydrachnidia in the country. This project provide the first integrative assessment of the geographical and environmental distribution of water mites in Pakistan through an taxonomic approach combining morphological identification with mitochondrial cytochrome c oxidase subunit I (COI) DNA barcoding. Representative freshwater habitats from the biogeographical regions of Pakistan will be surveyed to document species richness and community composition. Specimens will be identified using morphological characters and analysed using DNA barcodes to confirm species identities, detect cryptic diversity, and establish the reference barcode library for the Pakistani water mite fauna. DNA sequences will be compared with international databases (BOLD and GenBank) to evaluate phylogenetic relationships and biogeographical affinities with neighbouring regions, particularly Iran, India, and Türkiye.

Key environmental variables, including water temperature, pH, dissolved oxygen, electrical conductivity, flow velocity, substrate composition, habitat type, and altitude, will be recorded to determine the factors governing species occurrence and distribution. Biogeographical analyses will identify biodiversity hotspots, habitat specialization, dispersal patterns, and centres of endemism across Pakistan's freshwater ecosystems. The project will generate the first comprehensive inventory of Pakistani water mites, reveal undocumented and potentially undescribed species, establish a national DNA barcode reference library, and provide knowledge of their ecological and geographical distribution. By integrating classical taxonomy, molecular systematics, and freshwater ecology, the study will establish a baseline for biodiversity assessment, biomonitoring, evolutionary research, and freshwater conservation in Pakistan.

Keywords: water mites; Hydrachnidia; Pakistan; biogeography; habitat; biodiversity.

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Tectonic History and Water Mite Diversity in Pakistan: A Biogeographical Hypothesis

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Abstract

Water mites (Acari: Hydrachnidia) are among the most diverse freshwater arachnids and are valuable indicators of freshwater biodiversity. Despite Pakistan's strategic location at the junction of the Western Palaearctic and Oriental biogeographical regions, its water mite fauna remains largely unexplored. This study proposes a conceptual biogeographical hypothesis that the present-day diversity and distribution of Pakistani Hydrachnidia have been strongly influenced by the tectonic evolution of South Asia. The collision of the Indian and Eurasian plates and the subsequent uplift of the Himalayas, Karakoram, and Hindu Kush created extensive environmental heterogeneity, isolated drainage systems, and numerous freshwater habitats that may have promoted vicariance, ecological differentiation, and allopatric speciation. Based on published knowledge from neighbouring countries, including Iran, India, and Türkiye, Pakistan is expected to harbour a mixture of Western Palaearctic, Oriental, and endemic water mite lineages. The hypothesis predicts that high-altitude mountain regions represent centres of endemism, whereas the Indus River basin functions as a major dispersal corridor connecting different faunal elements. Future research integrating comprehensive field surveys, morphological taxonomy, DNA barcoding, phylogenetic analyses, and historical biogeography is required to test these predictions and document the true diversity of Hydrachnidia in Pakistan. This framework provides a basis for understanding the evolutionary history of freshwater biodiversity in tectonically active regions and supports future conservation of Pakistan's freshwater ecosystems.

Keywords: Hydrachnidia; Pakistan; biogeography; tectonic history; freshwater biodiversity.

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Competitive Effects of the Invasive Nile Tilapia *Oreochromis niloticus* (Actinoptyrigii: Cichlidae) on Growth and Survival of the Endemic Moroccan Barbel *Carasobarbus fritschii* (Actinoptyrigii: Cyprinidae)

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Abstract

The introduction of invasive Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) threatens native freshwater fishes through competition for resources, yet experimental evidence quantifying its effects on the endemic Moroccan barbel *Carasobarbus fritschii* (Günther, 1874) remains scarce. This study evaluated the impact of cohabitation with *O. niloticus* on the growth, physiological condition, and survival of *C. fritschii* under controlled conditions. Individual fish were monitored weekly over five weeks using biometric measurements (weight, standard length, and total length) supported by an AI-assisted photographic identification system for non-invasive individual tracking. In monoculture, *C. fritschii* exhibited positive growth, stable Specific Growth Rate (SGR), and maintained good physiological condition throughout the experiment. In contrast, individuals reared with *O. niloticus* showed reduced or negative growth, lower SGR and Thermal Growth Coefficient (TGC), progressive deterioration of body condition, and altered weight–length relationships, indicating impaired physiological performance. The Scaled Mass Index (SMI) proved to be a sensitive indicator of this decline. The most striking result was the cumulative mortality of *C. fritschii*, which exceeded 60% within 25 days in co-culture, whereas no comparable mortality occurred in monoculture. Conversely, *O. niloticus* maintained high growth and good physiological condition under both experimental conditions, demonstrating strong competitive ability and ecological plasticity.
These findings provide direct experimental evidence that *O. niloticus* exerts strong asymmetric competitive pressure on *C. fritschii*, reducing its growth, physiological condition, and survival. The study highlights the conservation risk posed by invasive Nile tilapia to endemic freshwater fishes and emphasizes the importance of managing invasive populations to protect the biodiversity of Moroccan freshwater ecosystems.

Keywords: invasive species; freshwater fish; interspecific competition; *Carasobarbus fritschii*; *Oreochromis niloticus*

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Phylogenetic Relationships of Pumpkinseed Sunfish (*Lepomis gibbosus*) from Water Bodies of the Northern Black Sea Region and across Its Native Range

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Abstract

The pumpkinseed sunfish (*Lepomis gibbosus*), a member of the family Centrarchidae native to North America, was introduced into Europe in the nineteenth century as an ornamental species. Owing to its ecological plasticity and invasive potential, it subsequently spread throughout major European river systems, ponds, and lakes. Since the late twentieth century, the species has also been recorded on the Crimean Peninsula, probably having colonized the region via the North Crimean Canal system. As an aggressive invasive species, pumpkinseed sunfish competes with native fishes, making phylogenetic studies particularly relevant. Specimens were collected in September 2024 from the Chernaya and Balaklavka rivers and a lake near Mount Gasfort. The work was carried out using the equipment and reagents of the Scientific and Educational Research Equipment Sharing Centre 'Phylogenomics and Transcriptomics' (A.O. Kovalevsky Institute of Biology of the Southern Seas). Genomic DNA was extracted using the DNA MAG Isolation Kit (Syntol LLC, Russia). The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced. Sequence alignment was performed in MEGA using the MUSCLE algorithm. Phylogenetic reconstruction employed the Maximum Likelihood method with 500 bootstrap replicates. Comparative analysis included COI sequences from the native and invasive ranges. The mitochondrial COI sequence of Centrarchus macropterus (Lacépède, 1801) was used as the outgroup. Phylogenetic analysis revealed that the Sevastopol populations formed a monophyletic clade with high statistical support (bootstrap ≥ 70%), indicating their origin from a common ancestor. Low genetic distances within the cluster (0.000–0.002) suggest recent colonization of Crimea or ongoing gene flow. The Crimean specimens clustered with North American samples, suggesting a close phylogenetic affinity with the native populations. In contrast, European populations formed a separate clade, indicating earlier divergence. Nevertheless, the mitochondrial COI marker appears to provide insufficient phylogenetic resolution to reconstruct the origin of the Crimean population. Therefore, additional molecular markers are required. Funded by state projects Nos. 125012100509-6 and 124022400148-4 of the A.O. Kovalevsky Institute of Biology of the Southern Seas.

Keywords: *Lepomis gibbosus*, invasive species, phylogenetic relationships, COI, Northern Black Sea region

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Seasonal Feeding Ecology of Endemic Barbels in a North African River: The Zat Basin Case

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Abstract

Understanding how endemic freshwater fish adjust their feeding behaviour to seasonal resource availability is central to predicting their resilience under environmental change. This study examined the feeding ecology of four barbel species endemic to the Zat basin, a tributary system of the Tensift watershed in central Morocco: Labeobarbus reinii, Luciobarbus magniatlantis, Luciobarbus ksibi, and Carasobarbus fritschii. Stomach contents from individuals sampled during dry and humid periods were analysed to characterize dietary composition, prey selection, and feeding intensity. All four species proved broadly omnivorous, relying primarily on aquatic vegetation, but each showed a distinct signature of seasonal prey switching. L. ksibi kept a fairly stable diet across seasons, with only slight declines in Baetidae and Heptagenidae mayfly larvae toward the humid period. L. magniatlantis, by contrast, sharply reduced its reliance on these same prey groups in the humid season, suggesting a shift toward alternative resources. L. reinii moved in the opposite direction, increasing its consumption of Baetidae as humidity rose. C. fritschii held Heptagenidae and Chironomidae in its diet across both seasons, albeit at reduced levels in the humid period. Feeding activity overall peaked during the dry season, when environmental conditions were most stable. Together, these species-specific dietary trajectories point to a form of trophic niche partitioning that may reduce interspecific competition and buffer the barbel assemblage against the Zat basin's marked hydrological seasonality a pattern with direct relevance to the conservation of Morocco's threatened endemic cyprinid fauna.

Keywords: Endemic Cyprinidae, Feeding ecology, Seasonality, Resource partitioning

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Rare Earth Element Bioaccumulation in Biota of the northern Black Sea (Crimea)

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Abstract

Rare earth elements (REEs), group IIIB elements of the Periodic Table, are increasingly used in high-tech and military production, medicine, agriculture, catalysis, and clean energy technologies. Their expanding application has made them emerging pollutants, including in marine environments. Inductively coupled plasma mass spectrometry is well suited for measuring low REE levels in marine organisms. Systematizing REE accumulation in Black Sea biota is important for biomonitoring, bioremediation, ecotoxicology, and health risk assessment. This report summarizes REE bioaccumulation in marine plants, including brown, red, and green algae and seagrass, and animals, including five mollusc and eight fish species, from the Black Sea coastal area of Crimea, in comparison with seawater and sediments. The highest REE concentrations occurred in sediments, 32–43 $mg \cdot kg^{-1}$, and the lowest in seawater, 14–55 $ng \cdot L^{-1}$. Excluding Sc, total REE concentrations were similar in plants and molluscs, 0.19–7.5 $mg \cdot kg^{-1}$ and 0.18–9.9 $mg \cdot kg^{-1}$, respectively, but much lower in fish muscle, 7–410 $\mu g \cdot kg^{-1}$. The highest values among plants and molluscs were found in seagrass rhizomes, brown algae, and the bivalve *Anadara kagoshimensis*, whereas the lowest occurred in *Ulva rigida* and *Rapana venosa*. In fish, REE concentrations decreased from the demersal detritivore *Chelon auratus* to the pelagic planktivore *Sprattus sprattus*. Sc concentrations in plants and molluscs generally followed total REE patterns but differed in fish, where they were higher, 0.15–1.5 $mg \cdot kg^{-1}$, especially in demersal species. Mean bioaccumulation factors ranged from 950 in *S. sprattus* to 200,000 in *Ericaria crinita*, largely mirroring organismal concentrations and emphasizing the role of seawater REE levels. Biota-sediment accumulation factors decreased monotonously from plants, 0.06–0.57, through molluscs, 0.014–0.13, to fishes, 0.0016–0.017. Sc BSAFs were generally higher than total REE BSAFs, exceeding 1 in some plants and *Mytilus galloprovincialis*. These patterns indicate trophic dilution of REEs, evident in the "bivalves–gastropod" food-chain link and in transfer from molluscs to fish.

Keywords: rare earth elements; scandium; bioaccumulation factor; biota–sediment accumulation factor; trophic dilution; Black Sea

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Introduction

Rare earth elements (REEs) comprise Sc, Y and the lanthanides and are essential to high-technology, energy, medical and agricultural applications. Their rapidly increasing production and use have intensified environmental releases and led to their recognition as emerging contaminants. In seawater, REEs are strongly affected by complexation, adsorption to organic and mineral particles, and scavenging to bottom sediments; consequently, dissolved concentrations are extremely low, whereas sediments can constitute a large reservoir (Balaram, 2019; Neira et al., 2022).

Marine organisms integrate exposure from water, suspended particles, sediments and food. Primary producers can accumulate REEs directly across their surfaces, rooted macrophytes can additionally interact with sediments, and suspension-feeding bivalves process large quantities of particulate material. In contrast, REE concentrations often decrease at higher trophic levels, and several marine and freshwater food-web studies report trophic dilution rather than biomagnification (Wang et al., 2022; Santos et al., 2023; Marginson et al., 2023). However, comparisons among plants, molluscs and fishes from one marine region remain scarce.

The Crimean coastal zone of the northern Black Sea contains diverse macrophyte communities, commercial and invasive molluscs, and fishes representing pelagic, benthopelagic and demersal habitats. Previous studies from this region quantified REEs in algae and seagrass, molluscs, sediments, seawater and fish muscle (Kapranov et al., 2021; Ryabushko et al., 2022, 2023, 2024a,b; Chelyadina et al., 2023; Nekhoroshev et al., 2025; Kapranov et al., 2026). The present short paper synthesizes these data for samples collected in 2017–2025. The objectives were to compare ΣREE (excluding Sc) and Sc among environmental matrices and major biotic groups, to evaluate BAF and BSAF patterns, and to assess whether the cross-taxon distribution is consistent with trophic dilution.

Materials and methods

The dataset combines separate sampling campaigns carried out in Crimean coastal waters from 2017 to 2025. The plant group comprised the seagrass *Zostera noltei* (leaves and rhizomes), the brown algae *Ericaria crinita*, *Gongolaria barbata* and *Padina pavonica*, the red alga *Ceramium ciliatum*, and the green alga *Ulva rigida*. Molluscs included the bivalves *Anadara kagoshimensis*, *Flexopecten glaber ponticus*, *Mytilus galloprovincialis* and *Crassostrea gigas*, and the predatory gastropod *Rapana venosa*. Fish muscle was analyzed in *Alosa immaculata*, *Chelon auratus*, *Engraulis encrasicolus*, *Mullus barbatus*, *Scorpaena porcus*, *Spicara flexuosum*, *Sprattus sprattus* and *Trachurus mediterraneus*. Full sampling designs, specimen characteristics and site descriptions are provided in the source studies.

Biological samples were rinsed, dried to constant mass and homogenized. The common analytical protocol used 0.1 g of dry material digested with 2 mL of sub-boiling-purified HNO_3 for 2 h in capped PTFE tubes at 2.2 bar. Digests were diluted with deionized water and analyzed using a PlasmaQuant MS Elite ICP-MS. The measured isotopes were ^{45}Sc, ^{89}Y, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb and ^{175}Lu. Five-point calibration covered 0.05–5 µg L^{-1} ($R^2 \geq 0.999$). Measurements included five scans and five analytical replicates; H_2 collision-gas checks were used to exclude polyatomic interference, and instrumental drift was corrected with repeated standards. Certified mussel tissue BCR-668 gave recoveries of 93–129% (Kapranov et al., 2026).

For the present comparison, ΣREE was defined as Y plus the lanthanides; Sc was treated separately because it dominated the REE burden in several fish species. BAF was calculated as

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C_{biota}/C_{water} after unit harmonization and is expressed as L kg^{-1} dry weight; the dimensionless BSAF was calculated as $C_{biota}/C_{sediment}$. Species means and standard deviations were plotted on logarithmic scales. Because the synthesis combines campaigns differing in year, exact location and replication, the resulting factors should be interpreted as regional comparative indices rather than site-matched constants.

Results and discussion

The environmental matrices differed by approximately six orders of magnitude. Regional sediments contained 32–43 mg kg^{-1} ΣREE, while seawater contained only 14–55 ng L^{-1}. Excluding Sc, plants and molluscs occupied a similar concentration range, 0.19–7.5 and 0.18–9.9 mg kg^{-1} dry weight, respectively, whereas fish muscle contained only 0.007–0.410 mg kg^{-1} (Fig. 1a). The highest values among primary producers occurred in *Z. noltei* rhizomes and brown algae; *U. rigida* had the lowest plant concentrations. Among molluscs, *A. kagoshimensis* was the strongest accumulator and *R. venosa* the weakest. In fishes, ΣREE excluding Sc broadly decreased from the demersal omnivore–detritivore *C. auratus* to the pelagic planktivore *S. sprattus*.

Sc followed the general plant and mollusc pattern but differed markedly in fishes (Fig. 1b). Fish-muscle Sc concentrations reached 0.15–1.5 mg kg^{-1} and generally exceeded ΣREE excluding Sc. Particularly high Sc occurred in demersal or benthopelagic species, consistent with their greater interaction with near-bottom particles and sediments. Nevertheless, habitat alone did not determine accumulation: the demersal predator *S. porcus* had comparatively low concentrations, indicating that diet, assimilation and tissue-specific retention also modify sediment-related exposure (Kapranov et al., 2026).

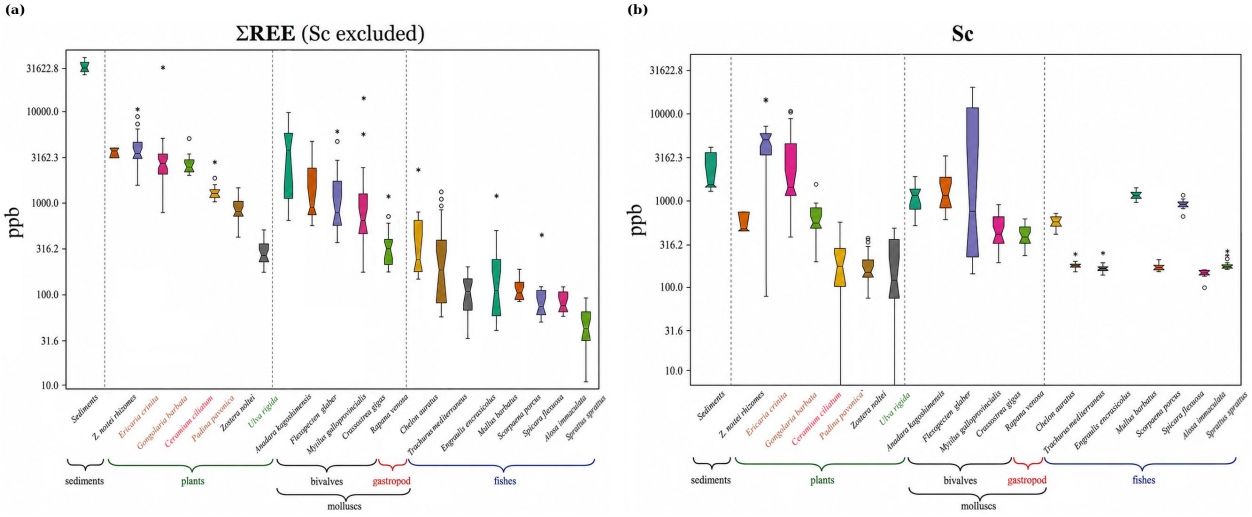


Figure 1. Concentrations of (a) ΣREE (Y plus lanthanides; Sc excluded) and (b) Sc in sediments and marine biota from Crimean coastal waters (ppb = μg kg^{-1}).

Mean ΣREE BAFs ranged from approximately 9.5×10^2 L kg^{-1} in *S. sprattus* to 2.0×10^5 L kg^{-1} in *E. crinita* (Fig. 2a). The ranking largely reproduced organism concentrations because seawater REE concentrations were uniformly very low. Plants and bivalves consequently showed the greatest apparent enrichment from water, while fishes showed the lowest. Sc BAFs were also high, approximately 3×10^3–1×10^5 L kg^{-1}, but species ordering differed from that of ΣREE, most conspicuously in *M. galloprovincialis* and several demersal fishes (Fig. 2b).

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These BAF values represent integrated exposure through water, particles and food and should not be interpreted as evidence of direct aqueous uptake alone.

BSAF provided a clearer cross-trophic gradient because sediment is the dominant regional REE reservoir. ΣREE BSAFs decreased almost monotonically from plants (0.06–0.57), through molluscs (0.014–0.13), to fishes (0.0016–0.017; Fig. 2c). The lower value in *R. venosa* than in its bivalve prey indicates dilution across the bivalve–gastropod link. Fish BSAFs were another order of magnitude lower, supporting inefficient transfer from benthic and planktonic food sources into muscle. This pattern accords with the particle-reactive nature of REEs: sedimentary binding promotes exposure but restricts biological assimilation and transfer (Neira et al., 2022; Santos et al., 2023).

Sc BSAFs were generally higher than ΣREE BSAFs (Fig. 2d). Values above one occurred in *Z. noltei* rhizomes, several brown and red algae, and *M. galloprovincialis*, indicating that these organisms or tissues can concentrate Sc above the bulk sediment concentration on a dry-mass basis. Such values do not by themselves demonstrate biomagnification, because uptake may include dissolved and suspended-particle pathways and the sediment denominator represents bulk material. In fishes, Sc BSAFs remained below those in plants and molluscs despite elevated absolute Sc concentrations in some demersal species.

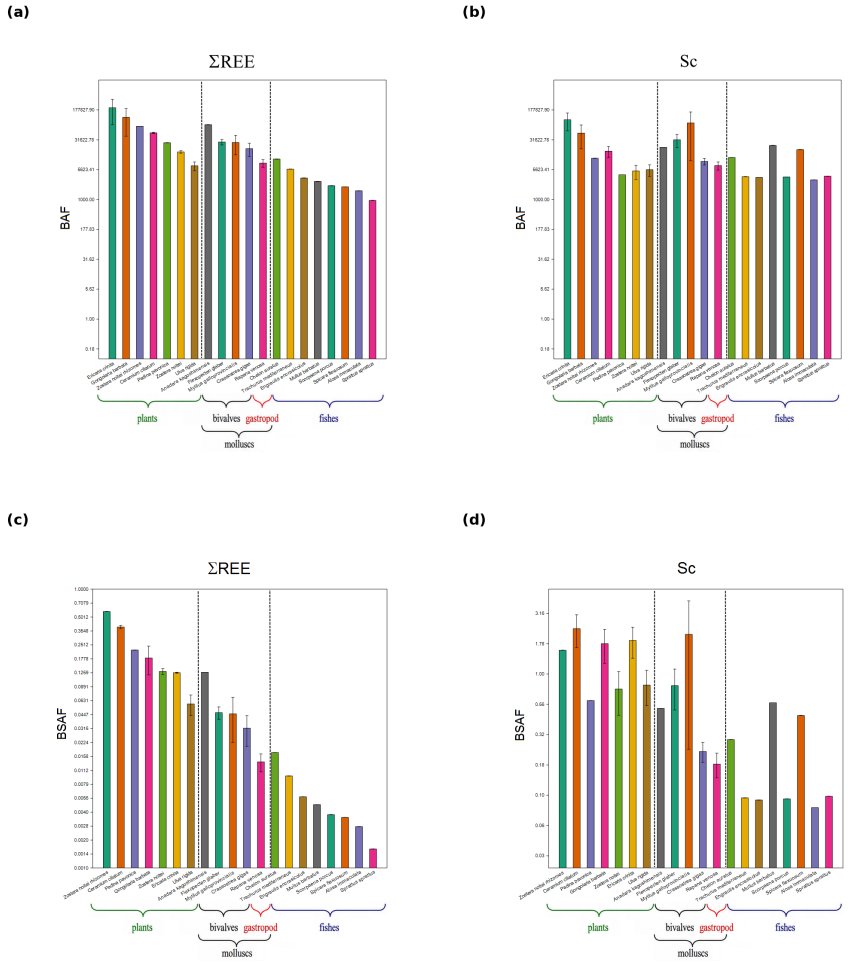


Figure 2. Mean bioaccumulation factors (BAF) for (a) ΣREE and (b) Sc, and biota–sediment accumulation factors (BSAF) for (c) ΣREE and (d) Sc. Error bars are standard deviations. Species are arranged in descending order within plants, bivalves, gastropod and fishes.

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BAF and BSAF describe complementary aspects of exposure. The very large BAF values arise partly from the extremely small dissolved denominator and therefore emphasize the capacity of organisms to integrate dilute seawater concentrations over time. They are useful for comparing potential biomonitors but cannot distinguish direct uptake from ingestion of suspended particles. BSAF is less sensitive to short-term variation in seawater and compares organisms with the principal environmental stock of REEs. Its orderly decline across ecological groups therefore provides stronger evidence for limited trophic transfer than BAF alone.

The high values in macrophytes are consistent with adsorption to cell walls and extracellular polysaccharides, interception of suspended matter, and, for *Z. noltei* rhizomes, direct contact with porewater and sediment particles. Brown and red algae may be especially effective because their surface chemistry offers abundant oxygen-containing binding sites. Bivalves accumulate REEs through filtration and ingestion of phytoplankton and detritus; correlations between Si and REEs in *M. galloprovincialis* have previously implicated ingested diatoms as a shared source (Chelyadina et al., 2023). The lower concentrations and BSAFs in *R. venosa* suggest that only a limited fraction of prey-bound REEs is assimilated across the digestive tract, while the remainder is rejected or eliminated.

Fish muscle represents a comparatively weak sink. Although REEs can associate with proteins and phosphate-containing fractions, muscle generally has lower metabolic and storage functions than liver, kidney or digestive tissues. The present cross-species pattern therefore combines low tissue affinity with differences in habitat and diet. Demersal contact increased exposure, particularly to Sc, but did not guarantee high accumulation, as shown by *S. porcus*. Pelagic planktivores had consistently low ΣREE and BSAF values, in agreement with published evidence that REEs frequently biodilute in marine fish assemblages (Wang et al., 2022; Marginson et al., 2023).

The combined evidence supports broad trophic dilution across the studied assemblage. Primary producers and suspension feeders are efficient integrators of dissolved and particulate REEs, whereas the predatory gastropod and fish muscle retain a smaller fraction of the environmental burden. The strongest and most consistent indicator is the decline in BSAF, rather than concentration or BAF alone. For regional biomonitoring, *E. crinita*, *Z. noltei* rhizomes, *A. kagoshimensis* and *M. galloprovincialis* appear particularly informative, whereas fish are better suited to evaluating habitat- and diet-related exposure than rapid changes in dissolved concentrations.

The main limitation is that the dataset synthesizes campaigns conducted across years and sites rather than a contemporaneous food-web survey. BAFs and BSAFs based on regional mean denominators may therefore include spatial and temporal variability unrelated to trophic position. Future work should pair water, suspended matter, sediments, prey and consumers at the same stations, quantify trophic position using stable isotopes, and compare fish muscle with metabolically active organs. Even with this limitation, the results demonstrate that Sc should be evaluated separately from the remaining REEs and provide a coherent regional baseline for the northern Black Sea.

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Ethics Approval

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This study did not involve human participants or animals requiring ethical approval. All work was conducted with the marine plant, invertebrate, and fish species, which are not subject to regulations for animal experimentation.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this work.

Data Availability

The data used in this work are available from the corresponding author on reasonable request.

Author Contribution

S.V. KAPRANOV: Conceptualization; Data curation; Investigation; Methodology; Writing – original draft; Writing – review & editing. E.V. GUREEVA: Resources. L.L. KAPRANOVA: Resources; Investigation. A.M. TOİCHKİN: Resources. J.D. DİKAREVA: Resources. V. I. RYABUSHKO: Conceptualization; Project administration.

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Morphometric Characterization of Cocoons in the Southern Medicinal Leech (*Hirudo verbana* Carena, 1820)

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Abstract

Medicinal leeches are clinically and pharmacologically important worldwide because of the therapeutic properties of their bioactive salivary compounds. Their reproductive success fundamentally depends on cocoons, which protect embryonic development. This study determined the morphometric characteristics of southern medicinal leech (*Hirudo verbana* Carena, 1820) cocoons and analyzed the relationships among the variables. The data collected from 103 cocoons encompassed various parameters, including cocoon weight, internal and external lengths and diameters, and the thickness of the sponge layer. Descriptive statistics for these parameters indicated a cocoon weight of 0.87 ± 0.19 g, an external length of 21.65 ± 2.04 mm, an external diameter of 14.14 ± 1.76 mm, and a sponge layer thickness of 3.74 ± 1.26 mm. Statistical analysis showed significant positive correlations among cocoon weight and dimensions, indicating the interdependence of structural features, whereas sponge layer thickness negatively correlated with internal cocoon length. Principal Component Analysis (PCA) revealed that the first two components explained 85.9% of the morphological variance (PC1:56.9%, PC2:29.0%). A strong positive correlation among cocoon weight, external length, and internal diameter primarily drove the variance in PC1. Overall, these findings offer a useful biometric evaluation of medicinal leech cocoon morphology, which may support future studies on leech reproduction and ecology.

Keywords: Cocoon morphology, Morphometrics, Reproductive biology, PCA analysis

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Assessment of Pond Productivity through Carp with *Macrobrachium rosenbergii* Culture at Rajshahi, Bangladesh

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Abstract

Integrated polyculture of freshwater prawn (*Macrobrachium rosenbergii*) with Indian major carps holds considerable promise for boosting pond productivity in Bangladesh, yet the optimal stocking density for prawns in such systems—particularly in the Rajshahi region—remains inadequately defined. To address this gap, a six-month trial (August 2024–January 2025) was conducted at the Department of Fisheries, University of Rajshahi, evaluating three prawn stocking densities: 20 (T_1), 30 (T_2), and 40 (T_3) individuals per decimal, equivalent to 4,940, 7,410, and 9,880 per hectare, respectively, while carp (Labeo rohita and Gibelion catla) densities were held constant across all treatments. Fortnightly monitoring confirmed that all key physicochemical parameters—including temperature, dissolved oxygen, pH, ammonia nitrogen, and alkalinity—remained well within the acceptable ranges for prawn carp co culture throughout the study. Biological performance analysis revealed significant variations ($p<0.05$) among treatments. M. rosenbergii exhibited the highest individual growth in T1, reaching a final average weight of 98.1 g, compared to 89.4 g in T2 and 62.6 g in T3. Similarly, G. catla achieved maximum growth in T1 (750.70 g). Conversely, L. rohita showed superior performance in T2, attaining 613.60 g. Survival rates were high across all treatments, exceeding 95% for prawns and 78% for carps. Total yield calculations (Kg/ha/6 months) indicated that T2 produced the highest combined biomass (2816.71 kg/ha), followed by T1 (2746.35 kg/ha) and T3 (2402.56 kg/ha). Economic analysis demonstrated that while T2 generated the highest net profit (Tk. 396,636/ha), T1 achieved the highest Cost-Benefit Ratio (CBR = 1.85), indicating superior return on investment. The study concludes that while moderate stocking density (T2) maximizes total production and profit, lower density (T1) offers the most cost-effective approach for resource-limited farmers.

Keywords: Indian majör carps, freshwater prawn, polyculture, economic analysis, Pond Productivity

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Macrofauna assemblages along the new Jetty in Iskenderun Bay, Mediterranean Sea, Türkiye

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Abstract

İskenderun Bay is an important area in the northeastern Mediterranean Sea for fisheries, maritime activities, and tourism. This study investigated the early colonization of macrofaunal communities on the piles of a newly constructed jetty using underwater photographs taken by scuba divers at upper, middle, and bottom depth zones. The results showed that artificial substrates were rapidly colonized, with well-defined macrofaunal assemblages established within approximately 90 days. Alien species, which are abundant in İskenderun Bay, comprised a significant proportion of the colonizing fauna, demonstrating their ability to quickly occupy newly available habitats. These findings highlight the rapid development of fouling communities on artificial structures and the important role of alien species in shaping early benthic community succession in the northeastern Mediterranean.

Keywords: Artifical reef, Macrofauna, biofouling, jetty, Türkiye

Introduction

The world's growing population and increasing trade activities are leading to significant changes to the coastline through human activity. This encompasses the construction of man-made marine structures (MMS), such as piers, docks, breakwaters, marinas, pipelines, oil and gas infrastructure, and offshore wind and wave energy facilities (Dafforn et al. 2015; Lemasson et al. 2021). These structures are typically placed in marine habitats composed of soft sediments. By introducing hard surfaces into the marine environment, they create ecological characteristics similar to those of natural reefs. This provides suitable surfaces for algae and various epibenthic organisms to attach to, enabling the development of a rich underwater community (biota/fouling) (Layman and Allgeier, 2020; Scanlon et al. 2024).

Located in the Mediterranean Sea in the south of our country, this bay has served as a major commercial port hub since ancient times due to its unique coastal features and geographical location. As one of the most important ports providing access to the Middle East, it is a major trade hub today and provides a natural breeding and feeding ground for many marine species (Özcan et al. 2005; Özcan and Özcan, 2025).

Vertical structures such as piers, jetties and terminal piers form hard substrates that allow different biological zones to develop depending on the water depth. As a result, various algae

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and invertebrates develop on these structures, while fish communities thrive in the surrounding areas. Over time, these structures can increase species diversity and organism abundance compared to soft-sediment habitats, as they provide living, feeding, shelter and spawning areas for many invertebrate and fish species (Perkol-Finkel et al. 2006; Lemoine et al. 2019; Layman and Allgeier 2020; Madgett et al. 2023). For this reason, these artificial hard surfaces can increase biodiversity at a local level by providing a habitat that soft sediments do not offer.

However, the effects of ecological changes are not always positive. While such structures initially support biodiversity, they can eventually result in the excessive accumulation of mussels, barnacles, tube worms and other fouling organisms. Excessive biofouling can put additional strain on structures and cause corrosion. To preserve the lifespan of the piers, this situation requires regular underwater cleaning. It can also cause ecological damage, which may result in the establishment of invasive species and the displacement of native species.

This study aimed to characterize the macrofaunal communities colonizing the piles of a newly constructed jetty in Iskenderun Bay, Levant Sea, through repeated underwater photographic surveys over time.

Material and Methods

On 18 May 2026, the identification of species in the macroinvertebrate communities developing on the steel pilings of a newly constructed jetty in the Iskenderun Bay was conducted using photographs. As part of the study, photographs were taken of the jetty's steel pilings and the species present on them were identified using the resulting images (Figure 1). Scuba divers photographed the jetty piles at three depth zones (upper, middle, and bottom) over the early colonization period following construction.

Results and Discussion

Expert divers took high-resolution photographs of the jetty piers from the water's surface to their deepest points. By targeting both newly installed piers and those installed some time ago, detailed images were obtained in order to determine the diversity of the macrofauna developing on these structures. These photographs have enabled us to reliably assess the diversity and distribution of species in macrofauna communities.

Based on the analysis of photographs, 11 species belonging to three systematic groups (Chordata Tunicata, Polychaeta and Crustacea) were identified. Four of the 11 identified species were determined to be exotic species (Table 1).

Table 1. Species of assambles jetty (*: Exotic species).

Species
BRYOZOA
**Schizoporella errata* (Waters, 1878)
CHORDATA TUNICATA
**Phallusia nigra* Savigny, 1816
**Didemnum vexillum* Kott, 2002
Styela plicata (Lesueur, 1823)
POLYCHAETA
**Branchiomma bairdi* (McIntosh, 1885)
Serpula vermicularis Linnaeus, 1767
Spirobranchus triqueter (Linnaeus, 1758)
CRUSTACEA

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Chthamalus stellatus (Poli, 1791)
Chthamalus montagui Southward, 1976
Perforatus perforatus (Bruguière, 1789)
Eriphia verrucosa (Forskål, 1775)

The Crustacea group represents 36.4% (4 species) of the total 11 species identified in the study area, followed by Polychaeta and Tunicata (3 species; 27.3%) and Bryozoa (1 species; 9.1%) (Figure 2).

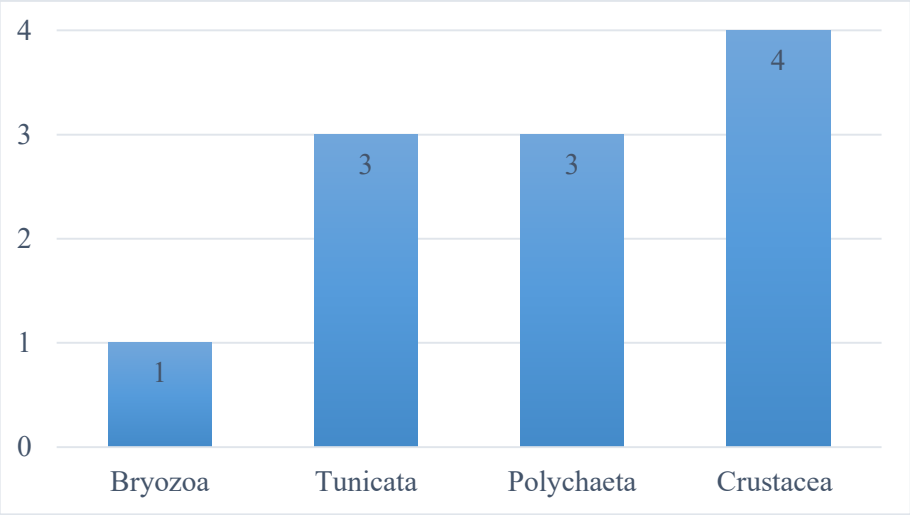


Figure 2. Number of species in the identified systematic groups

In conclusion, the study revealed the following: Biofouling has begun to form on the new jetty piers, as observed in photographs, as a result of increased maritime trade activities in the Iskenderun Bay. A total of 11 macroinvertebrate species belonging to four systematic groups were identified. Four exotic species were identified in the study area: *Schizoporella errata* (Waters, 1878), *Phallusia nigra* Savigny, 1816, *Didemnum vexillum* Kott, 2002 and *Branchiomma bairdi* (McIntosh, 1885). The proportion of exotic species detected on the jetty piers was found to be 36.4%.

The construction of ports, breakwaters, and other such facilities planned for the bay or elsewhere is expected to increase existing vessel traffic within the bay in the coming period and, as a result, may facilitate the introduction of new species in the form of ballast water or fouling organisms (e.g., *Eurycarcinus integrifrons*) (Özcan et al., 2010). As any activity within a marine ecosystem can impact it, it is important to continue conducting regular ecological monitoring studies to protect and sustainably use marine ecosystems.

The results showed that sessile and mobile organisms rapidly colonized the artificial substrate, with well-established macrofaunal assemblages developing within approximately 90 days. In addition, alien species, which are widespread in İskenderun Bay, constituted a substantial proportion of the macrofaunal community on the jetty piles.

Based on these data, it is clear that the species has become established in the Mediterranean ecosystem and expanded its range within just three years. Therefore, its impact on the ecosystem, as well as its potential to displace other species, must be monitored closely.

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Ethics Approval

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No need to ethical approval for this study.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data used in this study can be made available upon request to the corresponding author.

Author Contribution

The authors conceived and designed the study, conducted the fieldwork, collected and analyzed the data, prepared the figures, wrote the manuscript, and approved the final version of the manuscript.

Acknowledgement

We respectfully commemorate the coastal people of the Iskenderun Bay. They are the traditional inhabitants of the region where this study was conducted.

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Jellyfish along the Tunisian Coasts: Occurrence Patterns and Stinging Cases

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Abstract

Jellyfish are ecologically important components of Mediterranean pelagic ecosystems, but high-abundance events may also affect fisheries, tourism, coastal activities, and human health. Tunisia occupies a central position in the Mediterranean and represents an important region for evaluating spatial and seasonal patterns of gelatinous organisms, including non-indigenous taxa. This study synthesizes published information, field observations, and available citizen-science records on jellyfish occurrence, seasonal patterns, and stinging potential along the Tunisian coast. In addition, a preliminary Lagrangian particle-tracking experiment was used to explore the potential eastward transport of *Physalia physalis* within the Mediterranean Sea. The documented 2010 Mediterranean intrusion was used as a reference hindcast, while a standardized 2021 experiment was used for comparison. Available records indicate marked spatial and seasonal variability in jellyfish occurrence along Tunisian coasts, with several species having direct implications for human health and coastal activities. The 2010 hindcast reproduced the entry of virtual *P. physalis* colonies through the Strait of Gibraltar; however, subsequent transport was episodic rather than continuously eastward. Under the main 4.5% windage scenario, approximately 10% of the released virtual colonies crossed Gibraltar, while only a small eastern branch progressed to approximately 6–7°E. In the standardized 2021 experiment, initial eastward transport during February was followed by westward displacement during March, and the simple February Gibraltar-entry scenario did not reproduce the observed Sicily–Tunisia pattern. These findings highlight the importance of integrating occurrence records, human-health observations, and physical transport processes when evaluating changes in Mediterranean jellyfish distributions.

Keywords: Mediterranean Sea; Cnidaria; jellyfish; Tunisia; stinging; *Physalia physalis*; Lagrangian transport

Introduction

Jellyfish are important components of pelagic marine ecosystems and may influence trophic interactions through predation on zooplankton, fish eggs and larvae, and other gelatinous organisms. Large aggregations may also interfere with fisheries, aquaculture, coastal infrastructure, tourism, and recreational activities. In addition to their ecological and socioeconomic effects, jellyfish are relevant to human health because cnidarians possess specialized stinging cells containing nematocysts. Contact with some species can produce local reactions including pain, burning, erythema, itching, edema, and urticaria, while severe

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envenomation may occasionally involve systemic manifestations (Mariottini & Pane, 2010; Cegolon et al., 2013).

Tunisia occupies a key biogeographical position in the central Mediterranean, close to the transition between the western and eastern Mediterranean basins and the Sicily Channel. Consequently, the Tunisian coast represents an important region for examining the distribution and potential dispersal of gelatinous organisms. Previous assessments have demonstrated substantial spatial variation in jellyfish composition and occurrence between the Tunisian Western Mediterranean and Tunisian Plateau and have emphasized the value of combining historical records, field monitoring, and citizen-science observations (Gueroun et al., 2022).

Tunisia occupies a key biogeographical position in the central Mediterranean, close to the transition between the western and eastern Mediterranean basins. Due to its central position and proximity to the Sicily Channel, the Tunisian coast represents an important transition zone for the dispersal of gelatinous organisms between the western and eastern Mediterranean. Therefore, in this study, we presented the aggregation characteristics of jellyfish species distributed along the Tunisian coast and some stinging cases.

A comprehensive assessment of Tunisian jellyfish biodiversity documented 66 species during 1994–2020, including 11 non-indigenous species. The study demonstrated substantial spatial variation between the Tunisian Western Mediterranean and the Tunisian Plateau and highlighted the value of combining historical records, field monitoring and citizen-science observations (Gueroun et al., 2022). Among the scyphozoans reported from Tunisian waters are C. tuberculata, R. pulmo and R. nomadica, while recent observations have also increased interest in C. hysoscella and also *Physalia physalis* which is very dangerous stinger.

Jellyfish are also important from a human-health perspective because cnidarians possess specialized stinging cells called cnidocytes. These cells contain cnidae, including nematocysts, which are highly specialized microscopic structures capable of rapid discharge following appropriate stimulation. The discharged tubule may penetrate the skin and deliver venomous components. The composition and biological activity of cnidarian venoms vary among species, and this contributes to differences in the severity of human reactions (Mariottini & Pane, 2010; Cegolon et al., 2013).

Materials and Methods

The study was conducted as a literature-based assessment of jellyfish occurrence, seasonal distribution, and reported human stinging cases along the Tunisian coastline. Published scientific articles, historical records, field observations, and available citizen-science observations were considered. Particular attention was given to conspicuous or medically relevant gelatinous taxa. The principal Tunisian biodiversity dataset used for comparison covered the period 1994–2020 and integrated literature records, field monitoring, and citizen-science observations (Gueroun et al., 2022).

To investigate the potential eastward transport of *Physalia physalis* within the Mediterranean Sea, a preliminary Lagrangian particle-tracking approach was implemented in Python. The documented 2010 Mediterranean intrusion was used as a reference hindcast event, and virtual *P. physalis* colonies were released in the southwestern Iberian region and advected using surface ocean currents combined with wind-induced drift. Particle velocity was calculated as the sum of surface-current velocity and a windage component derived from 10-m wind velocity. A windage coefficient of 4.5% was used as the main scenario, while alternative coefficients of 2.66%, 7.5%, and 10% were examined to assess sensitivity to wind forcing. Particle positions were updated at hourly time steps using a forward-Euler integration scheme, and trajectories were followed to evaluate passage through the Strait of Gibraltar and subsequent eastward transport within the Mediterranean. Land encounters were identified using the ocean-model

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land mask and classified as strandings. Following the 2010 hindcast, a standardized experiment was conducted using 2021 atmospheric and oceanographic forcing to examine whether differences in circulation and wind conditions could reproduce the documented occurrence pattern of *P. physalis* in the central Mediterranean and along the Tunisian coast. These simulations were designed to assess physical transport potential rather than biological establishment or future occurrence probability.

Results and Discussion

Available records demonstrate a diverse and spatially heterogeneous gelatinous fauna along the Tunisian coast. High-abundance events have been reported from several regions, including Bizerte Lagoon and Bay, the Gulf of Tunis, Hammamet Bay, and the Gulf of Gabès. *Pelagia noctiluca* and *Rhizostoma pulmo* are among the taxa reported in conspicuous aggregations, while other taxa show more restricted or seasonal occurrence. These observations indicate that jellyfish distribution is not uniform along the Tunisian coastline and is likely influenced by the combined effects of temperature, food availability, reproductive cycles, circulation, and local hydrographic conditions.

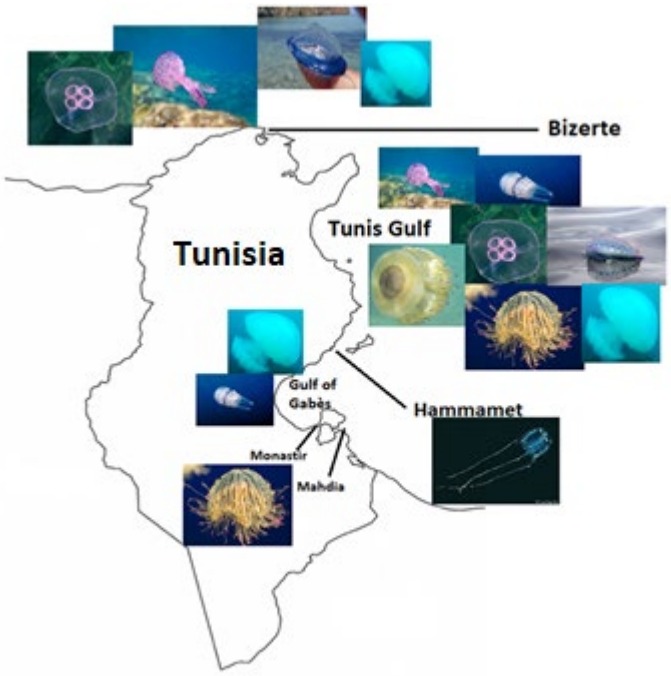


Figure 1. Swarmed Cnidaria species in the Tunisian coasts.

In 2012, 2014-2015 Pelagia noctiluca swarms were seen in Bizerte lagoon. Also the same year it was seen hundreds of Carybdea marsupialis in Hammamet Bay. Holidaymakers complained about painful stings. Aurelia solida showed swarm in 2014 in the Bizerte Lagoon. In 2008, Velella velella had swarms in the Bizerte Bay. Cotylorhiza only swarmed in Gabes Bay in 2021. *R. Pulmo* and *P. noctiluca* are the most swarmed species. *R. pulmo* swarms of 10–100 individuals/1000 m^3 have been reported, and individuals have been observed at a density of

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approximately 1 individual/m² in Gulf of Tunis, in 2013. And then 2014, 2015 and 2016 It existed from July to February in Bizerte Lagoons in high numbers for example 4.4 ind./per square km (Gueroun et al. 2020, Gueroun et al. 2015, Jouin et al. 2022). Ben Salah et al. (2025) reported a case on six years old child was stung by Rhizostoma pulmo while swimming in the Mediterranean Sea; 36 hours later, numerous pustules had developed around her mouth.

From a human-health perspective, the species considered differ substantially in their stinging potential. *Cotylorhiza tuberculata* is generally associated with relatively mild effects, whereas *R. pulmo* can produce clinically relevant local skin reactions. *C. hysoscella* has documented cutaneous toxicity in Mediterranean waters despite its comparatively uncommon occurrence in the Tunisian record. *R. nomadica* is particularly important because it is a non-indigenous scyphozoan with documented expansion along the Tunisian coast and clinically important stinging effects.

The first official Tunisian record of *R. nomadica* was reported from the Gulf of Gabès in 2008. The species was subsequently observed in the Gulf of Tunis and Bizerte, with regular observations reported from the Gulf of Tunis during summer and autumn. High local abundances have also been documented; for example, swarms of 10–100 individuals per 1000 m³ were reported near Ghar El Melh in September 2013, together with stranded individuals along the coast (Daly-Yahia et al., 2013). Saadaoui et al. (2025) reported a case of many blisters and scars by oral arms appendages on her legs and hands caused by *R. nomadica.*

There are blisters and scars by oral arms appendages on her legs and hands. The Tunisian gelatinous fauna also includes *Physalia physalis*, commonly known as the Portuguese man-of-war. Taxonomically, *P. physalis* is not a true jellyfish (Scyphozoa), but a colonial siphonophore within Hydrozoa. Nevertheless, it is highly relevant to studies of stinging gelatinous organisms because its long tentacles contain numerous nematocysts and contact can result in painful and potentially severe envenomation.

The documented 2010 Mediterranean intrusion provided an opportunity to investigate the physical transport of *P. physalis*. The 2010 hindcast successfully reproduced the passage of virtual colonies through the Strait of Gibraltar into the Mediterranean. Under the main 4.5% windage scenario, approximately 10% of released virtual colonies crossed the Strait. However, trajectories did not show persistent eastward displacement. Instead, repeated eastward and westward movements occurred in response to changing wind and surface-current conditions. During the extended simulation, a small eastern branch reached approximately 6–7°E, but continuous transport beyond 8°E was not observed by the end of April.

These results indicate that eastward dispersal within the Mediterranean is not necessarily a simple continuous process. Rather, successful progression toward the central and potentially eastern Mediterranean may depend on relatively short-lived favorable combinations of atmospheric and oceanographic conditions. Thus, specific transport windows may be more important than persistent eastward circulation.

The standardized 2021 experiment produced a contrasting pattern. Initial eastward transport during February was slightly stronger than in the corresponding 2010 comparison; however, the simulated particles subsequently shifted westward during March. Consequently, the simple hypothesis of a February entry through Gibraltar did not reproduce the documented Sicily–Tunisia occurrence pattern. This discrepancy may indicate that the actual 2021 event involved an earlier Atlantic entry, a different source or release location, alternative windage behavior, or circulation processes not fully represented in the present simplified model.

Importantly, the failure of this particular scenario to reproduce the 2021 observations should not be interpreted as evidence that eastward transport is impossible. Instead, it demonstrates the sensitivity of *P. physalis* dispersal to the timing of entry, wind forcing, and regional surface circulation. The modelling results should therefore be considered a preliminary hypothesis-testing framework rather than a forecast.

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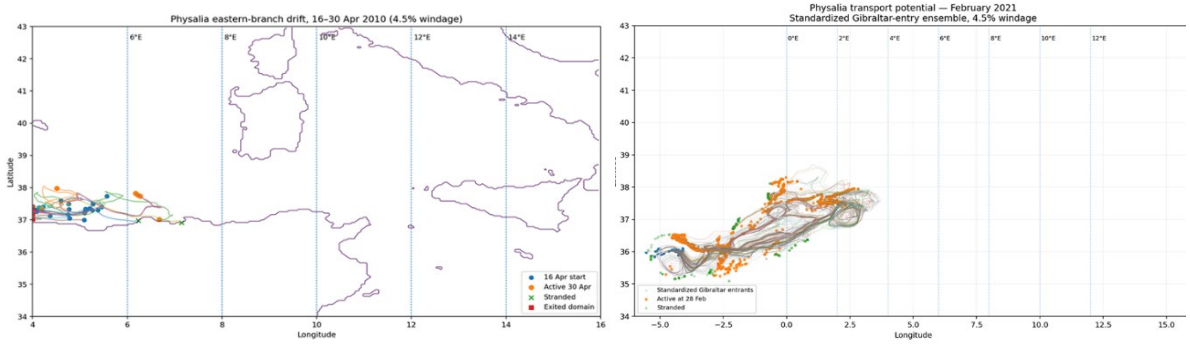


Figure 2. Preliminary Lagrangian particle-tracking simulations of *Physalia physalis* transport in the Mediterranean Sea. (A) 2010 hindcast showing the transport of virtual colonies through the Strait of Gibraltar and subsequent episodic eastward displacement. (B) Standardized 2021 experiment showing initial eastward transport followed by westward displacement during March. Simulations represent physical transport potential and should not be interpreted as predictions of biological establishment.

Taken together, the occurrence records and preliminary transport simulations emphasize that changes in Mediterranean jellyfish distributions cannot be interpreted from occurrence data alone. Transport, persistence after arrival, and establishment are distinct processes. Multi-year simulations, improved biological parameters, and independent observations will be required to determine how frequently favorable eastward-transport conditions occur and whether their frequency or duration changes among years. Accordingly, the possible transport of *P. physalis* toward the Aegean Sea and Turkish coasts should currently be regarded as a testable dispersal hypothesis rather than a prediction.

Conclusion

Jellyfish occurrence along the Tunisian coast is characterized by substantial spatial and seasonal variability and includes both native and non-indigenous taxa with differing ecological and human-health relevance. Among these species, *Rhopilema nomadica* warrants particular attention because of its documented expansion along the Tunisian coast and its comparatively important stinging potential.

The preliminary Lagrangian simulations further demonstrate that the Mediterranean transport of *Physalia physalis* may be highly dependent on temporal variations in wind forcing and surface circulation. Although the 2010 hindcast reproduced entry through the Strait of Gibraltar, subsequent eastward transport was episodic, and the standardized 2021 experiment did not reproduce the observed Sicily–Tunisia pattern using a simple February Gibraltar-entry scenario. These findings highlight the importance of transport timing and physical forcing and demonstrate the need for multi-year modelling before conclusions can be drawn regarding possible future eastward expansion.

An integrated monitoring strategy combining standardized jellyfish observations, confirmed species identification, environmental measurements, human-sting records, citizen-science observations, and physical transport modelling would provide a stronger basis for evaluating future changes in Mediterranean gelatinous fauna and their implications for human health and coastal activities.

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A Vision for Sustainable Aquaculture Development in Eastern Libya: Opportunities, Challenges, and Pathways to a Resilient Blue Economy

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Abstract

Due to large stretches of an unoccupied coastline in addition to the diversity of marine habitats, Eastern Libya has a wealth of untapped natural resources available for developing aquaculture. This paper provides an overview of the status of the aquaculture industry in Eastern Libya at present, identifies principal opportunities and obstacles to expansion, and provides a strategic framework that will lead to sustainable development. Using recent policy evaluations, empirical evidence, and field research, this study concludes that through appropriate investment, reform of business practices, and inter-communal cooperation, Eastern Libya could become the regional leader in climate-resilient aquaculture production. The results of this research indicate a necessary response to integrate aquaculture into national and regional climate adaptation strategies through the use of new technologies such as recirculating aquaculture systems (RAS) and offshore cage farming, as well as to build institutional capacity to develop the blue economy. The proposed integrated vision will increase food security, create jobs, and diversify economies while enhancing environmental sustainability and addressing climate issues in the Mediterranean.

Keywords: Aquaculture, Eastern Libya, Sustainable Development, Blue Economy, Climate Adaptation, Recirculating Aquaculture Systems (RAS).

Introduction

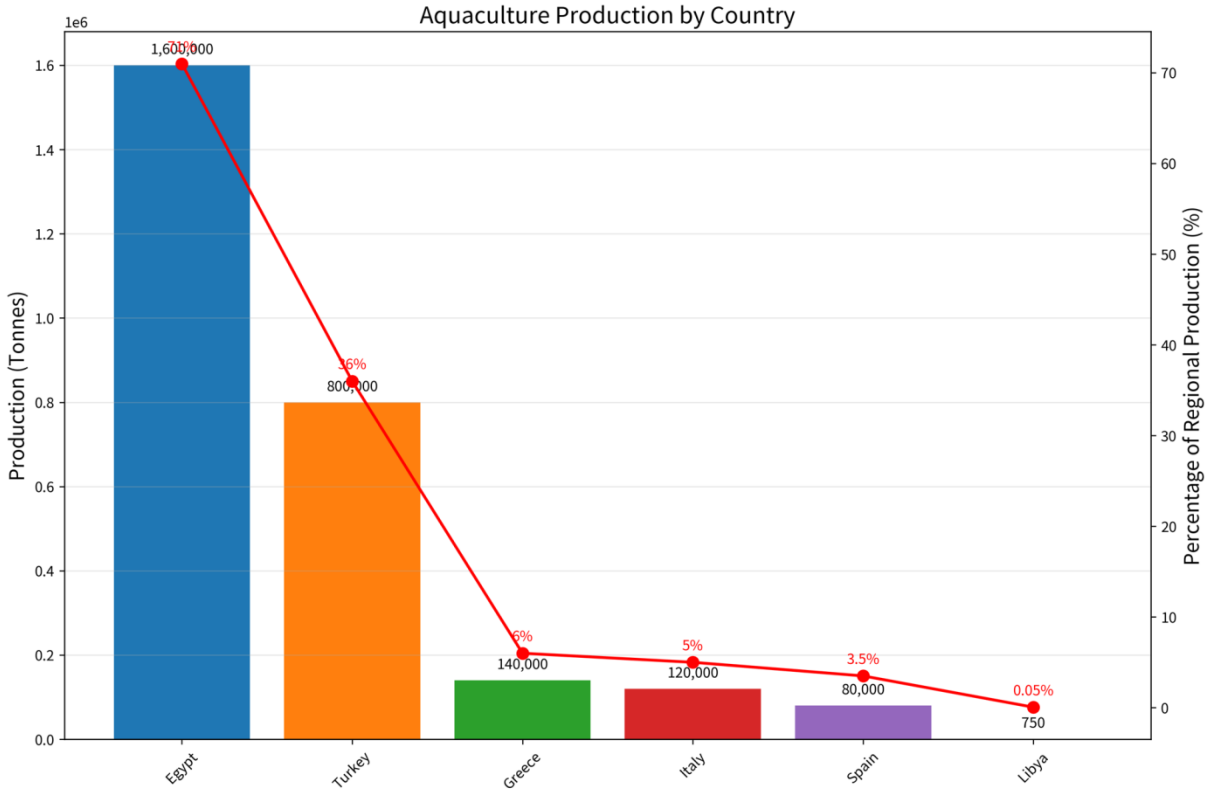
Aquaculture is one of the food production sectors that is developing very fast and now provides more than half of all aquatic animals consumed by man. The Food and Agriculture Organization (FAO, 2024) states that global aquaculture continues to develop as a response to the high demand for highly nutritious animal protein, declining wild fish stocks, and the necessity of more sustainable food production methods. Besides food production, aquaculture is important in employment generation, the fight against poverty, export revenue generation, and economic diversification in developing coastal countries. It should be mentioned that the Mediterranean region has also shown a significant increase in marine aquaculture over the last 20 years as countries such as Türkiye, Greece, Egypt, Italy, and Spain have been able to build competitive industries based on European sea bass, gilthead sea bream, meagre, mollusks, and other high-value organisms (Figure 1). These countries manage to show that policy support, innovation, good governance, and investment from the private sector are the main factors for sustainable aquaculture development.

In spite of having roughly 2,000 kilometers of coastline along the Mediterranean Sea, Libya is one of the less-developed aquaculture producers in the region. Although conditions on land and

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in the sea are favorable for aquaculture development in the country and the demand for fish products continues to grow, aquaculture production in Libya is still quite limited, compared to the neighboring countries. Such situation can be explained by multiple factors such as instability politics, poor infrastructure, split governance, lack of qualified specialists, lack of investments, and absence of environmental impact evaluation of the most suitable areas for aquaculture. Eastern Libya is considered one of the most promising destinations for aquaculture development because its waters are relatively clean and the bays are sheltered. Regions such as Susa, Ras Al-Hilal, Ain El-Ghazala, Derna, and Tobruk possess the geographical characteristics that are good for such types of aquaculture as offshore cage farming.

Figure 1. Comparative Aquaculture Production in Mediterranean Countries (2022)



New global efforts targeting Blue Economy suggest harmonization of aquaculture with environmental protection, climate protection, and socio-economic development. Sustainable aquaculture can provide food security, job opportunities, biodiversity protection, and creation of wealth in coastal areas. For Libya, adopting Blue Economy principles means that the national economy can be expanded and the pressure on overfished natural waters can be reduced. This article analyzes the current situation of aquaculture in Eastern Libya, pinpoints the key chances and challenges, and presents a model for environmentally friendly aquaculture that will comply with the best practices worldwide.

Materials and Methods

A qualitative review and policy analysis approach were taken in this study. Gathered data from peer-reviewed studies, international reports, government publications, and institutional databases was completed between 2017 and 2025. Major sources include the Food and

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Agriculture Organization (FAO), the General Fisheries Commission for the Mediterranean (GFCM), the WestMED Initiative, the World Bank, the United Nations Environment Programme (UNEP/MAP), and the Intergovernmental Panel on Climate Change (IPCC). The analysis of gathered data was structured into five components: (1) current scenario of aquaculture production and infrastructure; (2) environmental suitability; (3) socio-economic opportunities; (4) governance and institutional frameworks; and (5) climate-related issues. The study included comparative analysis with the leading aquaculture producers in the Mediterranean region to identify best practices in the Eastern Libya context.

This strategic framework has been developed based on the well-interconnected synthesis of scientific evidence, policy recommendations, and regional development experiences while taking into account the specifics of Eastern Libya in terms of environment, economy, and institutions.

Current Status of Aquaculture in Eastern Libya

Aquaculture is one of the most undeveloped areas of Libya's blue economy. Current production consists of small freshwater farming and a few marine cage culture projects focused mainly on Nile tilapia (*Oreochromis niloticus*), European sea bass (*Dicentrarchus labrax*), and gilthead sea bream (*Sparus aurata*). National production remains below average for the region as a whole; consequently, Libya continues to depend on seafood imports. Limited infrastructure serves as one of the main challenges in developing this sector. Hatchery capacity is insufficient to fulfill the domestic demand for fish fry, while the absence of local aquaculture feed production makes production costlier. Cold-chain infrastructure, fish processing plants, and marketing systems deserve attention as well.

In eastern Libya, a number of coastal sites have suitable conditions for aquaculture. There are places such as Susa, Ras Al-Hilal, Ain El-Ghazala, Derna and Tobruk, which have the right level of water quality, favorable hydrodynamic conditions and a proper temperature for cage aquaculture production. Despite this, the environmental carrying capacity assessment is not carried out in very detail, which results in deficit of effective basis of site selection. The aquaculture governance in Libya is split among many government institutions with similar tasks, which leads to a lack of clarity in the current regulation. The existing legislation provides only a little evidence regarding aquaculture licensing, environmental monitoring, biosafety and marine spatial planning. Expressing interest in the Blue Economy principles and new international cooperation initiatives make institutions in Eastern Libya aware of such opportunities.

Opportunities for Sustainable Aquaculture Development in Eastern Libya

Environmental Opportunities

The environmental conditions in Eastern Libya are suitable for the development of sustainable marine aquaculture. The coastline of the region is about 1,000 km long and includes several protective bays, natural bends, and semi-closed coastal zones, which are very good for offshore cage aquaculture. Compared to other Mediterranean coastal areas, the waters of Eastern Libya are not affected much by industrial pollution, and this means the water quality is good for high-value aquaculture species (UNEP/MAP, 2023). The average temperature is about 18-28°C, which is sufficient for the cultivation of European seabass and gilthead bream. The salinity is around 36-39‰, and the oxygen saturation is more than enough (GFCM, 2023). Several coastal places like Susa, Ras Al-Hilal, Ain El-Ghazala, Derna, and Tobruk have been noted as helpful sites according to good bathymetry, moderate wave exposure, and good water exchange.

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Additionally, the area provides options for diversification apart from fish farming activities. Shellfish aquaculture, algal cultivation and Integrated Multi-Trophic Aquaculture (IMTA) are examples of eco-friendly production approaches that can improve nutrient recycling and the conditions in ecosystems while generating more profits.

Technological Opportunities

Aquaculture innovations offer new possibilities for developing the industry in Eastern Libya. The recirculating aquaculture system allows fish production to happen under comfortable conditions with little water consumption and environmental damages. This technology is effective in hatcheries and juvenile production of expensive fish species. The offshore cage farming has become one of the most rapidly developing technologies of marine production. Modern cages function well at deeper sites and in stronger currents, improving water circulation, limiting waste build-up and disease outbreaks. The use of such high-tech gadgets as remote sensing, satellite monitoring, water-quality visual sensors, and even automated feeding systems may boost the profitability of aquaculture, as well as help manage climate-smart aquaculture. Renewable energy technologies, especially solar energy, also offer ways of cutting down costs on aquaculture in remote areas with no electrical systems.

Socioeconomic Opportunities

The growth of the aquaculture industry provides massive economic and social benefits for Libya. The expansion of the aquaculture sector creates job opportunities in various processes such as hatchery operation, seafood production, feed manufacturing, fish processing, transporting and selling fish and fish feed, and providing of the equipment and research services. Furthermore, aquaculture directly contributes to national food security by increasing fish production locally and reducing the imports of seafood products. Increasing local production enables the trade balance of the country to change for the better due to decreasing seafood prices. Aquaculture creates favorable conditions for young people and women to engage in small businesses and open their businesses in aquaculture, processing industry, and hatchery operations. Moreover, sustainable aquaculture supports the task of Libyan economy and helps country to be diversified, including different industries apart from oil and gas.

Policy and Investment Opportunities

In recent times international organizations have acknowledged Libya as having considerable potential when it comes to aquaculture development. Various initiatives in which the FAO, GFCM, WestMED Initiative, and the Union for the Mediterranean are involved provide assistance in many forms such as investment opportunities, technical and institutional support. Moreover, mechanisms linked to regional funding, such as the Blue Mediterranean Partnership, make investments possible for implementing projects connected to hatchery building, fish feed production, construction of marine facilities, research, and climate change-resistant aquaculture technologies. As for governance improvements, simplifying the licensing process, and developing public-private partnerships, all of that will contribute to boosting investor confidence and increasing the sector's lucrative menus over time.

Challenges Facing Sustainable Aquaculture Development

Institutional and Governance Challenges

Institutional fragmentation is one of the key obstacles facing aquaculture development in Libya. There are several governmental institutions involved in fisheries management, environmental protection, coastal zone planning, and investment promotion, with little coordination among them. Lack of a national aquaculture plan has resulted in investment stagnation and, ultimately,

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poor aquaculture sector planning. Current legislation does not address many of the requirements modern aquaculture presents, including marine spatial planning, environmental impact assessment, biosecurity measures, fish health, and licensing.

Infrastructure Constraints

The current state of aquaculture structures makes it impossible to achieve commercial-scale production. There are no adequate hatcheries in the country, failing to deliver a significant number of high-quality fingerlings. There are practically no manufacturing plants for producing feed, forcing consumers to purchase feed from other countries. Cold storage, seafood processing plants, means of transportation, and laboratory services are still very much underdeveloped, making the production process inefficient and uncompetitive.

Climate Change and Environmental Risks

Changes in climate are one of the most serious long-term challenges for aquaculture in coastal areas. Higher surface temperatures of the oceans can influence the growth, breeding of fish, and their susceptibility to diseases. Natural disasters - storms, floods, and rising ocean level endanger aquaculture infrastructure. The devastating consequences of Storm Daniel that struck in September 2023 proved the vulnerability of coastal infrastructure in Eastern Libya and emphasized the necessity of adapting climate-proof solutions to future planning.

Economic Challenges

instability in the political arena keeps domestic and foreign investors away from the market. Limited access to the financing system, increasing production costs, fluctuating exchange rates, and dependence on imported material negatively affect the profitability of the business and raise investment risk. In addition, the seafood market in the country is poorly organized, while the export certification must be improved before Libya can compete in the Mediterranean seafood market.

Technical and Human Capacity Challenges

One of the constraints here is the lack of trained professionals in aquaculture that thus needs to be addressed. It is imperative that research institutions receive modern labs, funding, and international collaboration in order to develop research programs. Extension services are another limitation since they prevent effective technology transfer from researcher to producer. Hence, continuous education increases the technical skills of professionals in this area.

Transition to the Next Section

Even with the above-mentioned obstacles, the eastern part of Libya has enough natural capital and geo-strategic features to set up viable aquaculture. This can be achieved through developing a coherent plan for development that unites reforms in the institutions, innovations in technology, adapting to the changes of climate, carrying out scientific research, and planning investments for the long run. The next part of this text presents a detailed plan for use in the process of sustainable development of aquaculture in eastern Libya.

ISBN: 978-625-91384-0-4 Publication of e-book date: 06.10.2026

Strategic Framework for Sustainable Aquaculture Development in Eastern Libya

Vision

In order to achieve food security, advance the Blue Economy, and enhance climate resilience in Eastern Libya, the proposed framework has the goal of creating a sustainable aquaculture industry that is resilient, competitive, and inclusive. This strategic approach incorporates elements of environmental sustainability, institutional reforms, technological innovation, collaboration in the private sector, and scientific research in one integrated plan that conforms to UN SDGs, FAO Blue Transformation Strategy, and Mediterranean Blue Economy initiative.

Strategic Pillars

Pillar I: Governance and Institutional Reform

Developing a competitive aquaculture sector requires effective governance supported by clear legislation and strong institutional coordination.

Priority actions include:

- Development of a National Aquaculture Strategy.
- Updating fisheries and aquaculture legislation.
- Implementation of Marine Spatial Planning (MSP).
- Simplification of licensing procedures.
- Establishment of a national aquaculture database.
- Strengthening environmental monitoring systems.

Pillar II: Sustainable Infrastructure Development

Infrastructure investment should prioritize:

- Modern hatcheries.
- Feed manufacturing plants.
- Fish health laboratories.
- Cold storage facilities.
- Fish processing plants.
- Modern landing sites.
- Climate-resilient coastal infrastructure.

Pillar III: Innovation, Research and Human Capacity

Long-term sustainability depends upon scientific innovation.

Priority investments include:

- Aquaculture research centers.
- University–industry partnerships.
- Capacity-building programs.
- Artificial Intelligence applications.
- Remote sensing technologies.
- Digital monitoring systems.

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- Disease surveillance programs (Figure 2).

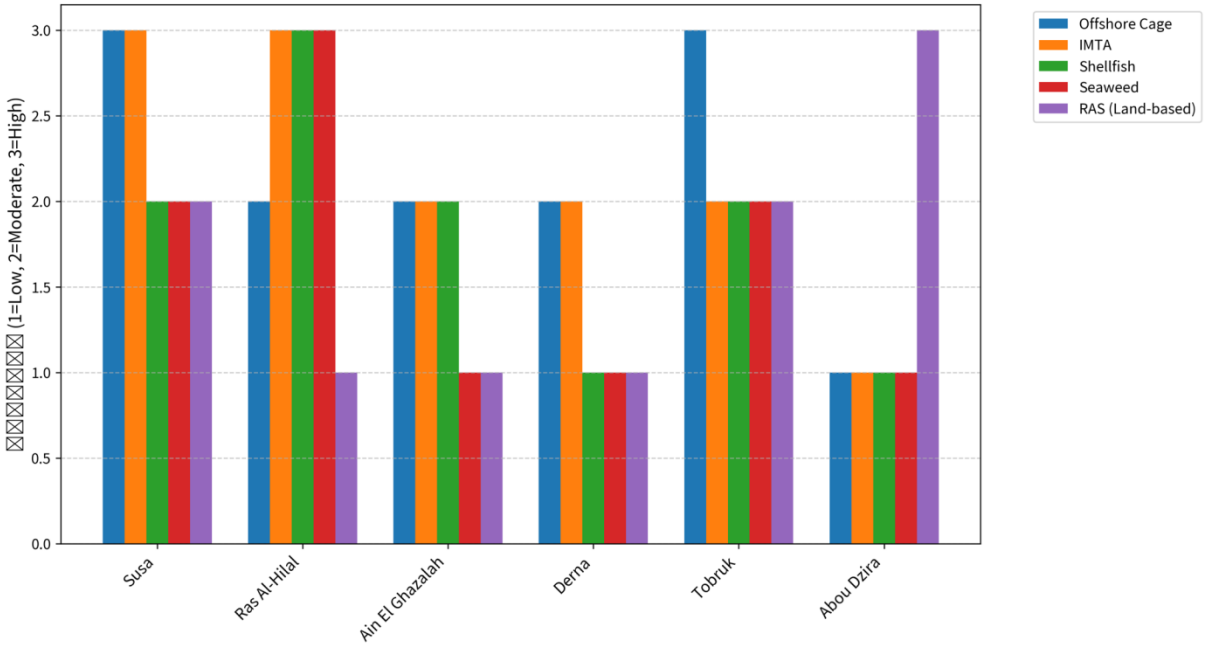


Figure 2. Estimated Investment Requirements by Pillar (Phase 1-3)

Pillar IV: Environmental Sustainability

Environmental management should focus on:

- Carrying-capacity assessment.
- Water-quality monitoring.
- Biodiversity conservation.
- Ecosystem-based management.
- Integrated Multi-Trophic Aquaculture (IMTA).
- Low-carbon production systems.
- Renewable energy applications.

Pillar V: Economic Development and Investment

Economic sustainability requires:

- Public-private partnerships.
- Investment incentives.
- Access to financing.
- Aquaculture insurance.
- Export-oriented production.
- Seafood quality certification.
- Regional market integration.

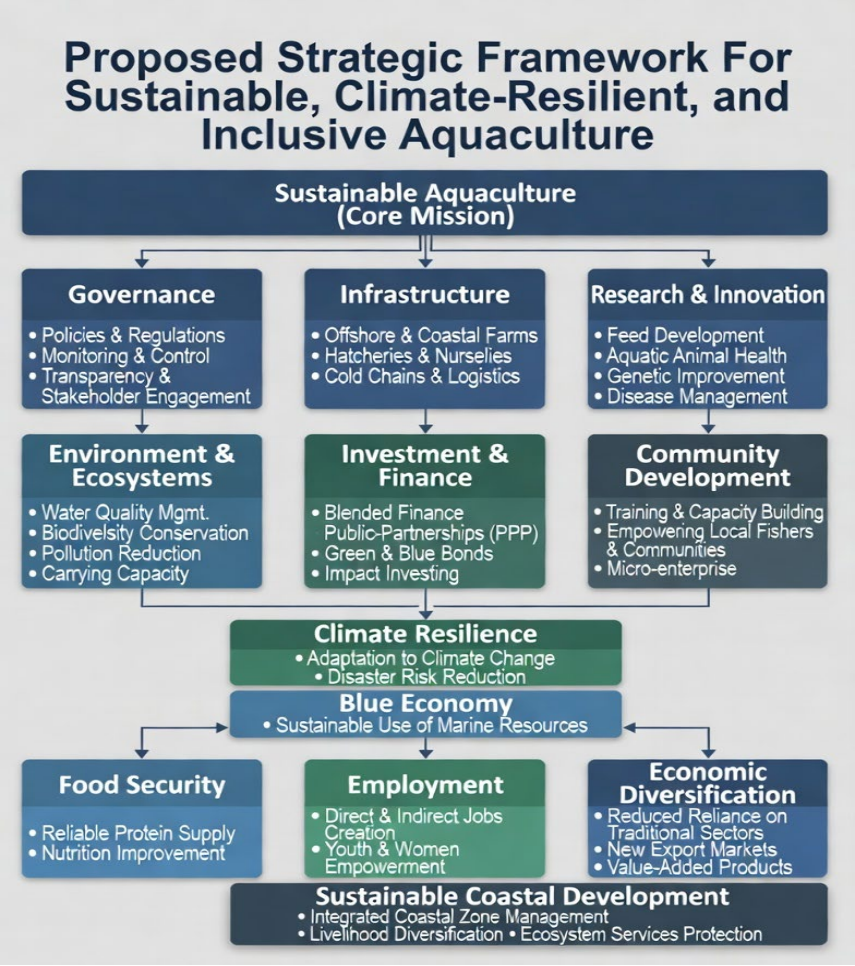
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Discussion

Eastern Libya is home to one of the biggest untapped marine aquaculture opportunities in the Mediterranean zone. The presence of appropriate shoreline ecosystems, relatively clean water, and strategic geographical proximity to the markets of Europe give substantial comparative advantages. Nonetheless, successful aquaculture development goes beyond positive environmental aspects. The international practice shows that successful long-term results require good governance, scientific research, technological innovation, nature conservancy, and continuous investments. Turkey serves as a good regional example of rapid aquaculture development through the presence of synchronized governmental strategy, private sector involvement, and continual technological innovation (Figure 3). Egypt has become Africa's top aquaculture producer thanks to substantial investments into hatcheries, feed production, extension services, and research institutes. Eastern Libya can significantly benefit from applying the successful practices and paying attention to its own unique environmental, socio-economic, and institutional conditions. Climate change makes it even more important to apply adaptive management practices since Storm Daniel demonstrated the vulnerability of coastal infrastructures and proved the necessity of disaster risk reduction in aquaculture.

Consequently, future development should integrate climate-smart technologies, ecosystem-based management, renewable energy applications, and continuous environmental monitoring to maximize resilience and sustainability.

Figure 1. Proposed Strategic Framework



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Conclusions

Eastern Libya has extraordinary natural and strategic resources that enable it to have a strong aquaculture industry. However, institutional fragmentation, poor infrastructure, a shortage of funds, obsolete laws, and many climate change risks hinder its development. The results of this paper confirm that sustainable aquaculture development is impossible without an integrated strategy based on reforms in governance, scientific research, technological changes, protection of environment, and investments by the private sector. The implementation of the strategic framework proposed in the paper can significantly improve food security, derive more jobs, minimize seafood imports, strengthen coastal economies, and help Libya enter the sustainable Blue Economy.

Recommendations: The following recommendations are proposed to accelerate sustainable aquaculture development in Eastern Libya.

Policy Makers

- Develop a National Aquaculture Development Strategy.
- Update fisheries and aquaculture legislation.
- Implement Marine Spatial Planning.
- Strengthen institutional coordination.
- Improve environmental monitoring.

Research Institutions

- Conduct site-selection studies.
- Develop climate adaptation research.
- Improve fish nutrition and disease-control programs.
- Establish regional research partnerships.

Private Sector

- Invest in hatcheries and feed production.
- Develop offshore cage farms.
- Introduce digital technologies.
- Promote seafood value chains.

International Organizations

- Support technology transfer.
- Provide technical training.
- Facilitate regional cooperation.
- Support climate adaptation projects.
- Expand investment opportunities.

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