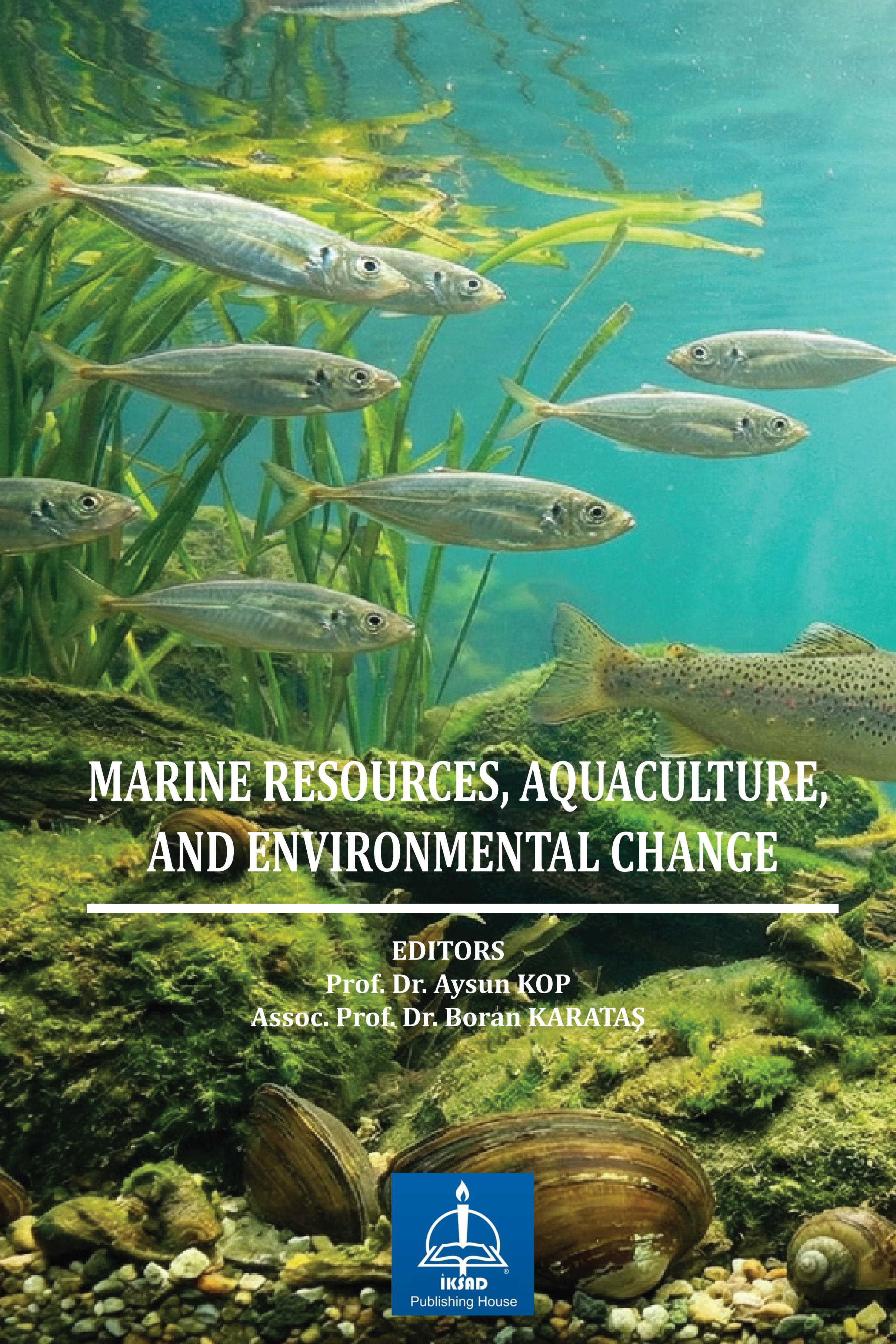

MARINE RESOURCES, AQUACULTURE, AND ENVIRONMENTAL CHANGE

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CONTENTS

PREFACE

The book Marine Resources, Aquaculture, and Environmental Change has been carefully prepared to bring together high-quality academic contributions that reflect both the scientific rigor and the evolving perspectives of modern aquaculture and aquatic sciences. While firmly grounded in scholarly research, the primary aim of this volume is not only to inform but also to connect researchers, practitioners, and students with the broader ecological and societal context in which aquatic systems operate.

The included chapters cover a wide and carefully structured range of topics, including acoustic environmental enrichment and fish welfare, epigenetic mechanisms in aquaculture, the ecological and economic value of marine macroalgae, climate-induced physiological stress, pesticide-related genetic and tissue alterations, and the growing importance of analytical tools such as remote sensing and time-series modeling. Taken together, these contributions highlight the complex interplay between biological systems, environmental change, and human intervention.

This book aspires to serve as a reliable academic reference while remaining accessible and engaging for its readership. We hope that the chapters will not only support scientific advancement but also inspire thoughtful dialogue, interdisciplinary collaboration, and a deeper appreciation of the complexity of aquatic environments.

As editors, we extend our sincere gratitude to all contributing authors for their invaluable efforts, academic dedication, and meticulous work throughout this process. We trust that this collective endeavor will make a meaningful contribution to the understanding and protection of aquatic ecosystems and will inspire future research in this vital field.

EDITORS

CHAPTER 1

INVASIVE NON-NATIVE SPECIES AND BIOSECURITY IN MARINE AQUACULTURE

Prof. Dr. Gürel TÜRKMEN[1]

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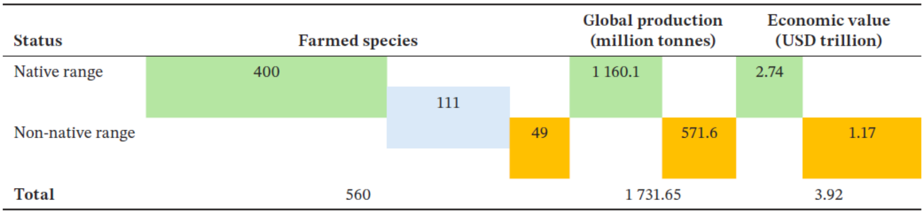
INTRODUCTION

Aquaculture is growing faster than many other agricultural sectors and is providing a source of protein for many people worldwide by compensating for shortages in fisheries. In 2022, global aquaculture production reached 130.9 million tonnes, valued at US$312.8 billion, accounting for 59 per cent of global fisheries and aquaculture production. Aquaculture surpassed capture fisheries for the first time in aquatic animal production, reaching 94.4 million tonnes and accounting for 51% of the global total (FAO, 2024). This rapid growth has resulted in species being transferred from their natural habitats to other regions. While the cultivation of non-native species has increased aquaculture production, the settlement of escaped species in non-native habitats has also led to environmental and socio-economic risks (Lins and Rocha, 2023; Oficialdegui et al., 2025; Zehra et al., 2025). Researchers estimate that invasive non-native species caused over 644 billion US dollars (612 billion euros) in costs worldwide between 1970 and 2020 (European Commission, 2025).

Since 1950, it has been determined that one-third (n = 160) of the 560 species used in aquaculture are farmed outside their natural habitats (Figure 1). The rate of increase in the number of non-native species in aquaculture has been greater than that of native species (Oficialdegui et al., 2025).



Status	Farmed species	Global production (million tonnes)	Economic value (USD trillion)
Native range	400	1 160.1	2.74
	111		
Non-native range	49	571.6	1.17
Total	560	1 731.65	3.92

Figure 1. Summary of the Number of Native/Non-Native Species in Aquaculture

Invasive non-native species (INNS) not only cause economic losses in aquaculture by spreading diseases and parasites, but also lead to the loss and degradation of biological diversity. This study addresses the biosecurity management and control methods that should be implemented in marine aquaculture to prevent or reduce the impacts of invasive non-native species.

2. INVASIVE NON-NATIVE SPECIES IN MARINE AQUCULTURE

2.1. Why Invasive Non-Native Species Are Problem?

Invasive Non-Native Species (INNS) can cause disease transmission and spread in marine aquaculture, structural defects in cage systems and associated economic losses, and prevent water exchange due to algal blooms in cages, resulting in fish being unable to obtain sufficient oxygen and dying. They also have serious negative effects on the environment, the economy and human health (Table 1) (Molnar et al., 2008; GloBallast, 2025).

Table 1. The Effects of Invasive Non-Navite Species on the Environment, Economy and Human Health.

Ecological Effects	It can reduce natural biodiversity and alter habitats.
	It may cause the extinction of native species.
	It may disrupt the structure and health of native species populations.
	It can alter the food web and the overall ecosystem.
	It may reduce water quality.
Economic Effects	It may restrict fishing and maritime activities.
	Algal blooms can affect fish farms.
	Negative physical impacts on coastal infrastructure, facilities and industry.
	It may reduce the value of coastal features and damage the natural heritage.
	Research, development, monitoring and management costs may increase.
	Secondary economic effects arising from impacts on human health.
	Secondary economic impacts resulting from the loss of biological diversity.
Effects on Human Health	Ballast water contains potential microorganisms that epidemiologists and invasion biologists have drawn attention to.
	There is evidence that cholera outbreaks may be directly related to ballast water discharges.
	Ballast water can carry microalgae species, including toxic types that can cause harmful algal blooms, in addition to bacteria and viruses.

2.2. Introduction to Invasive Non-Native Species (INNS)

If a species is endemic, it is classified as native. If a species overcomes a barrier by human activities or enters a new habitat, then it is an alien/non-native species, not all alien species are perceived negatively or pose a threat to natural biodiversity (Carlton, 1996). When these alien/non-native species have a negative impact on biodiversity, ecosystem services, human health or economic impact, they are referred to as Invasive Non-native Species (INNS) (Figure 2).

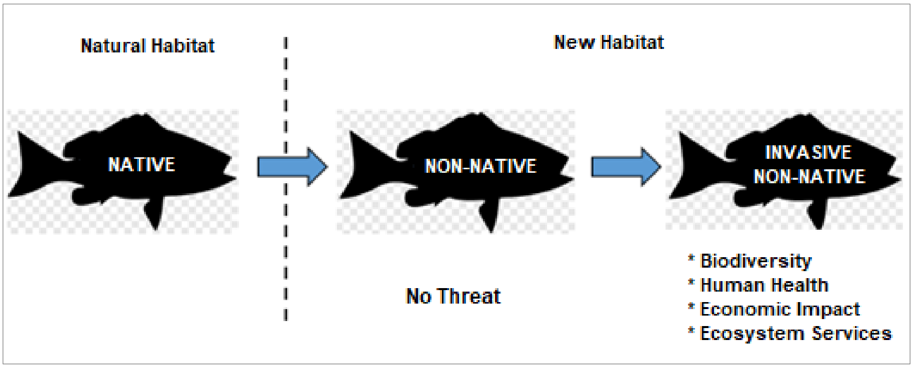


Figure 2. Native, Non-Native and Invasive Non-Native Species

2.2. Invasive Non-Native Species in Marine Aquaculture

In marine aquaculture, invasive non-native species are commonly transported via livestocks, equipment (Figure 3), cage systems, tools, and marine vessels and various mechanisms (Figure 4) (Katsanevakis et al., 2019).

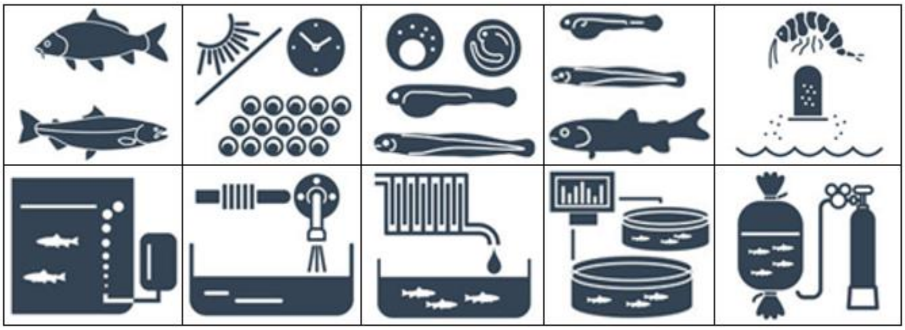


Figure 3. Livestocks and Equipment in Aquaculture

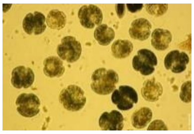
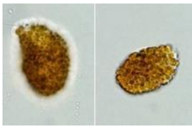
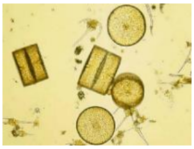
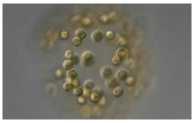
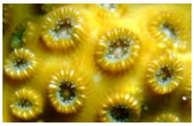
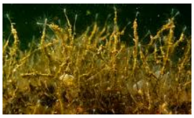
Algal Blooms • Economic losses • Fish deaths • Shellfish poisoning • Damage to cages • Growth disorders in fish	 *Alexandrium minutum*	 *Karenia mikimotoi*	 *Gymnodinium catenatum*
	 *Pseudochattonella verruculosa*	 *Coscinodiscus wailesii*	 *Phaeocystis pouchetii*
Degradation of Important Habitats • Excessive consumption plants • Algal imbalance • Desertification • Algal bloom	 *Siganus luridus*	 *Sargassum muticum*	 *Acrothamnion preissii*
	 *Caulerpa taxifolia*	 *Gracilaria vermiculophylla*	 *Oculina patagonica*
Predation and Competition • Decline zooplankton • Economic losses • Decline in mussel and oyster stocks • Species competition • Decrease in natural stocks	 *Mnemiopsis leidyi*	 *Rapana venosa*	 *Homarus americanus*
	 *Neogobius melanostomus*	 *Crassostrea gigas*	 *Urosalpinx cinerea*
Contamination in Shellfish/Equipment • Contamination in shellfish habitat • Economic loss in shellfish production • Biofouling in cages and equipments	 *Undaria pinnatifida*	 *Microcosmus squamiger*	 *Ficopomatus enigmaticus*
	 *Cercopagis pengoi*	 *Amphibalanus improvisus*	 *Cordylophora caspia*
Disease Transmission • White spot disease in shrimp farming • Transmission disease to European lobsters	 *Rhithropanopeus harrisii*	 *Homarus americanus*	 *Homarus gammarus*

Figure 4. Mechanisms of Harm Caused by INNS in Aquaculture

2.3. Biofouling in Marine Aquaculture

Biological fouling is the accumulation of aquatic organisms such as microorganisms, plants and animals on surfaces and structures immersed in or exposed to the aquatic environment (IMO, 2012). In marine aquaculture, intensive biological fouling may accumulate on cultivation systems (shellfish cultivation racks, baskets, nets and ropes, cage systems and nets (Figure 5), feed platforms, marine vessels and equipment (Figure 6) (Fitridge et al., 2012). Non-Native species colonising these environments may be invasive.

Cage

Buoys

Anchor

Collector

Hole-Type Collector

Ropes

Chain

Shackle

Oval Ring

Cage Net

Cage Net

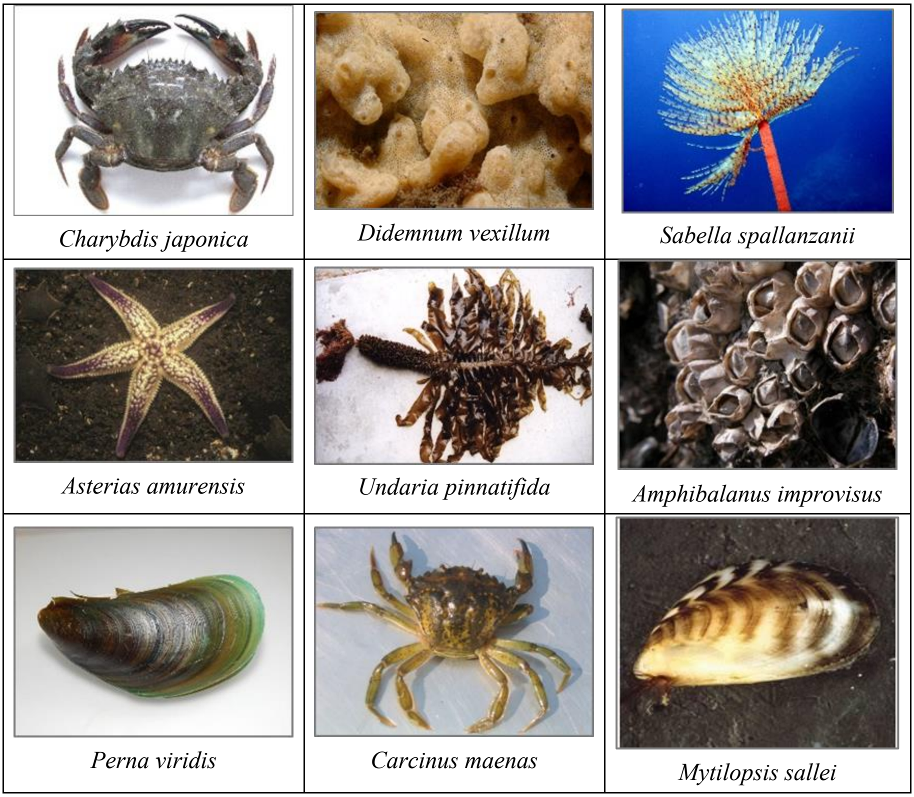
Cage Net

Figure 5. Cage and Equipment

Figure 6. Examples of Marine Aquaculture with High Biofouling
A: *Ciona intestinalis* B: *Ectopleura crocea*
C: *Mytilus edulis* D: *Ectopleura larynx*

Aquatic organisms can be transferred to different locations as biofouling. They can be harmful and invasive in places where they do not occur naturally. The transfer of invasive non-native species can threaten the seas, human, animal and plant life, and economic and cultural activities. Once invasive aquatic species have established themselves in a new habitat, their eradication is often impossible. The International Maritime Organisation (IMO) as "the accumulation of unwanted pollution" defines Biofouling (IMO, 2012).

Biofouling is classified as microfouling (which refers to a layer of microscopic organisms, including bacteria and diatoms and the slimy substances they produce) and macrofouling (which refers to multicellular organisms visible to the naked eye, such as barnacles, tubeworms, mussels, algae, etc.). In cage aquaculture, the hulls of marine vessels (boats/ships) and feed barges are the areas where biological fouling is most prevalent and should be monitored for unwanted INNS (Figure 7) (IMO, 2025).



Charybdis japonica

Didemnum vexillum

Sabella spallanzanii

Asterias amurensis

Undaria pinnatifida

Amphibalanus improvisus

Perna viridis

Carcinus maenas

Mytilopsis sallei

Figure 7. Unwanted INNS in Marine Aquaculture, Marine Vessels and Barges

3. BIOSECURITY IN MARINE AQUACULTURE

Biosecurity is the prevention, elimination or effective management of harmful organisms and diseases that threaten economy, environment, human health, and social and cultural values (DAFF, 2024). In marine aquaculture, biosecurity refers to all measures taken to prevent the introduction of pests, diseases and invasive non-native species and to eliminate or minimise the risk of their spread.

3.1. Why is Biosecurity Important in Marine Aquaculture?

In marine environments, the control and eradication of Invasive Non-Native Species is technically almost impossible or economically unfeasible. Biosecurity, which includes preventive measures, is an important component (Cook et al., 2016). Thanks to biosecurity in marine aquaculture:

- Improved animal health and performance,
- Reduces the transmission and spread of disease within and between farms,
- Enables early disease detection and mitigation,
- Limits or eliminates diseases that affect marketability,
- Increases trade and market potential in national or international markets,
- Enables integration with Hazard Analysis and Critical Control Points (HACCP) systems,
- Facilitates movement within and between jurisdictions,
- Enables farms to meet international trade requirements (health accreditation).
- Contributes to the environment, human health and the economy by reducing the risk of invasive non-native species ıntroduction and spreading.
- Reduces the risk of development, growth, disease and marketability of livestock stocks due to INNS.

3.2. Biosecurity Practices in Aquaculture Sector

It is important to consider aquaculture biosecurity practices across farms, as well as within each aquaculture farm because farming practices in one farm may affect the biosecurity of other farms, and some farmers cultivate more than one species on the same site. The biosecurity practices generally recommended for net cage farming and abalone, oyster, and mussel farms are listed below (Smith et al., 2016; Invasive Species, 2021).

- Prevent boat/vessel sharing between farms
- Prevent equipment sharing between farms
- Prevent movement of personnel between farms
- Control, clean/remove biofouling from cage systems/marine vessels
- Apply antifouling to boats/vessels and underwater structures
- Prevent movement of livestock between farms
- Isolate or quarantine newly arrived livestock
- Clean the stocks, cage systems and sea vessels before moving them to a new area.
- Remove the unused cage system, the vessel and the livestock (shellfish) from the marine environment.
- Apply disease prevention treatments to fish
- Regularly disinfect equipment
- Train your personnel
- Manage facilities by dividing them into separate units
- Restrict access to the facility
- Manually remove and dispose of harmful species
- Wash/clean spats before transfer
- Monitor/control algal blooms
- Treat/improve water entering the facility
- Treat/improve water leaving the facility
- Implement best management practices for animal welfare and health
- Separate production groups
- Conduct regular observation and monitoring

3.3. Biosecurity Zone and Access Requirements in Marine Aquaculture

The biosecurity zones will represent areas that are both physically and functionally separate, so you should be able to define the location and type of biosecurity measure(s) separating the areas. Separate zones should have infrastructure and/or sanitary protocols to prevent transfer of disease from one area to the other with movement of water, people, stock and equipment. These zones are listed in Table 2 (Landos et al., 2019). The main patways of introduction and spread for pests and diseases within and outside the farm are: Water, Wildlife, Equipment, Boats, Vehicles, Feed, People and Stock (Figure 8) (MPI, 2016).

Table 2. A Summary of Farm Biosecurity Zones Classifications

Biosecurity Zone	**Access Requirements**
High (most biosecure)	Highly restricted. Authorised personnel only. Entry must not occur after visiting other areas of the farm. For example: • fully enclosed broodstock room on RAS with sanitised water source • hatching room • algae room • larval rearing room
Moderate (moderately biosecure)	Limited access. Authorised personnel only. Can move from the highest security zone into this area, but not back again. Accompanied visitor access only after biosecurity entry assessment and approval. For example: • nursery
Low (low-level biosecurity)	No restricted access for staff. Visitor access only after biosecurity entry assessment and approval. For example: • grow-out ponds • raceways • sea-cages

Figure 8. Main Risk Pathways for Pests and Diseases to Spread On to, Within and From a Farm

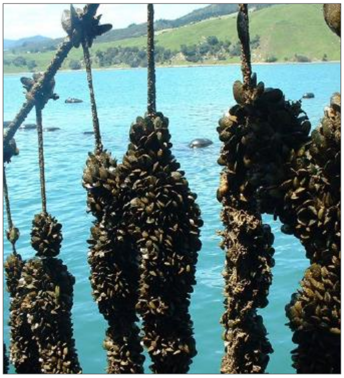
3.4. Biosecurity Management Objectives and Recommended Practices

Every marine aquaculture operation must plan its biosecurity objectives and goals according to its own scale and characteristics. Biosecurity policies and targeted management strategies aimed at countering invasions in their early stages (interception limits to and secure keeping) require careful assessment. Despite the implementation of recommendations, guidelines and emergency measures aimed at reducing the impact of escaped aquatic animals from farms in the past, stricter biosecurity protocols and assessments have become increasingly important to prevent the establishment of INNS populations (Oficialdegui, 2025). The following sections present biosecurity management and recommended practices in marine aquaculture (MPI, 2016).

3.4.1. Stock Health Management

The aim of stock health management is to maintain stock health under optimal conditions. Recommended practices are presented in Table 3.

Table 3. Recommended Practices in Stock Health Management

• Keep livestock stress-free • Ensure optimum water quality • Appropriate stocking densities • Feeding with appropriate feed • Avoid unnecessary handling • Minimise stressful transfers • Monitor and keep records livestock • Remove dead or dying individuals	

3.4.2. Stock Movements and Containment

The objective of Stock Movements and Containerisation is to minimise the risk of pests and diseases in stock movements within and outside the farm. Recommended practices are presented in Table 4.

Table 4. Recommended Practices in Stock Movements and Containment

• Only use healthy stock • Only move stock to optimum locations • New livestock must be quarantined • Euthanize and dispose in risky groups • Stock movements between healthy units • Monitor new stock and treat them • Keep records of all stock movements • Prevent stock escapes	

3.4.3. Water

The aim is to minimise the risk of pests and diseases entering the water in the area where the farm is located. It is difficult to minimise the risk of harmful organisms and diseases entering or leaving the farm. Therefore, greater importance should be placed on animal movements, people, equipment, continuous monitoring and supervision. Recommended practices are presented in Table 5.

Table 5. Recommended Practices in Water

• Regional risks should be determined based on epidemiological criteria. • The risk of disease transmission between farms should be considered	

3.4.4. Equipment, Vehicles and Vessels

The aim is to minimise the risk of equipment, vehicles and vessels introducing and spreading pests and diseases onto the farm. Recommended practices are presented in Table 6.

Table 6. Recommended Practices in Equipment, Vehicles and Vessels

• Biosecurity risk management for equipment, vehicles and marine vessels. • Cleaning/disinfection of equipment, vehicles and marine vessels. • Well-designed delivery and loading areas. • Prevention and disposal of biofouling • Restriction on the use of equipment, vehicles and marine vessels between farms. • Non-operational marine vessels no enter farms.	

3.4.5. People Management

The aim is to minimise biosecurity risks for staff and visitors, prevent unauthorised access by visitors or unauthorised persons to restricted areas, and ensure that they receive training and take responsibility in these matters. Recommended practices are presented in Table 7.

Table 7. Recommended Practices in People Management

• Manage access for staff and visitors in accordance with biosecurity measures • Inform visitors about biosecurity • Plan special changing, waiting, footbath and handwashing facilities away from production areas • Restrict personnel and visitor access to high-risk areas • Maintain visitor and personnel training records. • Consider biosecurity in the location and design of the farm • Establish a biosecurity zone and prevent unauthorised visitor access • Assess water, stock, personnel, equipment, and feed entries for biosecurity risks and take necessary precautions • Appoint a member of staff responsible for biosecurity • All staff must understand the farm's biosecurity plan and their responsibilities regarding its implementation • Provide staff with training on biosecurity	  

3.4.6. Feeds and Feeding

The aim is to minimise the risk of pests and diseases being transported within the farm, outside the farm, and away from the farm. Recommended practices are presented in Table 8.

Table 8. Recommended Practices in Feeds and Feeding

• Feed should be assessed for biosecurity risks • High-quality feed should be used • Feed containing aquatic organisms should be used with caution • Feed should be stored in clean, dry and cool areas. • Feed should be regularly checked for mould, pests and other unwanted organisms	

3.4.7. Wildlife, Scavengers and Vermin

The aim is to minimise the risk of wildlife, scavengers, vermin and domestic animals carrying harmful insects and diseases to the farm, within the farm or from the farm. Recommended practices are presented in Table 9.

Table 9. Recommended Practices in Wildlife, Scavengers and Vermin

• Control or remove predatory animals and wild animals • Remove or prevent predatory animals and wild animals from cage systems • Regularly inspect farms for biosecurity and take corrective measures when necessary • Keep records of biosecurity breaches and the preventive/corrective measures taken.	

3.4.8. Record Keeping

The aim is to Record all information necessary to trace and determine the origin of pest or disease. Recommended practices are presented in Table 10.

Table 10. Recommended Practices in Record Keeping

• Keep records of the health status of live animals • Keep records of all aspects of the biosecurity plan • Some important data that must be record: – stock transfers on to, within, and off farm – stock health – purchases and sales – monitoring and surveillance activities – testing and declarations – stocking densities – stock performance – feed schedules – environmental parameters (water and air temperature, water quality, pH, rainfall, dissolved oxygen levels) – stock disease and mortalities – treatments and vaccinations administered – cleaning and disinfection procedures – breaches in containment – security breaches (intruders and thefts)	  

3.4.9. Monitoring / Surveillance

The aim is to minimise biosecurity risks on farms by implementing appropriate monitoring and surveillance practices. Recommended practices are presented in Table 11.

Table 11. Recommended Practices in Monitoring / Surveillance

• Regularly check and monitor your stock. • Investigate any suspected health issues within your stock. • Observe and record any abnormalities and deaths among wild stock in the vicinity of your farm. • Ensure access to a qualified veterinarian. • Determine normal and abnormal farm mortality rates by monitoring deaths.	

3.4.10. Waste (Other Than Water)

The aim is to minimise the pest and disease risks associated with removal and disposal of farm waste. Recommended practices are presented in Table 12.

Table 12. Recommended Practices in Waste (Other Than Water)

• All waste must be assessed for biosafety risks • Biosafety rules must be applied in the storage, transport and disposal of waste • Waste storage areas must comply with biosafety rules • Waste disposal must be carried out at an approved waste site or waste management company	

3.5. Biofouling Management / Control in Marine Aquaculture

Biofouling on aquaculture infrastructure and stocks leads to increased maintenance costs and production losses (reduced growth rates/lower quality). Biofouling varies according to environmental factors such as salinity (increases at 25-36 ppt), water temperature (increases at ≥25°C), water depth (increases in shallow waters) and distance (increases in coastal areas) from the coast (Georgiades, et al., 2016).

In cage aquaculture, biofouling restricts water exchange, increases disease risk, and deforms cages and structures. The following methods are recommended for managing and controlling biofouling in cage aquaculture:

- Net change, cleaning and inspection (depending on the amount of biofouling)

- Nets with a mesh size of 5-10 mm should be changed every 15-45 days
- Nets with a mesh size of 14-16 mm should be changed every 4-6 months
- Nets with a mesh size of 16-18 mm should be changed every 8-10 months

- Antifouling paint application
- Biological control measures (encourage herbivorous species)
- Mechanical and chemical measures
    - Wash with water at 2000 psi pressure
    - Dry nets in the sun for 7 days
    - For soft-bodied organisms, 30-40 °C; for barnacles and shellfish, 50-70 °C;

    10 minutes at 65 °C (nylon nets may deteriorate above 71 °C)

    - Soak equipment in fresh water for at least 12 hours, preferably 72 hours
    - Treat with acetic acid, slaked lime, sodium hypochlorite, or alkaline ammonia (immersion in 2% bleach solution and 30 minutes of freshwater treatment immersion in 4% acetic acid solution and 10 minutes of freshwater soaking) (Georgiades, et al., 2016).

4. CONCLUSION

The marine aquaculture sector is considered one of the vectors playing a role in the transport of invasive alien species. Structures used in marine aquaculture systems, such as cage farming, mussel farming on stakes and ropes, and oyster farming on rack systems, create suitable habitats for the attachment, development and settlement of invasive non-native species (INNS). These invasive non-native species, which are intentionally transported for breeding purposes and unintentionally transported by other vectors or natural means, can become invasive species when suitable environments and conditions arise, thereby causing adverse effects on biological diversity, ecosystem services, human health and the economy. The Pacific oyster's invasion of all of Europe, the Mediterranean Sea, and the Black Sea is one of the most striking examples of this issue.

Aquaculture and marine aquaculture in particular, is a sector that continues to grow worldwide. Today, over 550 species have been cultured/farmed worldwide. The constant pursuit of development and cost-effectiveness in the sector has led producers to turn to the farming of alien/non-native species that do not occur naturally in their regions. Indeed, interest in alternative species has increased in recent times. As a result, the introduction/transportation of alien species is taking place in many different regions.

Although research and studies conducted worldwide have revealed that INNS is the greatest threat to the world's oceans after pollution, it is evident that robust legal regulations aimed at preventing and implementing measures specifically for marine aquaculture have not been established. The spread of INNS is a global phenomenon, and the scale of its impacts can range from local to transboundary or global. Therefore, the issue must be addressed at regional and global levels with consistent and coordinated approaches, and at national levels with numerous specific and targeted approaches.

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CHAPTER 2

TEMPORAL ANALYSIS OF SURFACE AREA CHANGES IN LAKE ERÇEK USING LANDSAT 8/9 OLI IMAGERY

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INTRODUCTION

Rising temperatures driven by global climate change are reducing water resources. The impacts are especially pronounced in mid-latitude, semi-arid countries such as Turkey, manifesting as shrinking lake surface areas and year-on-year declines in streamflow. Turkey is rich in lakes, which play a vital ecological and economic role. As key components of the hydrological cycle, lakes serve as natural reservoirs that store freshwater within terrestrial environments (Gao et al., 2012). These water bodies are complex systems in which evaporation and runoff processes are in dynamic balance. In particular, evaporation and runoff rates in lakes located within closed basins play a crucial role in determining variations in lake surface area. Lakes provide habitats for numerous species, particularly fish and waterfowl. The reduction in lake surface area has adverse effects on regional biodiversity. Owing to their rich biological diversity, lakes play a vital role in sustaining ecological balance as well as supporting the economic and cultural livelihoods of local communities. The reduction in the surface area of many lakes in recent years has been linked to factors such as climate change, increased evaporation, the mismanagement of water resources, and agricultural irrigation. (Schulz et al., 2020). As lake surface areas decrease, fish populations lose their habitats, waterfowl breeding grounds contract, and regional biodiversity declines. Moreover, the reduction in water volume leads to increases in salinity and temperature, which disrupt the balance of aquatic ecosystems and adversely affect their overall health. Lakes, whether freshwater or saline, are reservoirs of great ecological and socioeconomic importance. As key components of the hydrological cycle, they are complex systems that naturally store water while maintaining a dynamic balance between evaporation and runoff. Lakes support rich biodiversity, serving as habitats for numerous aquatic organisms, and are also vital for human activities such as drinking water supply and fisheries. However, in recent decades, rising temperatures, declining precipitation, and increased evaporation driven by global climate change have led to a significant reduction in lake surface areas. (Yao et al., 2023). This trend is particularly evident in lakes with closed-basin characteristics and in shallow lake systems. Declining water levels lead to shoreline retreat and disrupt the dynamic balance of these aquatic ecosystems (Wang et al., 2021). The changing shorelines of lakes resulting from water loss delineate the boundary between aquatic and terrestrial environments. Such changes indicate that the balance between water inflow and outflow has been disrupted, favoring increased water loss from the lake (Alesheikh et al., 2007). Therefore, the regular monitoring of surface area and shoreline changes in lakes is essential for understanding both the lake's water budget and the hydrological balance within its basin (Gao et al., 2012). Lakes are ecosystems with large surface areas. This makes monitoring their surface areas difficult. In particular, the fact that shoreline changes are not uniform across the lake makes it difficult to understand this change. In recent years, the most common method used to monitor lake surface areas is remote sensing. The capability of satellite imagery to detect water bodies across different spectral ranges has facilitated the development of algorithms that effectively distinguish water surfaces from land areas. Remote sensing techniques based on satellite data are among the most effective methods for monitoring changes in lake surface area and shoreline position. In particular, the contrast in reflectance between water and land surfaces enhances the accuracy of such analyses. Satellite sensors that are sensitive to various spectral bands can differentiate water and land surfaces according to their reflectance characteristics across the electromagnetic spectrum. Water bodies exhibit very low reflectance in the shortwave infrared (SWIR) and near-infrared (NIR) bands, while showing high reflectance in the visible green band. These spectral characteristics are commonly used to distinguish water surfaces from land areas. (Xu, 2006). The separation of water and land surfaces is a fundamental step in monitoring environmental change. The Normalized Difference Water Index (NDWI) and Modified Normalized Difference Water Index (MNDWI) are spectral indices developed to delineate lake surfaces and shorelines. NDWI enhances water–land discrimination by emphasizing the strong reflectance of water in the green band while suppressing the high near-infrared (NIR) reflectance of terrestrial surfaces (McFeeters, 1996). MNDWI, on the other hand, replaces the NIR band with the shortwave infrared (SWIR) band, producing more accurate results in built-up and sediment-affected areas (Xu, 2006). The biggest advantage of remote sensing lake monitoring is the ability to analyze long-term images from different dates and to monitor lakes with large areas with high accuracy at low cost (Donchyts et al., 2016). The continuous data stream provided by satellite missions such as Landsat, Sentinel, and MODIS has overcome many of the limitations associated with ground-based measurements in challenging terrain conditions. Gao et al. (2012) monitored large reservoirs and lakes worldwide using satellite remote sensing. The study utilized MODIS imagery to model the relationship between water surface area and water level and to estimate volume changes between 1992 and 2010. The research evaluated the role of reservoirs in the hydrological cycle and assessed the impacts of climate change and human activities on the water budget. It highlighted that the regular monitoring of lake and reservoir surface area changes using satellite data is crucial for understanding hydrological balance and improving water resource management. Pekel et al. (2016) mapped global surface water dynamics at a 30-meter spatial resolution using Landsat satellite imagery, capturing both spatial and temporal variations. They reported that changes in water bodies were largely driven by climate change, drought, and anthropogenic activities such as dam construction, water abstraction, and river diversions. Similarly, Tuygun et al. (2023) analyzed chlorophyll-a (Chl-a) concentrations and the trophic state index (TSI) in Lake Burdur from 1984 to 2021 using Landsat 5, 7, and 8 imageries. Their findings indicated a significant increase in Chl-a levels, particularly after the 2000s, suggesting that the lake had shifted toward a hypertrophic state. Monitoring changes in lake surface areas is therefore essential for future climate projections, identifying ecologically sensitive zones, and understanding the rate of environmental change. The Lake Van Basin is a closed drainage system that includes lakes of various sizes, the largest of which is Lake Van, Turkey's biggest lake. Lake Erçek is the second-largest lake within the basin. The water budgets of these lakes are primarily controlled by the balance between precipitation and evaporation. Water input is supplied by rainfall and snowfall throughout the year, whereas water loss occurs mainly through evaporation. Consequently, in years with high precipitation and low evaporation, the surface areas of the basin's lakes expand, while in years of low precipitation and high evaporation, they contract. In recent years, declining precipitation and above-average temperatures observed across Turkey have contributed to annual fluctuations in the surface area of Lake Erçek. Lake Erçek also represents one of the basin's most important fishing areas (Figure 1). Lake Erçek supports the second-largest stock of pearl mullet in the basin, following Lake Van, and therefore serves as an important fishing area. Commercial fishing activities in the lake are centered on the pearl mullet (*Alburnus tarichi* (Güldenstädt, 1814)), the only fish species inhabiting its waters. This species represents one of the main sources of livelihood for local communities and plays a significant role in the regional economy.

Figure 1. Commercial fishing activity in Lake Erçek

In addition to supporting fisheries, Lake Erçek provides suitable breeding and feeding habitats for a wide variety of bird species. The shallow zones of the lake offer ideal foraging and nesting areas for birds that visit the lake during different seasons (Figure 2).

Figure 2. Feeding and resting waterbirds along the shoreline of Lake Erçek

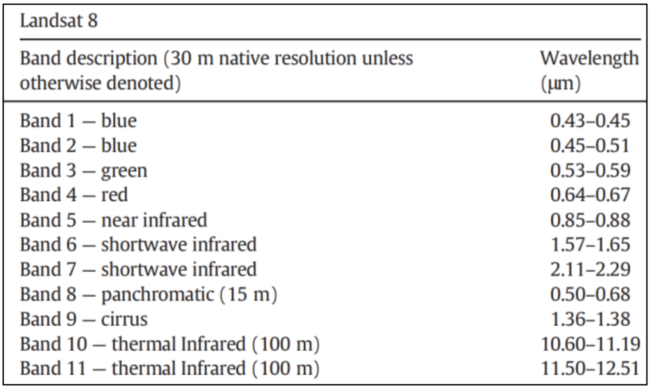
This study employed Landsat satellite imagery to assess changes in the surface area of Lake Erçek between 2019 and 2025. Remote sensing analyses revealed temporal trends in the lake's surface area and evaluated their relationship with climatic factors. The findings provide valuable data for the sustainable management of lakes and the preservation of the region's hydrological balance. Moreover, the study demonstrates that remote sensing is a powerful and effective tool for monitoring lake ecosystems.

MATERIALS AND METHODS

Lake Erçek, located east of Lake Van, is the second largest lake in the basin. Located approximately 30 km east of Van city center, it is a closed basin lake. Its water quality is similar to Lake Van's, with a salty and alkaline composition. While the lake is naturally devoid of fish, pearl mullet taken from Lake Van were introduced into the lake to create a stock. Seasonal streams and rainfall feed the lake. Therefore, the lake's water level and surface area are extremely sensitive to climatic changes. Rising temperatures and decreasing rainfall in the Van Basin, particularly in recent years, have caused a noticeable decrease in the lake's area. Its water quality is similar to Lake Van's, with a salty and alkaline composition. In 1985, pearl mullet fingerlings taken from Lake Van were introduced into the lake, which is devoid of native fish. Repeating this process has allowed the adapted pearl mullet to establish a stock

Figure 3. Location of Lake Erçek

The study utilized Landsat 8 Operational Land Imager (OLI) and Landsat 9 OLI-2 satellite imagery obtained from the United States Geological Survey (USGS). The Landsat 8/9 satellites acquire data across 11 spectral bands with a spatial resolution of 30 meters (Roy et al., 2014). These sensors provide long-term data continuity, making them particularly valuable for monitoring water surfaces, land-cover changes, and coastal ecosystems.



Landsat 8

Band description (30 m native resolution unless otherwise denoted)	Wavelength (μm)
Band 1 – blue	0.43–0.45
Band 2 – blue	0.45–0.51
Band 3 – green	0.53–0.59
Band 4 – red	0.64–0.67
Band 5 – near infrared	0.85–0.88
Band 6 – shortwave infrared	1.57–1.65
Band 7 – shortwave infrared	2.11–2.29
Band 8 – panchromatic (15 m)	0.50–0.68
Band 9 – cirrus	1.36–1.38
Band 10 – thermal Infrared (100 m)	10.60–11.19
Band 11 – thermal Infrared (100 m)	11.50–12.51

Figure 4. Landsat 8 spectral bands (Roy et al., 2014)

Landsat 8 and 9 satellite imagery used in this study was obtained from the USGS EarthExplorer platform (https://earthexplorer.usgs.gov/). The images, downloaded as Level-2 Science Products (Surface Reflectance), were atmospherically corrected using the LaSRC (Landsat Surface Reflectance Code) algorithm, which converts top-of-atmosphere reflectance values to bottom-of-atmosphere (BOA) surface reflectance. For each year, images with the lowest cloud cover (≤10%) were selected to construct a dataset representing the entire lake for the 2019–2025 period. August scenes were chosen for analysis because this month generally exhibits minimal cloud cover and corresponds to a period when water level fluctuations are most pronounced. All preprocessing and analysis of satellite imagery were conducted using ArcGIS Pro 3.5.4 software. The Modified Normalized Difference Water Index (MNDWI) was applied to delineate the lake's surface area from surrounding land. This method employs the green and shortwave infrared (SWIR) bands to create a strong contrast in reflectance between water and land surfaces, thereby enhancing the accuracy of water–land separation (Xu, 2006).

$$\text{MNDWI} = \frac{(Green - SWIR)}{(Green + SWIR)}$$

Green = Band 3 (0.53 – 0.59 μm)
SWIR = Band 6 (1.57 – 1.65 μm)

The resulting MNDWI raster images were classified and converted into polygon format to calculate the lake's surface area for each year.

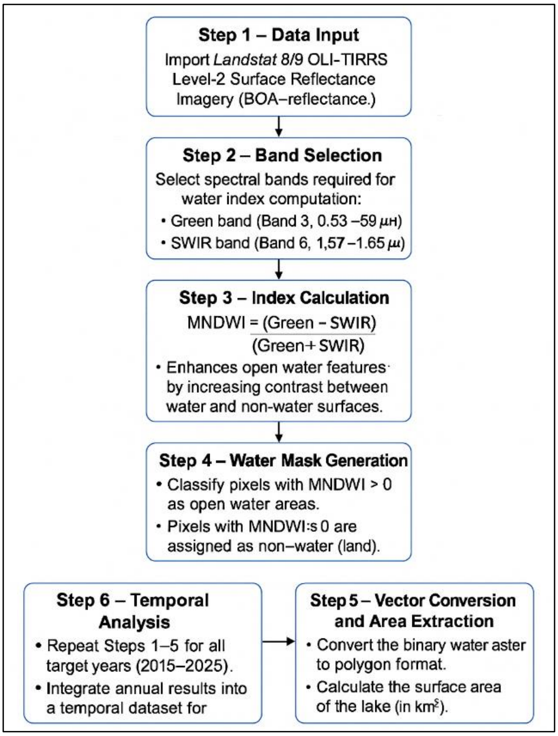


Figure 5. Data processing workflow for Lake Erçek

RESULTS

Because Lake Erçek has no natural outflow, its water level depends entirely on the balance between precipitation and evaporation. Consequently, the lake's water level and surface area are highly sensitive to climatic variations. In recent years, rising air temperatures and declining precipitation across the Van Basin have resulted in a noticeable reduction in the lake's surface area. This sensitivity underscores the strong dependence of the lake’s hydrological regime on climatic factors such as temperature, rainfall, and evaporation.

Table 1. Surface Area of Lake Erçek (2019–2025)

No	Year	Surface area (km^2)
1	2019	109.6
2	2020	109.4
3	2021	108.0
4	2022	106.6
5	2023	105.3
6	2024	106.7
7	2025	105.4

The analysis results derived from Landsat 8 and Landsat 9 OLI (Operational Land Imager) satellite imagery are presented in Table 1. According to the results, a clear decreasing trend was observed in the surface area of Lake Erçek between 2019 and 2025. At the beginning of the study period in 2019, the lake’s surface area was calculated as 109.6 km^2, representing the largest extent within the analyzed timeframe (2019–2025). By 2025, the lake’s surface area had decreased to 105.4 km^2, indicating a total loss of approximately 4.2 km^2 equivalent to a 3.8% reduction over six years. When annual surface area values are examined, the lake shows a consistent decrease from 2019 to 2023. A temporary increase occurred in 2024, when the lake’s surface area rose slightly from 105.3 km^2 to 106.7 km^2. This brief expansion likely reflects short-term seasonal or climatic variability rather than a long-term recovery trend. This increase is likely a short-term fluctuation caused by the specific seasonal conditions of that year. However, following this temporary rise, the lake’s area decreased again to 105.4 km^2 in 2025, continuing its overall downward trend (Figure 5). This trend indicates that, due to its closed-basin structure and endorheic nature, Lake Erçek is highly sensitive to climatic parameters such as precipitation, temperature, and evaporation. The absence of an outlet means that even small changes in water level are directly reflected in the lake’s surface area. High evaporation rates, particularly during the summer months, appear to be an important factor accelerating the observed reduction in surface extent. Consequently, fluctuations in the lake's surface area are primarily governed by climatic parameters. Water inflow to Lake Erçek occurs through rainfall and snowmelt, while outflow is limited solely to evaporation. In this study, surface area changes were determined exclusively from Landsat 8/9 OLI imagery using the Modified Normalized Difference Water Index (MNDWI) method. Incorporating climatic data such as precipitation and evaporation into future studies will help clarify the causes of the observed increases and decreases in the lake's surface area, providing a more comprehensive cause-and-effect understanding of these changes.

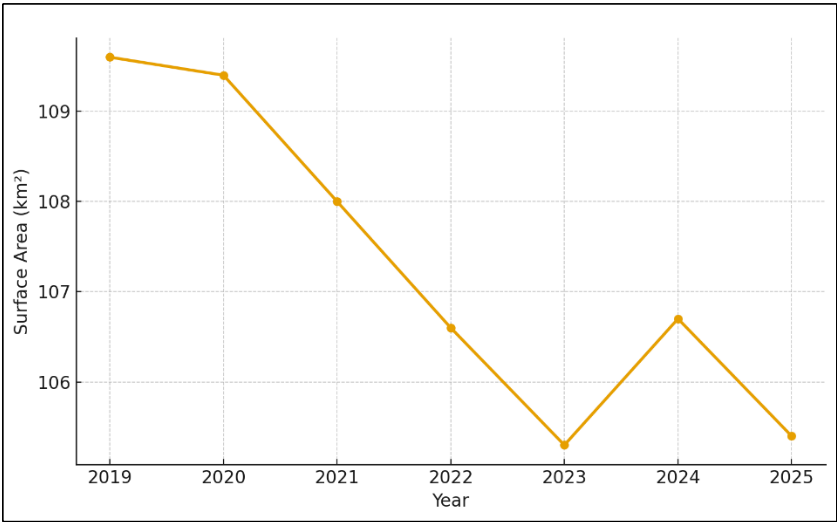


Figure 6. Yearly Surface Area Trend of Lake Erçek

The excess of evaporation over precipitation within the basin directly contributes to the reduction of the lake's surface area. High evaporation rates, particularly during the summer months, are considered a major factor accelerating the decline in the lake's surface extent.

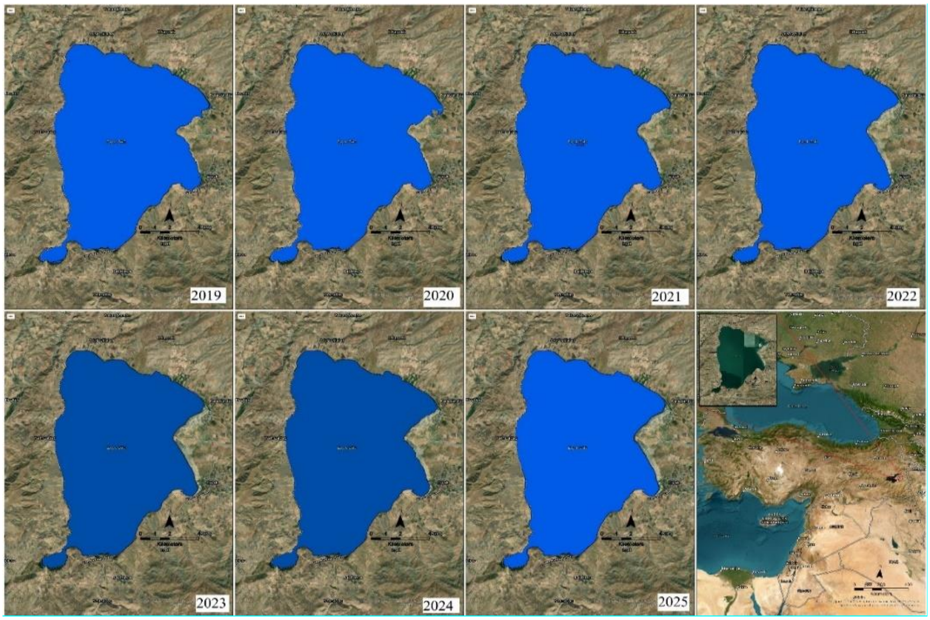


Figure 7. Yearly Variation in Lake Erçek Surface Area (2019–2025)

DISCUSSION

In this study, changes in the surface area of Lake Erçek were analyzed using Landsat 8 and 9 OLI satellite imagery for the period between 2019 and 2025. The results revealed an overall decreasing trend in the lake's surface area throughout the study period. The surface area, which was 109.6 km² in 2019, declined to 105.4 km² by 2025. This reduction of approximately 4.2 km² corresponds to a 3.8% decrease in the total surface area. When the interannual variation is examined, a continuous decline is observed from 2019 to 2023, followed by a short-term increase in 2024. However, the temporary increase observed in 2024 did not reach the maximum surface area recorded in 2019, and the decline in lake extent continued into 2025. Analysis of the lake's surface area between 2019 and 2025 indicates that, as a closed-basin system, Lake Erçek is highly sensitive to evaporation and precipitation, which are the primary components governing its water balance. The absence of an outlet means that even small variations in the equilibrium between evaporation and precipitation are directly reflected in the lake's surface area. Consequently, the hydrological balance of the lake is considered highly vulnerable to climatic fluctuations. This study is based solely on surface-area estimations derived from Landsat imagery; meteorological variables such as precipitation, evaporation, and temperature were not included in the analysis. Therefore, broader datasets are required to more precisely distinguish the extent to which surface-area fluctuations are driven by climatic processes, human influences, or other factors. The shrinkage trend observed in Lake Erçek is consistent with patterns reported in other closed-basin lakes across Turkey. Similar declines in water levels have been documented in other closed-basin systems such as Lake Burdur and Lake Acigöl (Ormeci and Ekercin, 2007; Sarp and Ozcelik, 2017). Accordingly, the reduction in Lake Erçek's surface area between 2019 and 2025 can be interpreted as a local manifestation of hydrological drought driven by climate change. From an ecological perspective, the contraction of the lake's surface area may lead to increased salinity, alterations in planktonic community structure, and the loss of habitats for waterfowl. In particular, the disappearance of shallow littoral zones, which are critical feeding and breeding areas for juvenile pearl mullets, poses a serious ecological concern. Future research should incorporate long-term meteorological parameters such as precipitation, temperature, and evaporation alongside satellite observations to more comprehensively evaluate the drivers of surface area fluctuations. Furthermore, monitoring the discharge of inflowing streams and assessing land-use changes through remote sensing analyses would provide a more accurate understanding of the lake's water budget dynamics.

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CHAPTER 3

INVASIVE NON-NATIVE SPECIES AND BIOSECURITY IN RECREATIONAL BOATING

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INTRODUCTION

The seas are under threat from overexploitation of resources, destruction of marine habitats, pollution, climate change, and biodiversity loss. Among these threats, marine invasive non-native species (INNS), which contribute to biodiversity loss, are expected to be among the most significant in the future. Recreational boating is a largely unregulated vector of INNS, contributing to both their initial introduction and secondary spread. Therefore, marinas and harbours are often hotspots for non-native species experiencing high propagation pressure (Vye et al., 2020).

Biofouling is the accumulation of microorganisms, plants, algae, or small animals on boat hull surfaces. Biofouling is a pathway that helps INNS to spread from one geographical area to another, where they can become invasive and have serious impacts on their adopting habitat (GloFouling, 2021). Boats of any type, size or class can have biofouling attached to their hulls (Carlton, 1985; Carlton, 2003). After an INNS' initial arrival, settlement is first dependent on surviving in the new environment, and then on their reproductive success (Galil et al., 20217). Once a new marine species establishes in a locality, eradication is often unfeasible as it is nearly impossible, thus prevention is universally considered the best management option for INNS (Ulman, 2018).

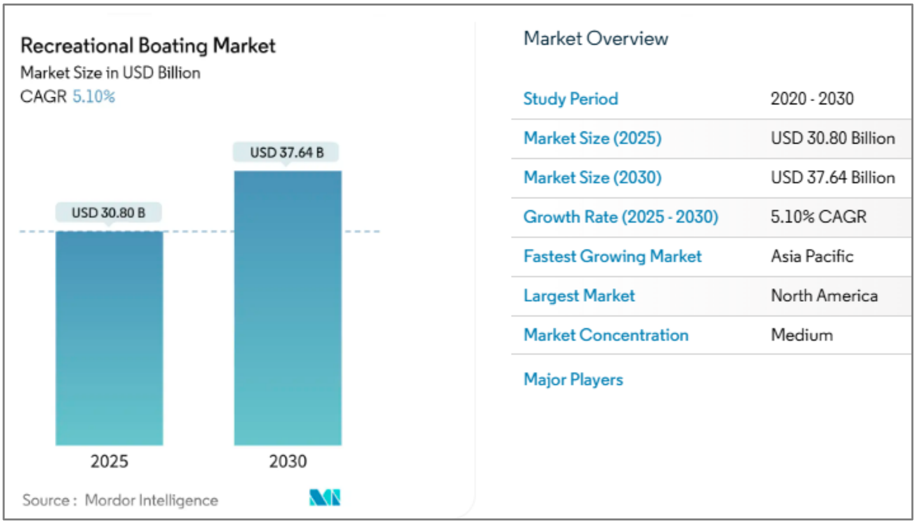
Biosecurity in recreational boating refers to all measures to be taken to minimize the risk of biofouling accumulation and the introduction of aquatic INNS. Actively minimising biofouling of recreational boats can greatly reduce the risk of spreading invasive alien species, while also improving boats' fuel efficiency, operating speeds and manoeuvrability (IMO, 2012). Prevention measures, such as biosecurity, are essential to reducing the introduction, spread of INNS, and are central to international and national INNS policy. A code of conduct has been developed with the motto "Check, Clean, Dry" based on the principle of volunteerism to prevent Invasive Non-Native Species in Recreational Boating around the world (Council of Europe, 2016).

2. WORLD RECREATIONAL BOATING INDUSTRY

There is no consensus on the terminology used to describe the types of boats used in recreational boating. Certain sections of EU legislation use terms such as "recreational boat" or "private pleasure craft" within the context of recreational boating. Recreational boating also includes the use of water recreational vehicles such as beach or sled-drawn sailboats, inflatable boats, and personal watercraft, such as windsurfers. Boats used for recreational boating can be small or large, sail- and/or power-driven, and used on inland waters and/or at sea. Recreational boating can range from close to shore to the ocean (Council of Europe, 2016).

- Recreational boating is a family-friendly activity regardless of age, gender, or mobility.
- It preserves maritime culture and traditions and promotes awareness of nature and its powers.
- Recreational boating has evolved from the traditional concept of "sea and sun" into a broad economic sector encompassing yacht tourism, yacht/boat charters, and water sports.
- Recreational boating provides an exceptional opportunity to maintain or revitalize the economies of smaller harbours, marinas, and moorings in rural areas, generating wealth and employment.
- Recreational boating has a significant commercial base, particularly in inland and coastal areas, generating high tourism returns and encouraging local hospitality, transportation, and construction.
- Boats choose less-visited locations away from cruise ship routes. Therefore, the socio-economic impact can be felt more broadly.
- While recreational boating activity fluctuates seasonally, it does not stop during the off-season, and boat maintenance continues to contribute to the economy.

The Recreational Boat Market is projected to reach $30.80 billion by 2025. This figure is expected to reach $37.64 billion by 2030, at a CAGR of 5.10% during the forecast period (2025-2030) (Figure 1). Despite inflationary pressures, participation in water sports is expected to increase, affluent buyers are moving to larger yachts, and marina infrastructure in Asia is expected to expand rapidly. North America holds the largest market share in the global recreational boat market, while Asia Pacific is reported to be the fastest-growing market (Mordor Intelligence, 2025).



Source : Mordor Intelligence

Market Overview

Study Period	2020 - 2030
Market Size (2025)	USD 30.80 Billion
Market Size (2030)	USD 37.64 Billion
Growth Rate (2025 - 2030)	5.10% CAGR
Fastest Growing Market	Asia Pacific
Largest Market	North America
Market Concentration	Medium
Major Players	

Figure 1. World Recreational Boating Market Size and Share

- Outboard motor boats lead the boat market with a 42.10% share in 2024. They are projected to grow at a compound annual growth rate (CAGR) of 7.80% through 2030.
- Fiberglass/GRP boats held the largest market share, accounting for 65.55% in 2024. However, boats made from advanced composites are projected to be among the fastest growing, with a compound annual growth rate (CAGR) of 10.1% through 2030.
- Boats under 20 ft accounted for 56.20% of the boat market in 2024. Boats over 50 ft are projected to have a compound annual growth rate (CAGR) of 8.90% between 2025 and 2030.
- İnternal combustion engines held a 92.10% share of the recreational boat market in 2024. Electric/hybrid propulsion systems are expected to grow at a CAGR of 12.50% to 2030.
- Water sports held a 38.15% share while cruising and coastal tourism are forecast to expand at 9.20% CAGR to 2030 (Mordor Intelligence, 2025).

It is estimated that there are at least 140 million active boating/yachting and water sports participants worldwide, including approximately 100 million in the United States and Canada, 36 million in Europe, and over 5 million in Australia. When looking at the leading countries in the global boating/yachting sector, the United States and Canada rank first, followed by European countries. In Europe, Germany, the United Kingdom, and France are the most prominent (Mordor Intelligence, 2021).

Europe holds a significant position in the boat and yacht sector, with 3,600 companies, 82,000 direct employees, 3.5 million indirect employment, and a total indirect business volume of €566 billion. With approximately 70,000 km of coastline, 37,000 km of inland waters, and a high level of economic development, it is reported that there are currently over 6 million boats and yachts in European waters. Furthermore, approximately 10,000 marinas have mooring capacity for over 1 million boats (European Boating Industry, 2021; Grand View Research, 2021).

The Mediterranean Basin is one of the global focal points for marine tourism. Marine tourism in the Mediterranean Basin is developing eastward, starting in the Western Mediterranean. However, due to the concreting and marine pollution seen along the coasts of Spain, France, and Italy, as well as the influence of radical movements and the closed societies of countries like Morocco, Algeria, and Tunisia, yachting routes have shifted to the relatively unexplored Eastern Mediterranean coast (Göbel, 2018).

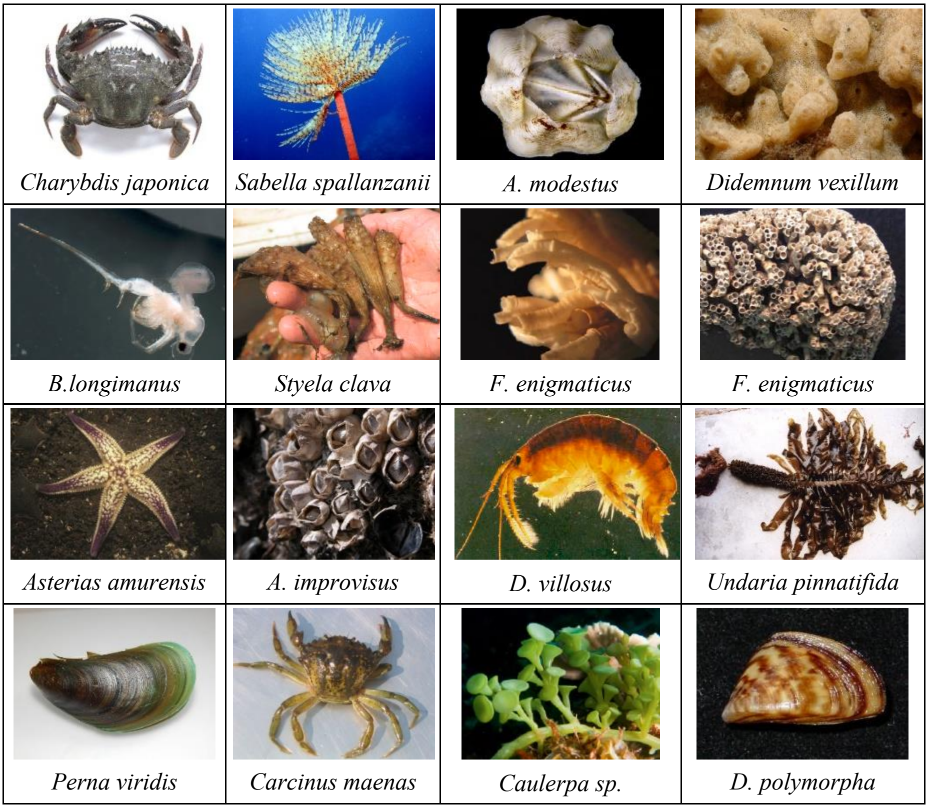
3. INVASIVE NON-NATIVE SPECIES IN RECREATIONAL BOATING

If a species is endemic, it is classified as native. If a species overcomes a barrier by human activities or enters a new habitat, then it is an alien/non-native species, not all alien species are perceived negatively or pose a threat to natural biodiversity (Carlton, 1996). When these alien/non-native species have a negative impact on biodiversity, ecosystem services, human health or economic impact, they are referred to as Invasive Non-native Species (INNS). However, the IYT definition adopted here assumes that the species is introduced through human intervention, whether intentionally or unintentionally (EUR-Lex, 2025). Therefore, new species transported and established in new habitats via recreational boats and yachts are considered invasive alien species if they have biodiversity, ecosystem services, human health, or economic impacts.

- Recreational boating is an important vector contributing to both the initial introduction and secondary spread of potential INNS (Vye, 2020).

- The main way IYT spreads in recreational boating is through boat hulls, propellers, bilges or engine cooling water systems (Council of Europe, 2016).
- Recreational boating is thought to account for over a third of recorded non-native introductions in England (Gallardo and Aldridge, 2013).
- There are more than 1000 alien species in the Mediterranean and it is one of the most invaded seas (GloFouling, 2021).
- The persistence of INNS depends first on their survival in the new environment and then on their reproductive success (Galil et al., 2017).
- INNS often replace native species (especially in artificial environments) and are almost impossible to eradicate from the ecosystem in which they established (Occhipinti-Ambrogi, 2007; Galil et al., 2015).

Some marine species have been introduced, either intentionally or unintentionally, because of human activities. When the adopted habitat shares similar characteristics, introduced alien species have a good opportunity to adapt and thrive. Due to certain competitive advantages, such as the absence of natural predators, some alien species have become dominant and disrupt the biodiversity of their newly adopted habitats (GEF-UNDP-IMO, 2022). These are often referred to as Invasive Non-Native Species (INNS). INNS range from fish, crabs, and mussels to seaweed and plants, to microscopic pathogens (Figure 2). This immense diversity can make invasive species difficult to detect (Payne et al., 2015; BIMCO, 2021). Eradicating INNS has proven difficult in most cases, and the best chance of success usually comes with early diagnosis, further highlighting the need for a preventative approach.



Charybdis japonica *Sabella spallanzanii* *A. modestus* *Didemnum vexillum*

B.longimanus *Styela clava* *F. enigmaticus* *F. enigmaticus*

Asterias amurensis *A. improvisus* *D. villosus* *Undaria pinnatifida*

Perna viridis *Carcinus maenas* *Caulerpa sp.* *D. polymorpha*

Figure 2. Common Invasive Non-Native Species Introduced by Biofouling

4. BIOSECURITY IN RECREATIONAL BOATING

Biosafety in recreational boating refers to all measures to be taken to minimize the accumulation of biofouling on boats and the associated risk of the introduction and spread of aquatic Invasive Non-Native Species (INNS) (Council of Europe, 2016). In short, biosecurity in recreational boating can be expressed as biofouling management.

4.1. Biofouling and Management in Recreational Boating

The International Maritime Organization (IMO) defines biofouling, often referred to as a hull fouling (Figure 3)*, as the undesirable accumulation of aquatic organisms such as micro-organisms, plants and animals on surfaces and structures immersed in or exposed to the aquatic environment* (GEF-UNDP-IMO, 2022).

Figure 3. Boat Hull with Heavily Biofouled

4.1.1. Biofouling Types

The types of biofouling are addressed in international guidelines using different approaches, categorized as soft biofouling and hard calcareous biofouling (Table 1), micro- and macro (Figure 4) or grading (Table 2). These definitions and grading's presented below (Cook et al., 2014; BIMCO, 2021; DAFF, 2024). Biofouling begins the moment a hull is placed in water, and its surface is rapidly colonized by various marine species, such as diatoms, bacteria, and microalgae. These species form a biofilm, commonly referred to as a slime layer. This is followed by the gradual proliferation and growth of larger macrofouling species, such as other algae, non-motile animals (sponges, anemones), motile benthic animals (worms, shrimp, crabs), and parasites (Figure 5) (GEF-UNDP-IMO, 2022).

Table 1. The Biofouling Types

Soft Biofouling		Hard Calcareous Biofouling
Mikro	**Makro**	**Makro**
Slime	Soft corals	Barnacles
	Sponges	Mussels
	Hydroids	Tube worms
	Anemones	Bryozoa
	Algae	Oysters
	Tunicates	

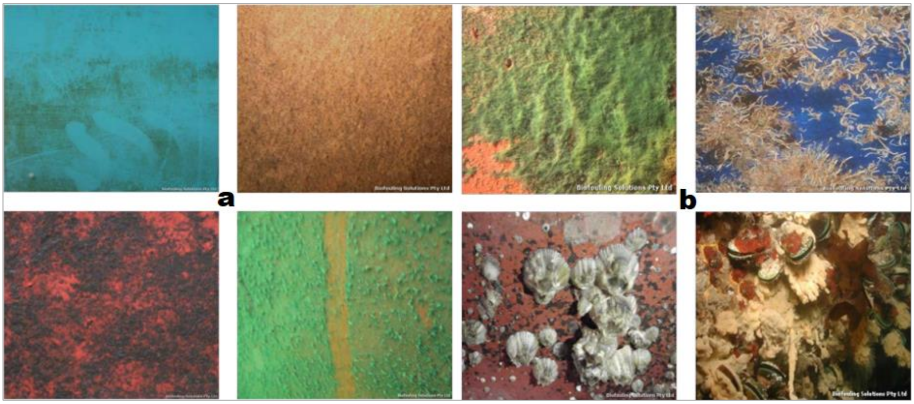


Figure 4. Micro (a) and Macro (b) Biofouling

Table 2. Monitoring and Rating Signs of Biofouling on the Boat Hull

Rank	Description	Visual estimate of biofouling cover
0	No visible fouling. Hull entirely clean no biofilm* on visible submerged parts of the hull.	Nil
1	Slime fouling only. Submerged hull areas partially or entirely covered in biofilm, but absence of any plants or animals.	Nil
2	Light fouling. Hull covered in biofilm and 1–2 very small patches of one type of plant or animal.	1–5 % of visible submerged surfaces
3	Considerable fouling. Presence of biofilm, and fouling still patchy, but clearly visible and comprised of either one or more types of plant and/or animal.	6–15 % of visible submerged surfaces
4	Extensive fouling. Presence of biofilm and abundant fouling assemblages consisting of more than one type of plant or animal.	16–40 % of visible submerged surfaces
5	Very heavy fouling. Many different types of plant and/ or animal covering most of visible hull surfaces.	41–100 % of visible submerged surfaces

* A thin layer of bacteria, microalgae, detritus, or other particles

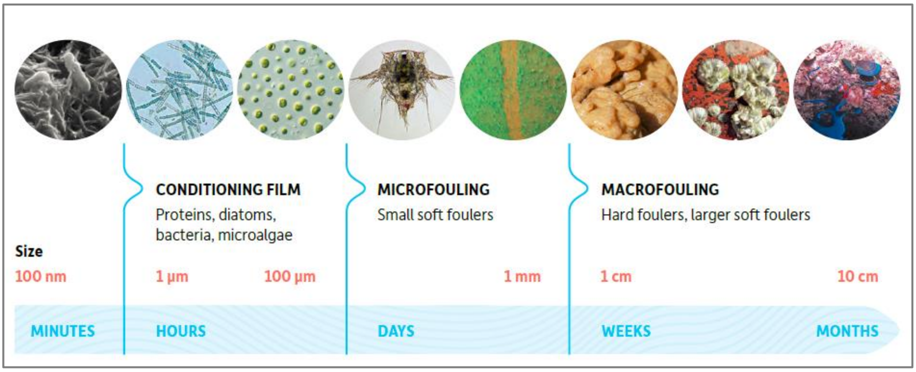


Figure 5. How Fast Biofouling Organisms Can Grow on a Surface

Microfouling refers to a film composed of bacteria, diatoms, and microscopic organisms and the mucus they produce. It poses relatively no biosecurity risk. Macrofouling refers to a variety of large, multicellular organisms visible to the human eye, such as balanids, tubeworms, mussels, oysters, algal mats, and other large, attached, or motile organisms. Macrofouling formations represent a greater biofouling and associated biosecurity risk. Boats must be beached, cleaned, maintained, repaired, and ideally, have an antifouling coating applied annually (DAFF, 2024).

4.1.2. What Affects Amount/Growth of Biofouling on Boat?

Aquatic organisms can be transferred to different locations as biofouling (Figure 6). They can be harmful and invasive in areas where they are not naturally found. The transfer of invasive aquatic organisms can threaten marine environments, human, animal, plant life, and economic and cultural activities. Once invasive aquatic species have established themselves in a new habitat, eradication is often impossible (IMO, 2012; GEF-UNDP-IMO, 2022).

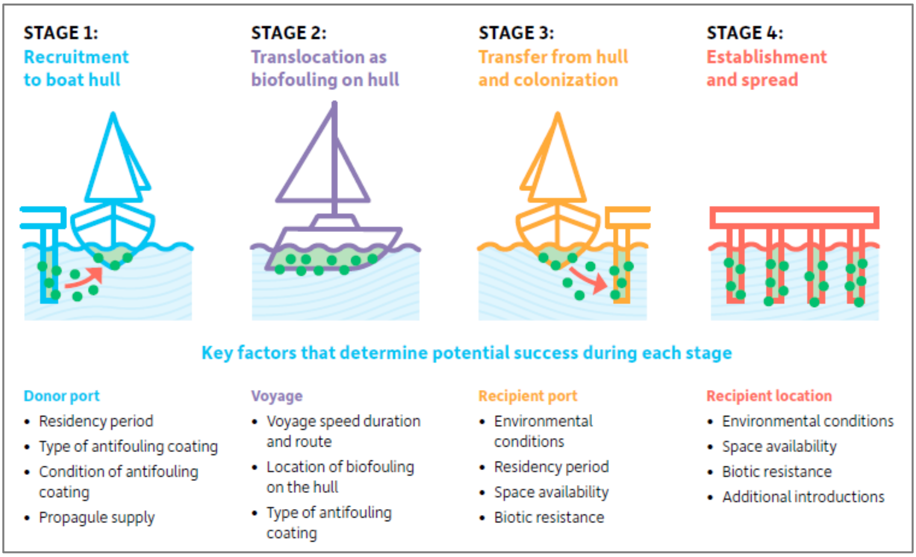


Figure 6. Stages in the Transfer and Establishment of INNS through Hull Biofouling

The amount and growth of biofouling on a boat are affected by environmental factors (Table 3), hull condition, niche areas, antifouling systems (Table 4), the boat's operating characteristics and design/structure (Table 5) (Council of Europe, 2016; DAFF, 2024). In some regions, marinas have been shown to host more Invasive Non-Native Species (INNS) than commercial ports. This may be explained by the longer time a recreational vessel spends in a marina or harbour than a commercial vessel. For sailboats and slower motorboats, the slower speed through the water creates more opportunities for INNS to remain attached to the hull and successfully establish in new areas with similar climatic conditions (GEF-UNDP-IMO, 2022). Factors affecting biofouling are presented below.

Table 3. Environmental Factors Affecting Biofouling.

Environmental Factors	Specific Related Risks
Salinity	The amount of biofouling and the risk of growth increases in waters of 25-36 ppt.
Water temperature	In waters where the water temperature is >25°C, biofouling and growth are observed throughout the year and slow down below 15°C.
Water depth and distance from shore	Water depth is low and the risk of biofouling increases in coastal areas.

Table 4. Hull Condition, Niche Areas and Anti-Fouling System Affecting Biofouling.

Boat Hull Condition Nich Areas Anti-Fouling Sytem	**Specific Related Risks**
Hull roughness	High roughness provides an ideal settling surface for biofouling organisms.
Type, age and application of antifouling coating	Depending on the type and application of the antifouling coating, its effectiveness decreases with age and the decrease in the active substance.

Table 5. Operating and Design/Structure of the Boat Affecting Biofouling

Operating and Design/Structure of the Boat	**Specific Related Risks**
Operation rate, anchorage/cruise time and location	Depending on local environmental conditions and AFS, long anchorage/marina residency may increase biofouling (calcareous organisms are established within 5-8 days).
Speed	Biofouling increases at low speeds
Damage to the antifouling coating (AFS)	Boats that frequently visit harbours and/or areas with high tides are more likely to have their anti-fouling coating damaged by friction.
Areas susceptible to biofouling	Rudders, propellers, propeller shafts and niche areas are areas more susceptible to biofouling.

4.1.3. The “Check, Clean and Dry” Protocol

To date, it has not developed a specific guide or code of conduct for recreation boating. Instead, it has published the "Code of Conduct for Recreational Fishing and Invasive Alien Species" (Council of Europe, 2016) in a general framework. It has adopted "Check, Clean, Dry" procedures based on public participation, education, awareness-raising, and voluntary work.

Check:

Check boats, equipment, and clothing for the presence of living plants and animals. Pay particular attention to areas that are damp/wet or difficult to inspect/control (Figure 7).

Figure 7. Routine Inspection After Use of Recreational Boats

Clean:

Clean and rinse all equipment with fresh water. Apply an antifouling coating annually. Remove any visible contamination and dispose of waste in a trash receptacle or other disposal area, not back into the water (Marine Pests, 2009; IMO, 2012; Council of Europe, 2016):

- Ideally, remove your boat from the water for cleaning at least once a year.
- Remove/clean any biofouling (e.g., algae and crustaceans) from the boat or trailer.
- Drain the hull, piping, and outboard motors.
- Use pressurized water to clean the hull and equipment (Figure 8).
- After use, treat wetsuits and other equipment with water at 45°C for approximately 15 minutes. This can kill some important INNS.
- If possible, remove boats to a single point on land, in facilities where biosecurity measures and practices (cleaning methods that capture and eliminate biological, chemical, and physical residues) can be implemented.

Figure 8. Use of Pressurized Water to Remove Biofouling on Boats.

The following practices are very important in boat hull cleaning (MAF, 2009; New York State Department of Environmental Conservation, 2025; DAFF, 2024):

- The ideal method for cleaning boat/yacht hulls is using pressurized (50-450 bar) hot water (60°C).
- The use of hot water (60°C) is especially important in niche areas of boats/yachts.
- The use of hot water (60°C) shortens the maintenance and lay-by times of boats/yachts in marinas.
- The use of hot water is an effective method for biosecurity and elimination of invasive alien species in marinas.
- It is recommended to use environmentally friendly, natural materials in abrasive blasting application (Mohit et al., 2023).

Niche areas are areas susceptible to biofouling due to varying water flow conditions, wear or damage to the antifouling coating, or inadequate coverage (Figure 9 and 10). For example, a rudder and any hull protrusion or recess can create turbulent flow with a high erosion factor. Niche areas: deck fittings, anchor well and anchor, hull surface, trailer, water inlets and outlets, propeller and shaft, burley bucket, sonar tubes or echo sounder booths, cooling pipes, sewage and bilge tanks, keel, rudder and marlin board and transducers

(Aquaculture Victoria, 2025). An antifouling coating, such as cathodic protection (CP) anodes, does not protect some niche areas. Biofouling associated with these anodes can be minimized if they are installed flush, a rubber-backing pad is placed between the anode and the housing, or the gap is filled. If your anodes are attached with countersunk bolts, the niches should be caulked. If your vessel is equipped with a Marine Growth Prevention System (MGPS), which uses electronic or chemical injections to minimise biological fouling in internal seawater systems, its correct operation should be checked regularly (Marine Pests, 2009; IMO, 2012; Council of Europe, 2016).

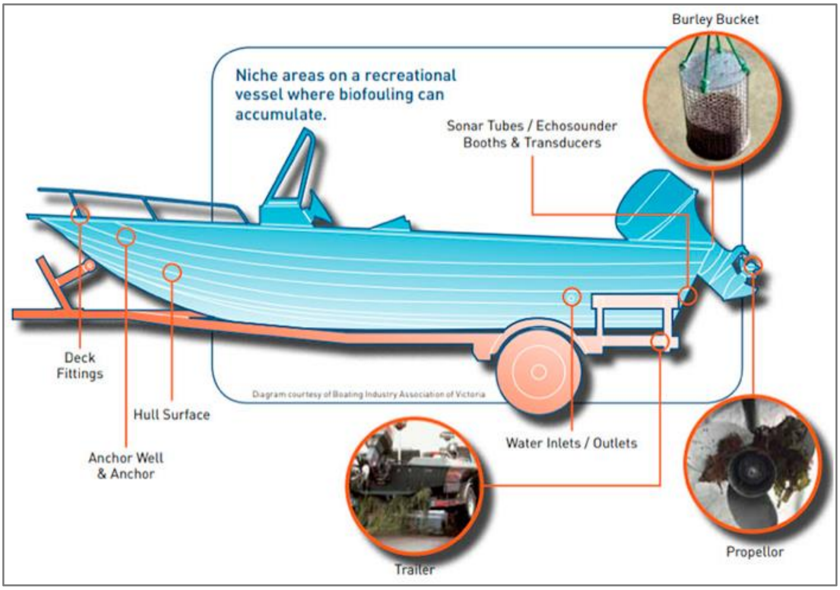


Diagram courtesy of Boating Industry Association of Victoria

Figure 9. Biofouling Niche Areas on a Trailered Recreational Boat

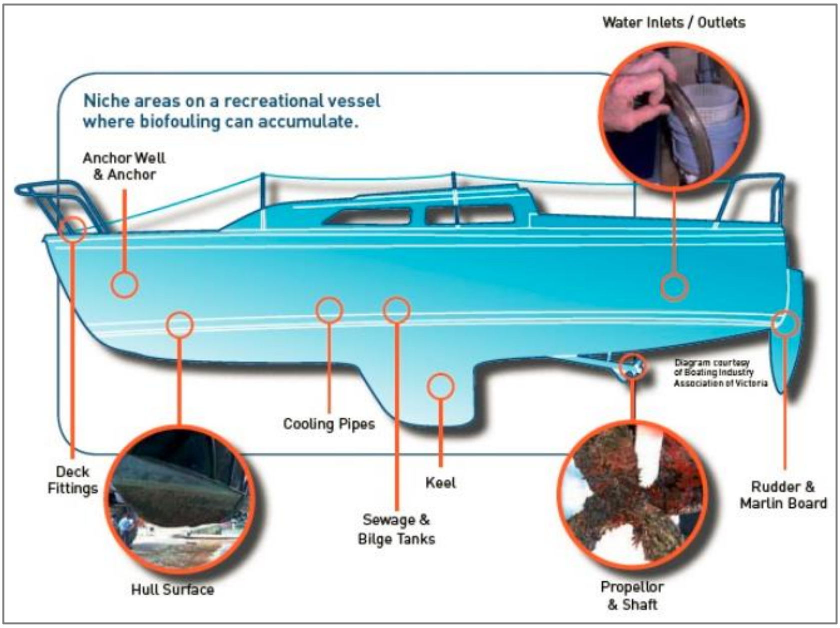


Figure 10. Biofouling Niche Areas on a Moored Recreational Boat

<u>Dry:</u>

When beaching boats, trailers, etc., and before leaving the site, drain and protect all equipment and any parts that could hold water, including any water accumulated in the bilges (Figure 11). Dry clothing and equipment as long as possible before using elsewhere. Drying is important! Some invasive alien species can survive for more than two weeks in humid conditions.

Figure 11. Recreational Boat Protected in Appropriate Conditions

4.1.4. Anti-Fouling System

Anti-Fouling System (AFS), any coating, paint, surface treatment, surface or device that is used on a boat to control or prevent the attachment of unwanted organisms as biofouling (GEF-UNDP-IMO, 2022).

- Reducing biofouling on boats minimizes the risk of spreading INNS.
- The best way to prevent biofouling is with appropriate AFS
- Routine annual hull cleaning and AFS increase fuel efficiency.
- The manufacturer's instructions should be followed when applying AFS.

The following points should be considered when applying AFS:

- The AFS application interval should be selected based on the vessel's use, location, type, and legal requirements.
- AFSs may not be effective against all species; therefore, a "Check, Clean, Dry" approach should be adopted along with routine applications.
- On regularly used boats, biofouling will be less and the effectiveness of the AFS will be increased.
- AFSs containing tributyltin (TBT) should not be used.
- TBT is toxic and consumption of affected seafood is harmful to humans.

When removing AFS:

- Choose facilities that have a closed-circuit discharge system at the application site or that contain toxic spills in a tarpaulin to prevent them from entering the water (sea, sewer, river).
- Use dust-free vacuum sanding or wet abrasion to reduce toxic dust and ensure user health.
- While the boat is on stilts, only remove the contamination, not the underlying paint.

When applying AFS:

- Choose the coating with the lowest biocide and copper levels. If possible, use water-based or low-VOC coatings.

- Apply the correct amount of coating and be mindful of paint spills/runs.
- Dispose of used brushes, rollers, and empty antifouling paint cans as hazardous waste.

5. CONCLUSION

- Oceans face threats such as overexploitation of resources, destruction of marine habitats, pollution, climate change, and biodiversity loss. Invasive non-native species (INNS) are among the most significant drivers of these threats.
- Recreational boating is a vector for INNS at global scales.
- Biofouling is the accumulation of animals and plants on boat hull surfaces and is a major pathway for the introduction of INNS.
- Actively minimising biofouling of recreational boats can greatly reduce the risk of introduction, while also improving boats' fuel efficiency, operating speeds and manoeuvrability.
- Biosecurity is crucial to reduce the introduction and spread of INNS and should be at the heart of international and national INNS policy.
- There are no legal measures in place to combat INNS within the sector. Instead, voluntary codes of conduct are being promoted.
- Collaborate with relevant government agencies and recreational boating associations and/or marinas regarding the implementation and adoption of the Code of Conduct (Guidelines) and its impact on recreational boating.
- Marinas are hotspots for INNS but biosecurity practices are generally voluntary.
- Marina operators consider the obstacles to biosecurity as cost and effectiveness. Considering that biosecurity approaches in combating IYT bring some advantages to marina management, operators can be more participatory.
- For this purpose, the "Check, Clean, Dry" Protocol adopted all over the world should be expanded. Information, education/training, and awareness of the regulations should be encouraged within the sector.

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CHAPTER 4

DETERMINATION OF TISSUE AND GENETIC EFFECTS CAUSED BY PESTICIDES IN FISH

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INTRODUCTION

The widespread use of pesticides in agriculture and public health has led to the contamination of aquatic ecosystems, raising global concerns about their ecological and toxicological consequences. Among aquatic organisms, fish serve as critical indicators of environmental health due to their sensitivity to waterborne pollutants, including pesticides. These toxic substances can reach aquatic habitats through agricultural runoff, industrial discharge, or atmospheric deposition, ultimately affecting non-target organisms such as fish (Kumar et al., 2025; Haque et al., 2025).

Fish possess highly vascularized gills and thin epithelial tissues that facilitate direct and efficient absorption of dissolved chemicals from the water, making them particularly vulnerable to xenobiotics. Once pesticides enter the fish's body, they can accumulate in vital organs such as the liver, kidneys, brain, and gills, triggering a cascade of physiological, cellular, and molecular changes (Bayır et al., 2025). Numerous studies have documented tissue damage in fish following exposure to common pesticides. For example, exposure to cypermethrin and malathion—both widely used insecticides—has been linked to hepatocyte necrosis, lamellar fusion in gills, and glomerular shrinkage in the kidneys of *Channa punctata* (Kumar et al., 2025). Similarly, glyphosate-based herbicides have been shown to cause anemia, leucocytosis, and liver degeneration in *Clarias gariepinus* (Ajisebiola et al., 2025). These observations are not only limited to acute exposure; chronic and sublethal doses can also lead to long-term physiological stress, reduced growth, immunosuppression, and reproductive impairment (Tahir et al., 2025). Beyond the tissue level, there is growing interest in understanding genotoxic and transcriptomic responses of fish to pesticide exposure. Molecular-level changes such as DNA strand breaks, micronucleus formation, and altered expression of detoxification genes (e.g., *GST*, *SOD*, *CAT*) have been reported in several fish species, including *Danio rerio*, *Labeo rohita*, and *Oreochromis niloticus* (Bayır et al., 2025; Zahan Etti, 2025). These genetic endpoints offer early-warning biomarkers and are particularly useful for detecting damage before overt symptoms appear.

The mechanisms through which pesticides cause damage often involve oxidative stress, wherein the balance between the production of reactive oxygen species (ROS) and the organism’s antioxidant defenses is disrupted. This can result in lipid peroxidation, protein oxidation, and DNA damage, culminating in apoptosis or carcinogenesis if left unchecked (Rasal et al., 2025). Moreover, the use of tools like Integrated Biomarker Response (IBR) allows researchers to synthesize multiple biochemical and genetic endpoints into a single index, enhancing the ecological relevance of toxicity assessments (Tahir et al., 2025). Fish-based ecotoxicological assessments play a pivotal role in environmental monitoring and policymaking. Given the growing number of pesticide compounds and their diverse modes of action, it is crucial to continuously update our understanding of how these chemicals affect aquatic life at both morphological and genetic levels. With advances in histological techniques, molecular biology, and omics-based tools, researchers can now explore pesticide toxicity with unprecedented resolution, aiding both conservation efforts and public health policies.

In summary, the determination of tissue and genetic effects of pesticides in fish is a multidimensional approach that bridges environmental science, toxicology, and genomics. It not only deepens our understanding of fish physiology under stress but also contributes to broader efforts in ecosystem protection and sustainable pesticide regulation.

2.TISSUE-LEVEL EFFECTS OF PESTICIDES IN FISH

Pesticide exposure in aquatic ecosystems often results in profound histopathological and physiological damage in fish. These tissue-level effects are widely used as biomarkers in environmental toxicology because they provide clear and quantifiable evidence of pollutant-induced stress. The primary target organs for pesticide-induced damage include the gills, liver, kidneys, spleen, and intestines, due to their high metabolic activity and direct exposure to waterborne contaminants (Kumar et al., 2025; Haque et al., 2025).

2.1. Gills: The First Line of Contact

Gills play a vital role in respiration, osmoregulation, and nitrogen excretion. They are also the first site of contact between the fish and its aquatic environment. Numerous studies have shown that pesticides can cause lamellar fusion, epithelial lifting, hyperplasia, and aneurysms in gill tissues, severely impairing gas exchange and ionic regulation (Tahir et al., 2025).

For example, exposure to chlorpyrifos, a commonly used organophosphate pesticide, caused extensive gill epithelial hyperplasia and clubbing of secondary lamellae in *Labeo rohita*, resulting in respiratory distress and hypoxia (Varma & Vasudevan, 2025). In another study, *Oreochromis niloticus* exposed to Sumithion exhibited degenerative gill alterations such as edema, necrosis, and hemorrhages, leading to compromised respiratory efficiency (Haque et al., 2025).

2.2. Liver: The Detoxification Hub

The liver is the central organ for metabolism and detoxification. Pesticides that enter the bloodstream via gills or ingestion accumulate in the liver and can disrupt its normal physiological function. Histopathological findings in the liver often include hepatocyte vacuolization, necrosis, sinusoidal dilation, and fatty degeneration (Ajisebiola et al., 2025; Kumar et al., 2025). In fish exposed to cypermethrin and malathion, hepatocytes showed extensive vacuolation and nuclear pyknosis, indicating oxidative damage and apoptosis (Kumar et al., 2025). Similarly, glyphosate exposure in *Clarias gariepinus* led to cellular swelling, bile duct proliferation, and fibrosis, all indicative of chronic hepatic stress (Ajisebiola et al., 2025). These hepatic lesions not only compromise liver function but can also reduce the fish's ability to metabolize other xenobiotics.

2.3. Kidneys: Impact on Excretion and Osmoregulation

Kidneys in fish are essential for waste excretion and maintaining electrolyte balance. They are highly vascularized and therefore vulnerable to bloodborne toxins. Common histopathological findings in kidneys include glomerular shrinkage, tubular degeneration, hyaline casts, and interstitial inflammation (Bayır et al., 2025). In a study involving zebrafish (*Danio rerio*) exposed to the herbicide dalapon, the renal tissues displayed reduced glomerular diameter and fragmented nephrons, suggesting impaired osmoregulatory function (Bayır et al., 2025). Such damage leads to inefficient excretion of metabolic waste and toxic compounds, exacerbating systemic toxicity (Figure 1.)

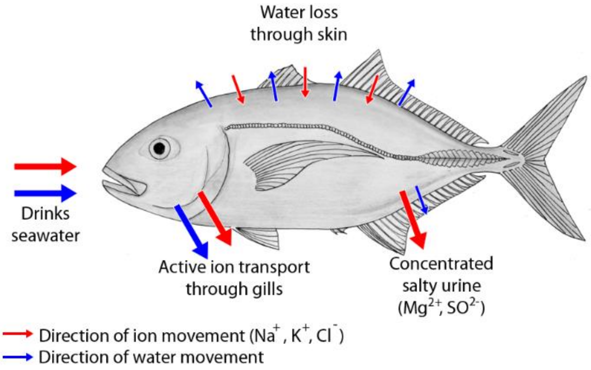

Figure 1. Osmoregulation And Excretory Systems on Fish

2.4. Spleen and Intestinal Tissues: Immunological and Digestive Impacts

The spleen is involved in hematopoiesis and immune regulation, and its exposure to pesticides can lead to splenic congestion, hemosiderosis, and atrophy of white pulp, all of which compromise immune competence (Rasal et al., 2025). Intestinal tissues, on the other hand, exhibit villous atrophy, epithelial erosion, and infiltration of inflammatory cells in fish exposed to insecticides like monocrotophos and abamectin. These changes interfere with nutrient absorption and may lead to malnutrition, reduced growth rates, and increased disease susceptibility (Rasal et al., 2025; Tahir et al., 2025).

2.5. Hematological and Biochemical Indicators

In addition to tissue-specific histopathological changes, pesticide exposure is also reflected in hematological parameters such as hemoglobin concentration, red and white blood cell counts, hematocrit values, and plasma glucose levels. Fish exposed to pesticides often show signs of anemia, hyperglycemia, leukocytosis, and thrombocytopenia, suggesting stress-induced metabolic disruptions (Haque et al., 2025; Ajisebiola et al., 2025).

Biochemical parameters such as elevated liver enzymes (ALT, AST), reduced total protein, and altered antioxidant enzyme activities (e.g., CAT, SOD) also serve as early warning signs of pesticide toxicity (Table 1).

Table 1. Common Histopathological Changes in Fish Exposed to Toxicants

Organ	Common Histopathological Changes	Implications
Gills	Lamellar fusion, hyperplasia, necrosis	Impaired respiration and osmoregulation
Liver	Hepatocyte vacuolization, necrosis	Disrupted detoxification and metabolism
Kidney	Glomerular shrinkage, tubular damage	Reduced excretion and ionic balance
Spleen	White pulp atrophy, congestion	Suppressed immune response
Intestine	Villous erosion, inflammation	Reduced nutrient absorption
Blood	Anemia, leukocytosis, glucose spike	Systemic stress marker

GENETIC AND MOLECULAR BIOMARKERS

While tissue-level biomarkers provide vital information on organ-specific damage, genetic and molecular assessments offer earlier, more sensitive indicators of pesticide-induced toxicity. These endpoints reflect sub-lethal cellular and biochemical alterations, often occurring before histopathological changes become visible. They are particularly useful for tracking chronic exposure and cumulative stress, providing insights into mechanisms of toxicity at the DNA and gene expression levels (Bayır et al., 2025; Rasal et al., 2025).

3.1. Genotoxicity: DNA Damage and Chromosomal Aberrations

Pesticides, especially organophosphates and carbamates, are known to cause genotoxic effects by inducing direct DNA damage, chromosome breaks, and point mutations. Genotoxicity can be assessed using various bioassays such as:

Micronucleus Assay (MN): Detects chromosomal fragments or whole chromosomes not incorporated into daughter nuclei during cell division.
Comet Assay (Single Cell Gel Electrophoresis):

Identifies DNA strand breaks and repair efficiency. Chromosomal Aberration Tests: Analyze structural abnormalities in metaphase chromosomes.

For example, fish such as Channa punctata and Oreochromis niloticus exposed to cypermethrin and malathion displayed significantly increased frequencies of micronuclei in erythrocytes, indicating genomic instability (Kumar et al., 2025). Similarly, the comet assay showed pronounced DNA fragmentation in liver cells of Danio rerio exposed to the herbicide dalapon (Bayır et al., 2025). Such genetic damage can interfere with cell replication and protein synthesis, leading to impaired development, carcinogenesis, or apoptosis (Figure 2).

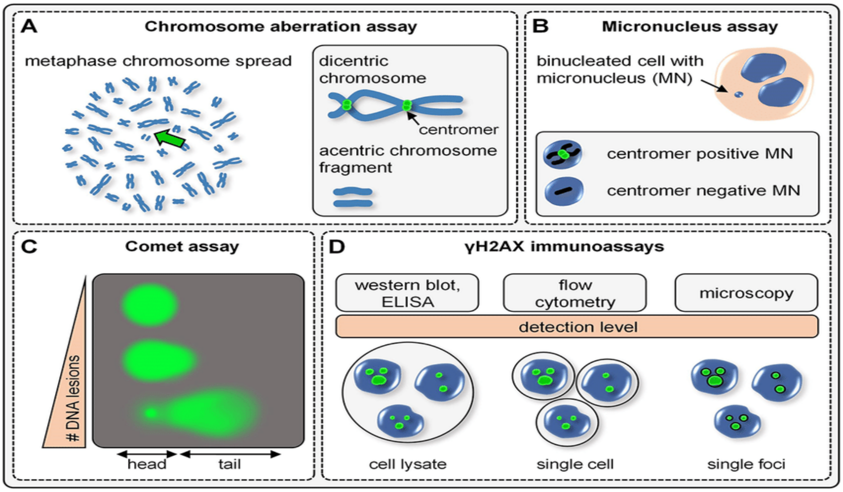

Figure 2. Schematic overview of genotoxicity assays.

3.2. Transcriptomic Alterations and Gene Expression Changes

Pesticide exposure alters the expression of several key genes involved in detoxification, antioxidant defense, inflammation, cell cycle control, and apoptosis. Among the most studied genes are:

Antioxidant defense: SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase)

Detoxification and xenobiotic metabolism: GST (glutathione-S-transferase), CYP450 enzymes

Heat shock and stress response: HSP70, HSP90

Inflammation and immune response: IL-1β, TNF-α, IFN-γ

In a transcriptomic study on Anabas testudineus exposed to monocrotophos, genes regulating oogenesis and estrogen signaling were significantly downregulated, highlighting the endocrine-disrupting potential of the pesticide (Rasal et al., 2025). Likewise, exposure to Sumithion in Oreochromis niloticus upregulated inflammatory cytokines and stress-related genes like HSP70, suggesting an active immune response (Zahan Etti, 2025). Modern techniques such as qPCR, RNA-seq, and microarrays are now commonly used to profile gene expression and reveal systemic biological responses to toxicants.

3.3. Epigenetic Effects and Heritable Changes

Though research in this area is still emerging, some studies indicate that pesticides can trigger epigenetic modifications, including DNA methylation and histone acetylation. These changes can alter gene expression without changing the DNA sequence, and in some cases, may be heritable across generations. For instance, methylation of promoter regions in antioxidant genes has been observed in fish exposed to chronic pesticide contamination, resulting in silencing of protective gene expression (Rasal et al., 2025). Such heritable changes raise significant concerns about the long-term ecological and evolutionary consequences of pesticide use in aquatic environments.

3.4. Mitochondrial Dysfunction and Apoptosis

Pesticides often target mitochondria, leading to reduced ATP production and the release of cytochrome c, which activates caspase cascades that culminate in programmed cell death (apoptosis). Mitochondrial swelling, altered membrane potential, and decreased expression of mitochondrial genes (e.g., COX, ATPase) have been documented in pesticide-exposed zebrafish and catfish (Bayır et al., 2025). Apoptotic pathways are often triggered in parallel with oxidative stress and DNA damage, forming a complex network of toxic responses. These responses not only impair tissue regeneration but also affect the immune and reproductive systems.

3.5. Integrated Biomarker Response (IBR) as a Tool

The Integrated Biomarker Response (IBR) is a quantitative framework used to combine multiple molecular and cellular biomarkers into a single index. This provides a comprehensive profile of organism health and enables cross-comparison between different pesticide treatments, time points, or species. For example, Micropterus salmoides (largemouth bass) exposed to abamectin showed strong activation of IBR pathways involving apoptosis, oxidative stress, and cardiac damage (Tahir et al., 2025). IBR analysis is increasingly being adopted in ecotoxicology to support environmental risk assessments and regulatory decisions (Table 2).

Table 2. Molecular and Cellular Biomarkers Used in Fish Toxicological Studies

Effect Type	Biomarker / Method	Implication
Genotoxicity	Micronucleus assay, Comet assay	DNA fragmentation, chromosomal aberrations
Gene expression	qPCR, RNA-seq, HSP70, SOD, CAT, GST	Oxidative stress, detoxification
Epigenetic changes	Methylation, histone marks	Heritable gene silencing or activation
Apoptosis	Caspase activity, cytochrome c, TUNEL assay	Cell death, tissue degeneration
Integrated assessment	IBR score	Holistic toxicity profile

4. CASE STUDIES: PESTICIDE-INDUCED TISSUE AND GENETIC EFFECTS IN FISH

Case studies offer real-world evidence of how pesticide exposure translates into physiological, histopathological, and genetic disruptions in various fish species. These studies also guide regulatory agencies and researchers in establishing risk thresholds and developing mitigation strategies.

4.1. Cypermethrin and Malathion Exposure in Channa punctata

In a controlled laboratory study, Channa punctata (snakehead murrel) was exposed to sublethal doses of two widely used pesticides—cypermethrin and malathion. Within 96 hours, the fish exhibited significant hematological disruptions, including reduced hemoglobin levels, leukocytosis, and thrombocytopenia. Tissue-level damage included vacuolar degeneration of hepatocytes, epithelial hyperplasia in gills, and glomerular shrinkage in kidneys. Genotoxic assessment via micronucleus assays revealed elevated frequencies of nuclear abnormalities in erythrocytes, indicating substantial chromosomal damage (Kumar et al., 2025).

4.2. Glyphosate-Based Herbicide in Clarias gariepinus

A chronic exposure study was conducted on Clarias gariepinus (African catfish), where fish were exposed to glyphosate-based herbicides over a 21-day period. Histological analysis revealed liver necrosis, splenic congestion, and intestinal inflammation. Hematological analysis reported anemia, hyperglycemia, and elevated white blood cell count. These alterations were indicative of immune suppression and systemic oxidative stress (Ajisebiola et al., 2025).

4.3. Dalapon-Induced Genomic and Biochemical Disruptions in *Danio rerio*

In this study, dalapon, a herbicide commonly used in rice farming, was shown to affect the antioxidant system and genomic integrity of Danio rerio (zebrafish). Post-exposure, fish displayed suppressed expression of detoxification genes (SOD, CAT) and increased apoptosis markers. DNA fragmentation and mitochondrial dysfunction were observed, confirming oxidative damage and cell death pathways activation (Bayır et al., 2025).

4.4. Abamectin Exposure in Micropterus Salmoides

Micropterus salmoides (largemouth bass) were exposed to environmentally relevant concentrations of abamectin. The study employed Integrated Biomarker Response (IBR) analysis and histological methods. The fish exhibited cardiac tissue necrosis, inflammatory infiltration, and upregulation of apoptosis-related genes. IBR indices confirmed multi-organ toxicity, particularly affecting cardiovascular function (Tahir et al., 2025).

4.5. Monocrotophos and Reproductive Gene Disruption in Anabas testudineus

A transcriptomic approach was used to study the effects of monocrotophos on the ovarian tissues of Anabas testudineus (climbing perch). RNA-seq analysis revealed downregulation of genes associated with folliculogenesis, oocyte maturation, and hormone signaling. These alterations suggest that monocrotophos acts as an endocrine-disrupting chemical (EDC), threatening reproductive success and population sustainability (Rasal et al., 2025).

4.6. Mitigation of Sumithion Toxicity in Oreochromis niloticus

In an intervention study, Oreochromis niloticus (Nile tilapia) were exposed to Sumithion, an organophosphate pesticide, and treated with probiotics and Spirulina. The fish exposed to Sumithion alone showed significant upregulation of stress genes (e.g., HSP70, TNF-α), while co-treatment groups exhibited restored antioxidant capacity and reduced inflammatory gene expression. This demonstrates the potential of dietary supplements to mitigate genetic and immunotoxic effects (Zahan Etti, 2025).

5. MECHANISMS OF TOXICITY

Understanding the mechanisms by which pesticides exert toxic effects on fish is essential for linking observed physiological damage to underlying molecular disruptions. These mechanisms are typically multifactorial and involve interactions at the cellular, biochemical, and genetic levels, often leading to tissue degeneration, metabolic dysfunction, and impaired reproduction or survival. Among the most critical pathways are oxidative stress, endocrine disruption, mitochondrial dysfunction, immune modulation, and apoptosis induction.

5.1. Oxidative Stress and ROS Generation

One of the most universal mechanisms of pesticide toxicity is the overproduction of reactive oxygen species (ROS). Pesticides such as organophosphates (e.g., malathion), pyrethroids (e.g., cypermethrin), and herbicides (e.g., dalapon) can disrupt the balance between ROS and the antioxidant defense system. ROS such as superoxide anions (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) react with DNA, lipids, and proteins, resulting in: Lipid peroxidation of cell membranes DNA strand breaks Protein denaturation and enzyme inactivation:

For example, Bayır et al. (2025) observed significant reductions in antioxidant enzyme activity (SOD, CAT, GPx) and elevated levels of malondialdehyde (MDA) in zebrafish exposed to dalapon, suggesting oxidative degradation of lipids and impaired antioxidant function.

5.2. Mitochondrial Dysfunction

Pesticides often target mitochondria—central hubs of cellular respiration—leading to:

- Disruption of electron transport chain (ETC)
- Reduced ATP production
- Mitochondrial swelling and membrane depolarization
- Release of cytochrome c, initiating apoptotic pathways

In Danio rerio, exposure to herbicides caused significant mitochondrial damage, including the collapse of the mitochondrial membrane potential ($\Delta\psi m$) and upregulation of caspase-3 activity, indicating initiation of programmed cell death (Bayır et al., 2025).

5.3. Endocrine Disruption

Several pesticides mimic or block the actions of natural hormones by binding to nuclear hormone receptors, interfering with gene transcription. These chemicals are termed endocrine-disrupting chemicals (EDCs).

- Common impacts include:
- Inhibition of estrogen or androgen synthesis
- Downregulation of hormone receptor genes (e.g., ERα, AR)
- Disruption of oocyte development, testicular structure, and steroid hormone balance

In the ovarian tissues of Anabas testudineus, Rasal et al. (2025) reported downregulation of folliculogenesis genes and altered transcriptomic profiles upon monocrotophos exposure. This reflects the hormone-mimicking effects of pesticides that jeopardize reproductive capacity.

5.4. Immune Suppression and Inflammation

- Pesticides can weaken the immune system in fish by:
- Damaging lymphoid organs (e.g., spleen, thymus)
- Suppressing expression of immune-related genes (TNF-α, IL-1β, IFN-γ)
- Increasing susceptibility to pathogens

Tahir et al. (2025) demonstrated significant immunosuppressive effects in Micropterus salmoides following exposure to abamectin, with marked reduction in macrophage activity and cytokine gene expression.

5.5. Apoptosis and Programmed Cell Death

Persistent oxidative stress and mitochondrial damage eventually lead to apoptosis, a regulated form of cell death. Hallmarks of apoptosis in pesticide-exposed fish include:

- DNA laddering
- Nuclear condensation and fragmentation
- Activation of caspases (especially caspase-3 and -9)

This was observed in Channa punctata exposed to cypermethrin, where nuclear fragmentation and cytoplasmic blebbing in liver tissues were accompanied by increased apoptotic markers (Kumar et al., 2025).

5.6. DNA Damage and Genotoxicity

As discussed earlier, many pesticides cause direct genotoxic effects:

- Single- and double-strand breaks
- Chromosomal aberrations
- Micronucleus formation

The Comet Assay and Micronucleus Test remain key techniques for evaluating DNA integrity. In studies on Clarias gariepinus and Oreochromis niloticus, genotoxicity was observed even at sublethal pesticide doses (Ajisebiola et al., 2025; Zahan Etti, 2025) (Table 3.)

Table 3. Mechanistic Pathways and Biological Consequences of Toxicant Exposure in Fish

Mechanism	Biological Targets	Consequences
Oxidative Stress	Lipids, DNA, proteins	Lipid peroxidation, mutations
Mitochondrial Dysfunction	ETC, cytochrome c	Energy failure, apoptosis
Endocrine Disruption	Hormone receptors, glands	Impaired reproduction
Immune Modulation	Cytokine genes, spleen	Immunosuppression
Apoptosis	Caspase cascade	Programmed cell death
Genotoxicity	DNA strands, nuclei	Mutations, carcinogenesis

These interconnected pathways show that pesticide toxicity in fish is rarely linear but often a synergistic process, where multiple biochemical disruptions converge to impair growth, immunity, reproduction, and survival.

6. APPLICATIONS IN ENVIRONMENTAL MONITORING

Fish-based bioassays are now part of many national environmental monitoring programs due to their sensitivity to aquatic pollutants. The combination of histopathology, hematology, and molecular biology offers a powerful toolset for assessing aquatic health. Additionally, studies involving probiotics and natural supplements (e.g., *Spirulina*) have explored protective strategies to mitigate pesticide-induced damage, with some success in reversing genetic and immunological impairments (Zahan Etti, 2025).

7. CONCLUSION

Pesticides pose a significant threat to fish health by inducing histological deformities and genetic mutations. These effects not only endanger aquatic biodiversity but may also pose risks to human health through biomagnification. Continued research using advanced histological and genomic tools is crucial for understanding the ecological consequences of pesticide exposure and for informing effective regulatory policies.

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CHAPTER 5

MARINE MACROALGAE AS SOURCES OF RARE EARTH ELEMENTS

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INTRODUCTION

Macroalgae are photosynthetic and benthic organisms that grow on substrates in coastal areas (Lobban & Harrison, 1994). According to their morphology and pigments, they are divided into three main groups as brown (Phaeophyceae), green (Chlorophyta), and red (Rhodophyta) (Bogdan et al., 2025). These groups function as primary producers and are important components of coastal biodiversity. Their distribution is influenced by environmental factors such as nutrients, temperature, light, and salinity. Red algae usually occur in deeper zones, brown algae dominate in temperate and cold regions, while green algae are common in shallow coastal areas with strong light (Lindsay et al., 2025). Throughout history, macroalgae have been used as food and raw materials. Macroalgae have historically been used as food and raw material. Today they are also applied in pharmaceutical, cosmetic, biotechnology, and agricultural fields (Holdt & Kraan, 2011). Polysaccharides such as alginate, carrageenan, and agar are important in food processing, where they function as stabilizers, thickeners, and gelling agents. Macroalgae also support environmental sustainability through carbon storage and habitat formation (Cotas et al., 2023). These features make macroalgae ecologically and economically important organisms.

Valuable elements are classified as strategic raw materials because of their role in technology and economic importance. These elements are geopolitically crucial because of their low natural abundance, limited geographic distribution, and dependence on external supply (Groves et al., 2025). Although traditionally linked with gold (Au), silver (Ag), and the platinum group metals (Ir, Os, Ru, Rh, Pt, Pd), rare earth elements (REEs) and related elements have gained attention due to recent technological and energy transitions (Golroudbary et al., 2022; Groves et al., 2025). REEs include the lanthanide series, as well as yttrium (Y) and scandium (Sc). They are grouped into light (La–Nd), medium (Sm–Dy), and heavy (Ho–Lu) REEs (Golroudbary et al., 2022). Their strategic applications have led to their classification as new-generation valuable elements (Li et al., 2021). These elements are used across various sectors due to their magnetic, catalytic, and optoelectronic properties, including electronics, defense technologies, renewable energy systems, and medical devices (Li et al., 2021; Golroudbary et al., 2022; Groves et al., 2025). In recent years, the global REE market has grown significantly in both production and consumption. International production, which was around 45 kt in 2015, increased by an average of 10% annually from 2021 to 2024, reaching approximately 80–85 kt in 2024. China accounts for 96% of this increase, reinforcing the concentration of global supply. Moreover, about 95% of global refining capacity is controlled by China-based companies, increasing market dependency (Humphries, 2010; IEA, 2025). On the consumption side, more than 60% of global demand in 2024 was from China, followed by the USA, Europe, and Japan as secondary markets. This concentration poses a long-term supply risk and accelerates the search for new sources. Over the past two decades, supply chain vulnerabilities and monopolized production have made it necessary to explore alternative and sustainable resources (Massari & Ruberti, 2013). REEs are technologically and geochemically important tracers that are also measured in biological matrices. Macroalgae have been investigated as potential natural concentrators of these elements, yet published concentrations vary widely across taxa and studies, and systematic comparisons remain limited. In this context, marine biomasses - especially macroalgae - have become a focus of research due to their low environmental impact and renewable nature. This chapter focuses on the relationship between marine macroalgae, particularly brown algae, and REEs. It examines the accumulation patterns of these elements in macroalgae and evaluates the accumulation capacities of different species in terms of their potential ecological and economic implications.

2. LIGHT RARE EARTH ELEMENTS

Light Rare Earth Elements (LREEs) consist of the first four lanthanides: lanthanum (La), cerium (Ce), praseodymium (Pr), and neodymium (Nd). They are more common in terrestrial crustal layers than heavy rare earth elements and show larger ionic radii in the Ln^{3+} state (Cotton, 2025; Gupta & Krishnamurthy, 1992). The +3 oxidation state is dominant; however, Ce readily oxidizes to +4, and CeO_2 is used as an oxygen-storage material in automotive three-way catalysts (Montini et al., 2016). La is used in fluid catalytic cracking (FCC) catalyst modification, La–Ni hydrogen storage alloys for Ni–MH batteries, and high-refractive-index optical glasses (Binnemans et al., 2013; Cotton, 2025). Pr and Nd are used in Nd–Fe–B permanent magnets, glass and ceramic coloring, and some laser and optical applications (Balaram, 2019;

Cotton, 2025). Nd plays a central role in Nd–Fe–B magnets that are applied in wind turbines, vehicle engines, and electronic equipment. Nd: YAG lasers are widely used in medical, industrial, and defense applications (Balaram, 2019; Dubey & Yadava, 2008; Saydjari et al., 2016). Because of higher demand in green technologies and supply concerns, LREEs are recognized as strategic or critical raw materials by the European Commission and the USGS (Bobba et al., 2020; Lederer et al., 2024).

These elements are examined in both geological and biological contexts because of their technological and chemical characteristics. Whether they can be obtained from natural biological sources, including macroalgae, has become relevant in the context of renewable and sustainable raw material exploration. Table 1 summarizes concentrations of La, Ce, Pr, and Nd in different macroalgae species. La concentrations were reported from 3.0×10^{-4} µg/g in *Undaria pinnatifida* (Sakamoto et al., 2008) to 2991.4 µg/g in *Cystoseira crinita* (Ryabushko et al., 2022). Ce concentrations ranged from 4×10^{-4} µg/g in *U. pinnatifida* (Sakamoto et al., 2008) to 560.9 µg/g in *C. crinita* (Ryabushko et al., 2022). Pr concentrations were as low as 1.0×10^{-4} µg/g in *Ulva pertusa* (Sakamoto et al., 2008) and as high as 8.1 µg/g in *C. crinita* (Ryabushko et al., 2022). Nd concentrations ranged from 1.0×10^{-4} µg/g in *U. pertusa* (Sakamoto et al., 2008) to 104.4 µg/g in *C. crinita* (Ryabushko et al., 2022). The values compiled in Table 1 illustrate marked interspecific variation, suggesting that the ability to concentrate LREEs is strongly species-dependent even within the same algal group. The data show that LREEs accumulate across a wide range in macroalgae. High accumulation is observed particularly in brown algae and in some green algae, with considerable interspecies variability.

Table 1. Concentrations of La, Ce, Pr, Nd in various macroalgae species (µg/g dry weight)

Species	**La**	**Ce**	**Pr**	**Nd**	**References**
Green Algae					
Ulva conglobata	$1.73\text{-}3.02\text{x}10^{-1}$	$4.56\text{-}7.61\text{x}10^{-1}$	$2.48\text{-}6.38\text{x}10^{-2}$	$2.4\text{-}8.3\text{x}10^{-2}$	(Kano et al., 2002)
Ulva rigida	$1.86\text{x}10^{-1}$	$7.6\text{x}10^{-2}$	$7.7\text{x}10^{-2}$	$2.49\text{x}10^{-2}$	(Ryabushko et al., 2024)
Ulva rigida	$1.7\text{x}10^{-1}$	$3.1\text{x}10^{-1}$	-	$1.1\text{x}10^{-1}$	(Milinovic et al., 2021)
Ulva fasciata	$1.11\text{x}10^{-1}$	$1.74\text{x}10^{-1}$	$2.56\text{x}10^{-2}$	$9.98\text{x}10^{-2}$	(Fu et al., 2000)
Ulva pertusa	$5.0\text{-}5.8\text{x}10^{-4}$	$2.6\text{-}5.4\text{x}10^{-3}$	$1.0\text{-}14.0\text{x}10^{-4}$	$1.0\text{-}33.0\text{x}10^{-4}$	(Sakamoto et al., 2008)
Chaetomorpha moniligera	$2.1\text{x}10^{-1}$	$5.1\text{x}10^{-1}$	$3.98\text{x}10^{-2}$	$1.65\text{x}10^{-1}$	(Kano et al., 2002)
Codium bursa	2.4	5.2	0.64	2.6	(Squadrone et al., 2018)
Codium bursa	0.76-3.5	1.6-6.3	0.18-0.81	0.73-3.1	(Squadrone et al., 2017)
Codium fragile	$3.29\text{x}10^{-2}$	$7.6\text{x}10^{-2}$	$8.6\text{x}10^{-3}$	$3.93\text{x}10^{-2}$	(Fu et al., 2000)
Codium tomentosum	$2.7\text{x}10^{-1}$	$5.3\text{x}10^{-1}$	-	$2.3\text{x}10^{-1}$	(Milinovic et al., 2021)
Flabella petiolata	1.6	3.6	0.44	1.5	(Squadrone et al., 2018)
Flabella petiolata	0.61-4.3	1.3-8.8	0.14-1.0	0.59-4.0	(Squadrone et al., 2017)
Acetabularia acetabulum	1.4	1.8	0.29	1.2	(Squadrone et al., 2018)
Caulerpa cylindracea	4.1	8.4	1.0	4.0	(Squadrone et al., 2018)
Caulerpa racemosa	1.1	1.8	0.24	1.0	(Squadrone et al., 2017)
Brown Algae					
Sargassum tortile	$1.81\text{-}1.96\text{x}10^{-1}$	$5.57\text{-}6.11\text{x}10^{-1}$	$3.08\text{-}4.42\text{x}10^{-2}$	$1.12\text{-}1.81\text{x}10^{-1}$	(Kano et al., 2002)
Sargassum hemiphyllum	$7.0\text{-}7.5\text{x}10^{-4}$	$1.6\text{-}9.7\text{x}10^{-3}$	$1.1\text{-}2.7\text{x}10^{-3}$	$5.0\text{-}5.1\text{x}10^{-4}$	(Sakamoto et al., 2008)
Padina pavonica	1.9	3.1	0.42	1.7	(Squadrone et al., 2018)

Table 1. *continued.*

Species	La	Ce	Pr	Nd	References
Padina pavonica	1.6-3.9	2.0-7.2	0.33-0.91	1.4-3.6	(Squadrone et al., 2017)
Padina sp.	1.86-12.4	3.39-26.2	0.35-2.89	1.78-14.2	(Mashitah et al., 2012)
Padina okadai	3.34x10^{-2}	7.8x10^{-2}	1.02x10^{-2}	4.55x10^{-2}	(Fu et al., 2000)
Dictyota dichotoma	1.0	1.8	0.28	1.0	(Squadrone et al., 2018)
Dictyota dichotoma	1.8	3.3	0.41	1.6	(Squadrone et al., 2017)
Halopteris scoparia	1.5	3.2	0.45	1.5	(Squadrone et al., 2018)
Halopteris scoparia	2.73	6.2	0.65	2.7	(Squadrone et al., 2017)
Halopteris filicina	2.9-43x10^{-1}	6.4-88x10^{-1}	7-110x10^{-2}	3.1-41x10^{-1}	(Squadrone et al., 2017)
Cystoseira sp.	1.0	1.5	0.23	0.99	(Squadrone et al., 2017)
Cystoseira crinita	2686.5-2991.4	89.3-560.9	7.7-8.1	73.2-104.4	(Ryabushko et al., 2022)
Cystoseira barbata	657.2-2551.6	55.3-147.2	3.7-4.1	24.3-25.3	(Ryabushko et al., 2022)
Gongolaria barbata	2.29x10^{-1}	1.01x10^{-1}	8.8x10^{-2}	3.8x10^{-2}	(Ryabushko et al., 2024)
Ecklonia cava	8.3x10^{-3}	2.02x10^{-2}	1.86x10^{-3}	7.8x10^{-3}	(Fu et al., 2000)
Gongolaria barbata	2.29x10^{-1}	1.01x10^{-1}	8.8x10^{-2}	3.8x10^{-2}	(Ryabushko et al., 2024)
Undaria pinnatifida	3.0-22.0x10^{-4}	4.0-40.0x10^{-4}	5.0-13.0x10^{-4}	2.0-20.0x10^{-4}	(Sakamoto et al., 2008)
Undaria pinnatifida	2.0x10^{-2}	3.0x10^{-2}	-	1.0x10^{-2}	(Milinovic et al., 2021)
Saccorhiza polyschides	3.0x10^{-2}	4.0x10^{-2}	-	2.0x10^{-2}	(Milinovic et al., 2021)

Table 1. *continued.*

Species	**La**	**Ce**	**Pr**	**Nd**	**References**
Fucus distichus	$7.88 x 10^{-1}$	1.214	$2.02 x 10^{-1}$	$9.05 x 10^{-1}$	(Lobus & Udalov, 2021)
Fucus serratus	$2.19 x 10^{-1}$	$1.8 x 10^{-2}$	$1.9 x 10^{-3}$	$8.0 x 10^{-3}$	(Panova et al., 2024)
Ascophyllum nodosum	$4.16 x 10^{-1}$	$4.08 x 10^{-1}$	$7.7 x 10^{-2}$	$2.7 x 10^{-1}$	(Panova et al., 2024)
Laminaria digitata	$2.95 x 10^{-1}$	2.085	$5.5 x 10^{-2}$	$2.48 x 10^{-1}$	(Lobus & Udalov, 2021)
Red Algae					
Desmarestia menziesii	$3.03 x 10^{-1}$	$7.2\text{-}19.6 x 10^{-2}$	$9.0\text{-}64 x 10^{-3}$	$4.1\text{-}28 x 10^{-2}$	(Runcie & Riddle, 2004)
Himantothallus grandifolius	$2.29 x 10^{-1}$	$5.5\text{-}27 x 10^{-2}$	$7.0\text{-}4.8 x 10^{-2}$	$3.2\text{-}20.1 x 10^{-2}$	(Runcie & Riddle, 2004)
Grateloupia filicina	$3.26 x 10^{-1}$	$9.43 x 10^{-1}$	$4.91 x 10^{-2}$	$1.91 x 10^{-1}$	(Kano et al., 2002)
Grateloupia elliptica	$1.02\text{-}1.59 x 10^{-1}$	$4.56\text{-}7.26 x 10^{-1}$	$2.57\text{-}2.78 x 10^{-2}$	$0.93\text{-}1.12 x 10^{-1}$	(Kano et al., 2002)
Grateloupia elliptica	$1.4\text{-}1.9 x 10^{-3}$	$2.6\text{-}5.0 x 10^{-3}$	$1.9\text{-}2.3 x 10^{-3}$	$1.6\text{-}5.0 x 10^{-3}$	(Sakamoto et al., 2008)
Grateloupia okamurai	$7.61 x 10^{-2}$	$3.85 x 10^{-1}$	$1.44 x 10^{-2}$	$5.95 x 10^{-2}$	(Kano et al., 2002)
Nemalion multifiduma	$7.48 x 10^{-1}$	1.582	$1.31 x 10^{-1}$	$4.55 x 10^{-1}$	(Kano et al., 2002)
Peyssonnelia squamaria	2.0	3.3	0.46	1.7	(Squadrone et al., 2018)
Peyssonnelia squamaria	1.52-1.6	2.6-2.9	0.32-0.35	1.3-1.4	(Squadrone et al., 2017)
Dudresnaya verticillata	0.52	0.8	0.09	0.35	(Squadrone et al., 2017)
Phyllophora crispa	1.2	2.3	0.31	1.1	(Squadrone et al., 2018)
Ganonema farinosum	2.5	4.8	0.55	2.2	(Squadrone et al., 2017)
Laurencia obtusa	1.3	2.3	0.28	1.2	(Squadrone et al., 2017)
Delisea fimbriata	5.4x10	9.5x10	9.4x10	5.8x10	(Fu et al., 2000)

Table 1. *continued.*

Species	**La**	**Ce**	**Pr**	**Nd**	**References**
Gracilaria gracilis	2.0x10^{-1}	3.9x10^{-2}	-	1.5x10^{-1}	(Milinovic et al., 2021)
Osmundea pinnatifida	3.9x10^{-1}	8.1x10^{-1}	-	3.5x10^{-1}	(Milinovic et al., 2021)
Porphyra sp	6.0x10^{-2}	7.0x10^{-2}	-	2.0x10^{-2}	(Milinovic et al., 2021)
Porphyra sp.	1.6-22.4x10^{-1}	2.68-42.5x10^{-1}	3.9-48.6x10^{-2}	2.03-21.0x10^{-1}	(Liu et al., 2018)
Iridaea mawsonii	2.41x10^{-1}	5.1-31x10^{-2}	8.0-4.6x10^{-2}	3.4-18.2x10^{-2}	(Runcie & Riddle, 2004)
Prasiola crispa	2.696	5.788	0.638	2.4	(Runcie & Riddle, 2004)
Neopyropia yezoensis	4.68-14.99x10^{-2}	7.68-25.37x10^{-2}	1.22-2.76x10^{-2}	5.58-11.55x10^{-2}	(Zhao et al., 2024)

3. MIDDLE RARE EARTH ELEMENTS

Middle rare earth elements (MREEs) include samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), and dysprosium (Dy), positioned in the central part of the lanthanide series. These elements show intermediate properties in terms of ionic radius and geochemical behavior between light and heavy lanthanides (Cotton, 2025; Gupta & Krishnamurthy, 1992). Each element has specific industrial applications. Sm is used in Sm–Co permanent magnets due to its high thermal stability, particularly in defense and aerospace sectors (Balaram, 2019). Eu, which can alternate between +2 and +3 oxidation states, is used in red phosphors for TV screens, fluorescent lamps, and LEDs (Binnemans et al., 2013). Gd is applied in control rods for nuclear reactors and as an MRI contrast agent because of its magnetic properties and neutron absorption capacity (Wang et al., 2023). Tb is utilized in magneto-optical materials and phosphors, and as an additive in Nd–Fe–B magnets to improve coercivity (Wu et al., 2021). Dy is used in high-performance magnets for wind turbines and electric vehicle motors (Heim & Vander Wal, 2023). These applications place MREEs among strategic raw materials in energy, electronics, medical, and defense industries.

The concentrations of MREEs in macroalgae are shown in Table 2. Sm concentrations ranged from 2.0×10^{-4} µg/g in *Sargassum hemiphyllum* (Sakamoto et al., 2008) to 13.5 µg/g in *Cystoseira crinita* (Ryabushko et al., 2022). Eu concentrations were reported at 4.2×10^{-5} µg/g in *Grateloupia elliptica* (Sakamoto et al., 2008) and up to 499.8 µg/g in *C. crinita* (Ryabushko et al., 2022). Gd concentrations ranged from 1.5×10^{-5} µg/g in *G. elliptica* (Sakamoto et al., 2008) to 13.4 µg/g in *C. crinita* (Ryabushko et al., 2022). Tb concentrations ranged from 6.0×10^{-5} µg/g in *Fucus vesiculosus* (Panova et al., 2004) to 36.6 µg/g in *C. crinita* (Ryabushko et al., 2022). Dy concentrations were reported as low as 0.4×10^{-5} µg/g in *G. elliptica* (Sakamoto et al., 2008) and as high as 16.2 µg/g in *C. crinita* (Ryabushko et al., 2022). The brown algae generally contains the highest MREE concentrations, particularly *Cystoseira* species, while green algae mostly remain low levels.

Table 2. Concentrations of Sm, Eu, Gd, Tb, Dy in various macroalgae species (µg/g dry weight)

Species	Sm	Eu	Gd	Tb	Dy	References
Green Algae						
Ulva conglobata	1.34-4.87x10^{-2}	0.480-1.28x10^{-2}	1.81-6.31x10^{-2}	2.97-9.17x10^{-3}	9.67-49.2x10^{-3}	(Kano et al., 2002)
Ulva rigida	5.8x10^{-3}	2.1x10^{-3}	6.5x10^{-3}	9.7x10^{-3}	6.1x10^{-3}	(Ryabushko et al., 2024)
Ulva rigida	-	3.0x10^{-2}	3.0x10^{-2}	1.02x10^{-2}	-	(Milinovic et al., 2021)
Ulva fasciata	2.38x10^{-2}	7.1x10^{-3}	2.91x10^{-2}	4.5x10^{-3}	2.78x10^{-2}	(Fu et al., 2000)
Ulva pertusa	4.0-45.0x10^{-4}	1.0-15.0x10^{-4}	1.0-69.0x10^{-4}	1.0-16.0x10^{-4}	1.0-49.0x10^{-4}	(Sakamoto et al., 2008)
Chaetomorpha moniligera	2.79x10^{-2}	0.7x10^{-2}	4.12x10^{-2}	0.574x10^{-3}	2.35x10^{-2}	(Kano et al., 2002)
Codium bursa	6.6x10^{-1}	7.2x10^{-2}	6.6x10^{-1}	1.2x10^{-1}	6.1x10^{-1}	(Squadrone et al., 2018)
Codium bursa	1.3-6.5x10^{-1}	2.2-17x10^{-2}	1.2-6.8x10^{-1}	1.7-14.0x10^{-2}	8.5-51.0x10^{-2}	(Squadrone et al., 2017)
Codium fragile	9.3x10^{-3}	2.81x10^{-3}	1.05x10^{-2}	1.28x10^{-3}	9.2x10^{-3}	(Fu et al., 2000)
Codium tomentosum	-	2.0x10^{-2}	4.0x10^{-2}	8.0x10^{-3}	-	(Milinovic et al., 2021)
Flabella petiolata	3.5x10^{-1}	6.4x10^{-2}	3.2x10^{-1}	4.4x10^{-2}	2.3x10^{-1}	(Squadrone et al., 2018)
Flabella petiolata	1.1-8.5x10^{-1}	2.1-16.0x10^{-2}	1.2-8.3x10^{-1}	1.6-160x10^{-3}	8.6-60x10^{-2}	(Squadrone et al., 2017)
Acetabularia acetabulum	2.5x10^{-1}	5.7x10^{-2}	2.7x10^{-1}	3.8x10^{-2}	2.1x10^{-1}	(Squadrone et al., 2018)

Table 2. *continued*

Species	Sm	Eu	Gd	Tb	Dy	References
Caulerpa cylindracea	9.5x10^{-1}	1.4x10^{-1}	9.6x10^{-1}	1.5x10^{-1}	8.0x10^{-1}	(Squadrone et al., 2018)
Caulerpa racemosa	2.2x10^{-1}	5x10^{-2}	2.4x10^{-1}	4.0x10^{-2}	2.0x10^{-1}	(Squadrone et al., 2017)
Brown Algae						
Sargassum tortile	1.95-4.78x10^{-2}	8.3-10.8x10^{-3}	3.21-44.9x10^{-2}	4.17-8.19x10^{-2}	1.79-3.94x10^{-2}	(Kano et al., 2002)
Sargassum hemiphyllum	2.0-20.0x10^{-4}	2.0-12.0x10^{-4}	2.0-23.0x10^{-4}	2.0-23.0x10^{-4}	1.0-26.0x10^{-4}	(Sakamoto et al., 2008)
Padina pavonica	3.6x10^{-1}	7.0x10^{-2}	3.6x10^{-1}	5.3x10^{-2}	2.9x10^{-1}	(Squadrone et al., 2018)
Padina pavonica	2.9-7.7x10^{-1}	6.0-16.0x10^{-2}	3.3-7.8x10^{-1}	4.6-13.0x10^{-2}	2.5-5.8x10^{-1}	(Squadrone et al., 2017)
Padina sp.	3.2-23.1x10^{-1}	3.2-23.1x10^{-1}	3.7-22.6x10^{-1}	4.0-20.0x10^{-2}	2.3-9.5x10^{-1}	(Mashitah et al., 2012)
Padina okadai	1.20x10^{-2}	3.6x10^{-3}	1.62x10^{-2}	2.0x10^{-3}	1.38x10^{-2}	(Fu et al., 2000)
Dictyota dichotoma	2.5x10^{-1}	6.0x10^{-2}	2.7x10^{-1}	4.2x10^{-2}	2.5x10^{-1}	(Squadrone et al., 2018)
Dictyota dichotoma	3.4x10^{-1}	9.0x10^{-2}	3.6x10^{-1}	6.0x10^{-2}	2.7x10^{-1}	(Squadrone et al., 2017)
Halopteris scoparia	4.1x10^{-1}	1.0x10^{-1}	4.7x10^{-1}	7.3x10^{-2}	4.3x10^{-1}	(Squadrone et al., 2018)
Halopteris scoparia	5.1x10^{-1}	1.0x10^{-1}	5.1x10^{-1}	7.0x10^{-2}	3.4x10^{-1}	(Squadrone et al., 2017)
Halopteris filicina	7.0-87x10^{-2}	1.0-17x10^{-2}	7.0-87.0x10^{-2}	1.0-22.0x10^{-2}	5.0-64x10^{-2}	(Squadrone et al., 2017)
Cystoseira sp.	2.2x10^{-1}	5.0x10^{-2}	2.5x10^{-1}	4.0x10^{-2}	2.0x10^{-1}	(Squadrone et al., 2017)

Table 2. *continued*

Species	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**References**
Cystoseira crinita	12.7-13.5	387.7-499.8	13.0-13.4	16.4-36.6	13.1-16.2	(Ryabushko et al., 2022)
Cystoseira barbata	3.4-6.2	41.5-218.3	6.0-9.4	6.2-19.0	5.1-12.4	(Ryabushko et al., 2022)
Gongolaria barbata	1.0x10^{-2}	1.49x10^{-2}	1.4x10^{-2}	5.6x10^{-3}	1.65x10^{-2}	(Ryabushko et al., 2024)
Ecklonia cava	3.34x10^{-3}	9.5x10^{-4}	4.7x10^{-3}	7.3x10^{-4}	4.61x10^{-3}	(Fu et al., 2000)
Undaria pinnatifida	5.0-24.0x10^{-4}	1.0-10.0x10^{-4}	3.0-25.0x10^{-4}	1.0-10.0x10^{-4}	4.0-15.0x10^{-4}	(Sakamoto et al., 2008)
Undaria pinnatifida	-	2.0x10^{-2}	1.0x10^{-2}	6.0x10^{-3}	-	(Milinovic et al., 2021)
Saccorhiza polyschides	-	2.0x10^{-2}	1.0x10^{-2}	4.0x10^{-3}	-	(Milinovic et al., 2021)
Fucus vesiculosus	1.3x10^{-2}	1.4x10^{-2}	1.2x10^{-2}	6.0x10^{-5}	9.0x10^{-3}	(Panova et al., 2024)
Fucus vesiculosus	0.09-0.12	0.017-0.032	-	0.046	-	(Truus et al., 2001)
Fucus distichus	1.7x10^{-2}	1.0x10^{-2}	1.1x10^{-2}	1.2x10^{-3}	9.0x10^{-3}	(Panova et al., 2024)
Fucus serratus	0.9x10^{-4}	5.1x10^{-3}	1.9x10^{-3}	0.2x10^{-4}	2.1x10^{-3}	(Panova et al., 2024)
Ascophyllum nodosum	5.2x10^{-2}	2.4x10^{-2}	5.3x10^{-2}	3.3x10^{-3}	3.7x10^{-2}	(Panova et al., 2024)
Laminaria digitata	2.3x10^{-2}	-	2.5x10^{-2}	-	2.0x10^{-2}	(Lobus & Udalov, 2021)
Red Algae						
Desmarestia menziesii	9.0-56.0x10^{-3}	-	-	-	-	(Runcie & Riddle, 2004)

Table 2. *continued*

Species	Sm	Eu	Gd	Tb	Dy	References
Himantothallus grandifolius	$8.0-42.0x10^{-3}$	-	-	-	-	(Runcie & Riddle, 2004)
Grateloupia filicina	$3.43x10^{-2}$	$8.58x10^{-3}$	$4.49x10^{-2}$	$6.57x10^{-3}$	$3.02x10^{-2}$	(Kano et al., 2002)
Grateloupia elliptica	$15.1-27.7x10^{-2}$	$4.38-7.45x10^{-3}$	$1.68-2.68x10^{-2}$	$2.28-4.16x10^{-3}$	$1.28-2.13x10^{-2}$	(Kano et al., 2002)
Grateloupia elliptica	$1.6-4.7x10^{-3}$	$4.2x10^{-5}$	$0.5-1.5x10^{-5}$	$2.6x10^{-5}$	$0.4-1x10^{-5}$	(Sakamoto et al., 2008)
Grateloupia okamurai	$1.14x10^{-2}$	$3.73x10^{-3}$	$1.71x10^{-2}$	$2.55x10^{-3}$	$1.52x10^{-2}$	(Kano et al., 2002)
Nemalion multifiduma	$7.62x10^{-2}$	$1.04x10^{-2}$	$5.57x10^{-2}$	$8.68x10^{-3}$	$3.73x10^{-2}$	(Kano et al., 2002)
Peyssonnelia squamaria	$4.0x10^{-1}$	$7.2x10^{-2}$	$3.4x10^{-1}$	$4.5x10^{-2}$	$2.3x10^{-1}$	(Squadrone et al., 2018)
Peyssonnelia squamaria	$2.5-2.6x10^{-1}$	$5.0-7.0x10^{-2}$	$2.6x10^{-1}$	$4.0-5.0x10^{-2}$	$1.8x10^{-1}$	(Squadrone et al., 2017)
Dudresnaya verticillata	$6.0x10^{-2}$	$1.0x10^{-2}$	$7.0x10^{-2}$	$1.0x10^{-2}$	$4.0x10^{-2}$	(Squadrone et al., 2017)
Phyllophora crispa	$2.6x10^{-1}$	$5.4x10^{-2}$	$2.6x10^{-1}$	$3.7x10^{-2}$	$1.9x10^{-1}$	(Squadrone et al., 2018)
Ganonema farinosum	$4.6x10^{-1}$	$1.0x10^{-1}$	$4.7x10^{-1}$	$8.0x10^{-2}$	$3.5x10^{-1}$	(Squadrone et al., 2017)
Laurencia obtusa	$2.5x10^{-1}$	$6.0x10^{-2}$	$2.7x10^{-1}$	$5.0x10^{-2}$	$2.1x10^{-1}$	(Squadrone et al., 2017)
Ceramium ciliatum	$3.9x10^{-2}$	$1.52x10^{-2}$	$4.0x10^{-2}$	$6.0x10^{-2}$	$4.0x10^{-2}$	(Ryabushko et al., 2024)
Delisea_fimbriata	$1.08x10^{-3}$	$4.8x10^{-4}$	$2.8x10^{-3}$	$3.8x10^{-4}$	$2.1x10^{-3}$	(Fu et al., 2000)
Gracilaria gracilis	-	$6.0x10^{-2}$	$5.0x10^{-2}$	$1.6x10^{-2}$	-	(Milinovic et al., 2021)

Table 2. *continued*

Species	Sm	Eu	Gd	Tb	Dy	References
Osmundea pinnatifida	-	4.0x10^{-2}	7.0x10^{-2}	1.2x10^{-2}	-	(Milinovic et al., 2021)
Porphyra sp	-	4.0x10^{-2}	2.0x10^{-2}	1.1x10^{-2}	-	(Milinovic et al., 2021)
Porphyra sp.	5.5-40.3x10^{-2}	1.5-8.7x10^{-2}	9.4-43x10^{-2}	1.4-5.7x10^{-2}	9.6-34.2x10^{-2}	(Liu et al., 2018)
Iridaea mawsonii	7.0-33x10^{-3}	-	-	-	-	(Runcie & Riddle, 2004)
Prasiola crispa	4.66x10^{-1}	-	-	-	-	(Runcie & Riddle, 2004)
Neopyropia yezoensis	2.03-3.23x10^{-2}	6.67-8.94x10^{-3}	3.91-6.56x10^{-2}	5.27-9.12x10^{-3}	3.64-6.5x10^{-2}	(Zhao et al., 2024)

4. HEAVY RARE EARTH ELEMENTS

Heavy rare earth elements (HREEs) comprise holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu), the final five lanthanides in the series. Because of their smaller ionic radii, these elements show distinct geochemical patterns compared to light and middle REEs, especially in binding behavior within minerals and biological systems (Cotton, 2025; Gupta & Krishnamurthy, 1992). Each element is associated with particular industrial uses. Ho is used in magneto-optical devices and some laser systems due to its high magnetic moment. Er is a key component in erbium-doped fiber amplifiers for telecommunications and is also used in surgical lasers (Binnemans et al., 2013). Tm is applied in X-ray devices and medical lasers, though its use is limited. Yb is used in fiber lasers, as an alloying element, and in some magnetic and catalytic processes (Balaram, 2019). Lu is one of the rarest and most expensive REEs and is used in PET scintillation crystals and catalysts (Runcie & Riddle, 2004). HREEs are more supply-restricted and are listed among the most critical REEs in strategic assessments by the EU and US (Bobba et al., 2020; IEA, 2025).

The concentrations of HREEs in macroalgae differed among algal groups, with the highest values reported in brown algae (Table 3). Ho concentrations ranged from 0.1×10^{-5} µg/g in *Ulva pertusa* and *Sargassum hemiphyllum* (Sakamoto et al., 2008) to 3.8 µg/g in *Cystoseira crinita* (Ryabushko et al., 2022). Er concentrations ranged from 0.1×10^{-5} µg/g in *S. hemiphyllum* (Sakamoto et al., 2008) to 16.1 µg/g in *C. crinita* (Ryabushko et al., 2022). Tm concentrations ranged from 1.0×10^{-5} µg/g in *S. hemiphyllum* and *Unidaria pinnatifida* (Sakamoto et al., 2008) to 2.9 µg/g in *C. crinita* (Ryabushko et al., 2022). Yb concentrations ranged from 0.2×10^{-5} µg/g in *Ulva pertusa* (Sakamoto et al., 2008) to 13.9 µg/g in *C. crinita* (Ryabushko et al., 2022). Lu concentrations ranged from 1.0×10^{-5} µg/g in *S. hemiphyllum* and *U. pinnatifida* (Sakamoto et al., 2008) to 22.0 µg/g in *C. crinita* (Ryabushko et al., 2022). The brown algae contain dominate with the highest HREE concnetrations, especially in *C. crinita* and *S. hemiphyllum*, whereas green algae record comperatively lower values. Red algae occupy an intermediate position, with some taxa showing moderate accumulation.

Table 3. Concentrations of Ho, Er, Tm, Yb, Lu in Various Macroalgae Species (µg/g dry weight)

Species	**Ho**	**Er**	**Tm**	**Yb**	**Lu**	**References**
Green Algae						
Ulva conglobata	2.43-10.5x10^{-3}	9.12-30.0x10^{-3}	1.0-4.62x10^{-3}	7.60-23.6x10^{-3}	1.21-4.59x10^{-3}	(Kano et al., 2002)
Ulva rigida	1.9x10^{-3}	4.1x10^{-3}	1.6x10^{-3}	2.7x10^{-3}	1.9x10^{-3}	(Ryabushko et al., 2024)
Ulva rigida	-	-	-	3.0x10^{-3}	-	(Milinovic et al., 2021)
Ulva fasciata	5.8x10^{-3}	1.81x10^{-2}	2.7x10^{-3}	1.65x10^{-2}	2.80x10^{-3}	(Fu et al., 2000)
Ulva pertusa	0.1-1.1x10^{-5}	35.5x10^{-5}	9.0x10^{-5}	0.2-1.5x10^{-5}	7.0x10^{-5}	(Sakamoto et al., 2008)
Chaetomorpha moniligera	5.78x10^{-3}	15.5x10^{-2}	2.29x10^{-3}	8.18x10^{-3}	2.24x10^{-3}	(Kano et al., 2002)
Codium bursa	1.2x10^{-1}	3.3x10^{-1}	4.0x10^{-2}	2.4x10^{-1}	3.2x10^{-2}	(Squadrone et al., 2018)
Codium bursa	1.8-15.0x10^{-2}	4.6-26.0x10^{-2}	7.0-50.0x10^{-3}	3.7-20.0x10^{-2}	6.0-43.0x10^{-3}	(Squadrone et al., 2017)
Codium fragile	2.04x10^{-3}	8.1x10^{-3}	1.17x10^{-3}	7.0x10^{-3}	1.2x10^{-3}	(Fu et al., 2000)
Codium tomentosum	-	-	-	8.0x10^{-3}	-	(Milinovic et al., 2021)
Flabella petiolata	4.1x10^{-2}	1.1x10^{-1}	1.4x10^{-2}	8.7x10^{-2}	1.3x10^{-2}	(Squadrone et al., 2018)
Flabella petiolata	1.8-1.6x10^{-2}	4.7-30x10^{-2}	7.0-60x10^{-3}	4.8-25.0x10^{-2}	8.9-5.10x10^{-3}	(Squadrone et al., 2017)
Acetabularia acetabulum	4.1x10^{-1}	1.1x10^{-1}	1.3x10^{-2}	7.6x10^{-2}	1.1x10^{-2}	(Squadrone et al., 2018)
Caulerpa cylindracea	1.5x10^{-1}	3.9x10^{-1}	4.9x10^{-2}	2.8x10^{-1}	4.0x10^{-2}	(Squadrone et al., 2018)
Caulerpa racemosa	5.0x10^{-2}	1.1x10^{-1}	-	8.0x10^{-2}	2.0x10^{-2}	(Squadrone et al., 2017)
Padina pavonica	0.056	0.15	0.019	0.11	0.010	(Squadrone et al., 2018)

Table 3. *continued*

Species	Ho	Er	Tm	Yb	Lu	References
Padina pavonica	5.0- $14.0x10^{-2}$	1.4- $3.0x10^{-1}$	1.8- $5.0x10^{-2}$	1.1- $2.4x10^{-1}$	1.6- $4.1x10^{-2}$	(Squadrone et al., 2017)
Padina okadai	$3.0x10^{-3}$	$8.5x10^{-3}$	$1.07x10^{-3}$	$6.3x10^{-3}$	$1.06x10^{-3}$	Fu et al., 2000
Padina sp.	4.0- $1.2x10^{-2}$	1.23- $3.6x10^{-1}$	1.0- $2.2x10^{-2}$	1.03- $2.9x10^{-1}$	1.0- $9.2x10^{-2}$	(Mashitah et al., 2012)
Sargassum tortile	5.11- $8.43x10^{-3}$	13.6- $24.1x10^{-3}$	1.45- $4.58x10^{-3}$	7.66- $22.1x10^{-3}$	2.99- $4.02x10^{-3}$	(Kano et al., 2002)
Sargassum hemiphyllum	$0.1\text{-}1.0x10^{-5}$	$0.1\text{-}1.9x10^{-5}$	$1.0\text{-}8.8x10^{-5}$	$1.2\text{-}8.8x10^{-3}$	$1.0\text{-}7.0x10^{-5}$	(Sakamoto et al., 2008)
Dictyota dichotoma	$5.0x10^{-2}$	$1.4x10^{-1}$	$1.9x10^{-2}$	$1.2x10^{-1}$	$1.9x10^{-2}$	(Squadrone et al., 2018)
Dictyota dichotoma	$7.0x10^{-2}$	$1.5x10^{-1}$	$2.0x10^{-2}$	$1.1x10^{-1}$	$2.0x10^{-2}$	(Squadrone et al., 2017)
Halopteris scoparia	$9.0x10^{-2}$	$2.5x10^{-1}$	$3.3x10^{-2}$	$2.1x10^{-1}$	$3.1x10^{-2}$	(Squadrone et al., 2018)
Halopteris scoparia	$7.3x10^{-2}$	$1.7x10^{-1}$	$2.5x10^{-2}$	$1.3x10^{-1}$	$2.2x10^{-2}$	(Squadrone et al., 2017)
Halopteris filicina	$1.0x10^{-2}$	$3.0x10^{-2}$	$3.1x10^{-2}$	$2.0x10^{-2}$	$3.0x10^{-3}$	(Squadrone et al., 2017)
Cystoseira sp.	$4.0x10^{-2}$	$1.2x10^{-1}$	$1.6x10^{-2}$	$9.6x10^{-2}$	$1.5x10^{-2}$	(Squadrone et al., 2017)
Cystoseira crinita	3.8	15.7-16.1	2.6-2.9	13.4-13.9	11.6-22.0	(Ryabushko et al., 2022)
Cystoseira barbata	1.5-2.2	9.5	0.9-1.9	5.9-9.1	3.2-13.0	(Ryabushko et al., 2022)
Gongolaria barbata	$3.8x10^{-3}$	$1.31x10^{-2}$	$2.2x10^{-3}$	$1.01x10^{-2}$	$2.5x10^{-3}$	(Ryabushko et al., 2024)
Ecklonia cava	$5.2x10^{-4}$	$1.69x10^{-3}$	$4.5x10^{-4}$	$2.08x10^{-3}$	$4.0x10^{-4}$	(Fu et al., 2000)
Undaria pinnatifida	1.0-7.0x10	0.5-1.5x10	1.0-4.0x10	0.5-2.5x10	1.0-5.0x10	(Sakamoto et al., 2008)

Table 3. *continued*

Species	Ho	Er	Tm	Yb	Lu	References
Undaria pinnatifida	-	-	-	$2.0x10^{-3}$	-	(Milinovic et al., 2021)
Fucus vesiculosus	$1.5x10^{-2}$	$1.0x10^{-2}$	$1.8x10^{-2}$	$8.0x10^{-3}$	$2.0x10^{-2}$	(Panova et al., 2024)
Fucus vesiculosus	-	-	-	-	<0,05	(Truus et al., 2001)
Fucus distichus	$1.2x10^{-2}$	$1.2x10^{-2}$	$1.4x10^{-2}$	$9.0x10^{-3}$	$1.6x10^{-2}$	(Panova et al., 2024)
Fucus distichus	$3.9x10^{-2}$	$1.15x10^{-1}$	$1.3x10^{-2}$	$9.7x10^{-2}$	$1.4x10^{-2}$	(Lobus & Udalov, 2021)
Fucus serratus	$7.0x10^{-3}$	$1.3x10^{-2}$	$1.0x10^{-2}$	$1.0x10^{-3}$	$1.3x10^{-2}$	(Panova et al., 2024)
Ascophyllum nodosum	$1.9x10^{-2}$	$3.2x10^{-2}$	$2.0x10^{-2}$	$1.8x10^{-2}$	$2.2x10^{-2}$	(Panova et al., 2024)
Laminaria digitata	$1.2x10^{-3}$	$4.0x10^{-3}$	$1.0x10^{-3}$	$3.0x10^{-3}$	$1.0x10^{-3}$	(Lobus & Udalov, 2021)
Red Algae						
Grateloupia filicina	$6.81x10^{-3}$	$15.7x10^{-3}$	$2.29x10^{-3}$	$8.35x10^{-3}$	$1.95x10^{-3}$	(Kano et al., 2002)
Grateloupia elliptica	3.31- $3.35x10^{-3}$	7.53- $13.3x10^{-3}$	2.03- $2.09x10^{-3}$	5.32- $12.7x10^{-3}$	1.32- $1.85x10^{-3}$	(Kano et al., 2002)
Grateloupia elliptica	$3.0x10^{-5}$	$1.3x10^{-5}$	$1.3x10^{-5}$	$1.3x10^{-5}$	$2.0x10^{-5}$	(Sakamoto et al., 2008)
Grateloupia okamurai	$3.82x10^{-3}$	$6.13x10^{-3}$	$1.89x10^{-3}$	$4.46x10^{-3}$	$1.23x10^{-3}$	(Kano et al., 2002)
Nemalion multifiduma	$8.44x10^{-3}$	$21.6x10^{-3}$	$3.34x10^{-3}$	$15.3x10^{-3}$	$3.12x10^{-3}$	(Kano et al., 2002)
Peyssonnelia squamaria	$4.3x10^{-2}$	$1.2x10^{-1}$	$8.3x10^{-2}$	$1.4x10^{-2}$	$1.2x10^{-2}$	(Squadrone et al., 2018)
Peyssonnelia squamaria	$4.0\text{-}5.0x10^{-2}$	$9.0x10^{-2}$	$1.3\text{-}7.0x10^{-2}$	$2.0\text{-}7.0x10^{-2}$	$1.0x10^{-2}$	(Squadrone et al., 2017)
Dudresnaya verticillata	$1.0x10^{-2}$	$2.0x10^{-2}$	$2.0x10^{-3}$	$1.3x10^{-2}$	$2.0x10^{-3}$	(Squadrone et al., 2017)
Ganonema farinosum	6.0x10[illegible]	1.9x10[illegible]	3.0x10[illegible]	1.5x10[illegible]	3.0x10[illegible]	(Squadrone et al., 2017)

Table 3. *continued*

Species	**Ho**	**Er**	**Tm**	**Yb**	**Lu**	**References**
Laurencia obtusa	5.0x10^{-2}	1.1x10^{-1}	2.0x10^{-2}	8.0x10^{-2}	2.0x10^{-2}	(Squadrone et al., 2017)
Ceramium ciliatum	1.4x10^{-2}	2.7x10^{-2}	1.0x10^{-2}	1.8x10^{-2}	1.1x10^{-2}	(Ryabushko et al., 2024)
Delisea_fimbriata	2.14x10^{-3}	5.9x10^{-3}	6.6x10^{-4}	3.8x10^{-3}	6.3x10^{-4}	(Fu et al., 2000)
Gracilaria gracilis	-	-	-	6.0x10^{-3}	-	(Milinovic et al., 2021)
Osmundea pinnatifida	-	-	-	1.4x10^{-2}	-	(Milinovic et al., 2021)
Porphyra sp	-	-	-	2.0x10^{-3}	-	(Milinovic et al., 2021)
Porphyra sp.	1.8-5.8x10^{-2}	5.7-17.2x10^{-2}	7.0-21.0x10^{-3}	4.9-12.9x10^{-2}	7.0-1.8x10^{-2}	(Liu et al., 2018)
Neopyropia yezoensis	6.88-13.04x10^{-3}	2.18-3.98x10^{-2}	2.12-4.46x10^{-3}	1.81-3.37x10^{-2}	2.23-4.82x10^{-3}	(Zhao et al., 2024)

5. YTTRIUM AND SCANDIUM

Yttrium (Y) and scandium (Sc) are chemically associated with rare earth elements but are not part of the lanthanide series. Y has a similar ionic radius to heavy lanthanides, particularly the Ho–Er group and is therefore often classified with HREEs in geochemical studies (Cotton, 2025; Gupta & Krishnamurthy, 1992). Sc belongs to group 3B of the periodic table and differs from lanthanides due to its smaller ionic radius and distinct binding behavior. Both elements have strategic industrial applications. Y is used in Y–Ba–Cu–O superconductors, laser crystals such as YAG (Yttrium Aluminium Garnet), and phosphor technologies due to its thermal resistance and optoelectronic properties (Binnemans et al., 2013). Y_2O_3-doped ceramics are applied in wear-resistant coatings and high-temperature materials. Sc is primarily used in aluminum–scandium alloys to improve strength and corrosion resistance, particularly in aerospace and defense industries (Balaram, 2019). Sc_2O_3-based materials are also used in cathode ray tubes, lamps, and some fuel cell components. Both elements have limited production sources and are monitored as critical raw materials due to supply risks (Bobba et al., 2020; IEA, 2025).

The concentrations of yttrium (Y) and scandium (Sc) in macroalgae are presented in Table 4. The brown algae, especially *Cystoseira* species, contained the most significant Y and Sc concentrations, while green and red algae exhibited considerably lower levels. The lowest Y concentration was measured in *Ulva rigida* at 7.3×10^{-2} µg/g (Ryabushko et al., 2024). The maximum was reported in *Cystoseira crinita*, ranging between 282.1 and 455.8 µg/g (Ryabushko et al., 2022). Sc concentrations were lowest in *Laminaria digitata*, with 6.0×10^{-2} µg/g and in *Fucus distichus*, with 2.95×10^{-1} µg/g (Lobus & Udalov, 2021). The highest Sc concentrations were found in *C. crinita* (2824.2–7724.6 µg/g) and *C. barbata* (2469.9–4283.5 µg/g) (Ryabushko et al., 2022). The brown algae exhibit the highest concentrations of Y and Sc by a wide margin. Green algae generally show lower to moderate levels, while red algae remain mostly at the low concentrations.

Table 4. Concentrations of Y, Sc in various macroalgae species (µg/g dry weight)

Species	**Y**	**Sc**	**References**
Green Algae			
Ulva rigida	7.3×10^{-2}	3.82×10^{-1}	(Ryabushko et al., 2024)
Codium bursa	2.9	0.74	(Squadrone et al., 2018)
Codium bursa	0.49-2.9	0.33-1.9	(Squadrone et al., 2017)
Flabella petiolata	0.97	1.8	(Squadrone et al., 2018)
Flabella petiolata	0.51-3.10	0.66-3.10	(Squadrone et al., 2017)
Acetabularia acetabulum	1.7	0.25	(Squadrone et al., 2018)
Caulerpa cylindracea	4.1	1.3	(Squadrone et al., 2018)
Caulerpa racemosa	1.3	0.5	(Squadrone et al., 2017)
Brown Algae			
Padina pavonica	0.7	2.2	(Squadrone et al., 2018)
Padina pavonica	1.74-3.3	0.45-0.94	(Squadrone et al., 2017)
Dictyota dichotoma	0.74	1.6	(Squadrone et al., 2018)
Dictyota dichotoma	1.61	1.13	(Squadrone et al., 2017)
Halopteris scoparia	1.2	2.4	(Squadrone et al., 2018)
Halopteris scoparia	1.87	0.871	(Squadrone et al., 2017)
Halopteris filicina	0.23-3.4	0.25-1.53	(Squadrone et al., 2017)
Cystoseira sp.	1.38	0.32	(Squadrone et al., 2017)
Cystoseira crinita	282.1-455.8	2824.2-7724.6	(Ryabushko et al., 2022)

Table 4. *continued*

Species	Y	Sc	References
Cystoseira barbata	84.9-221.9	2469.9-4283.5	(Ryabushko et al., 2022)
Gongolaria barbata	$3.09x10^{-1}$	$4.0x10^{-1}$	(Ryabushko et al., 2024)
Fucus distichus	1.146	$2.95x10^{-1}$	(Lobus & Udalov, 2021)
Laminaria digitata	$9.8x10^{-2}$	$6.0x10^{-2}$	(Lobus & Udalov, 2021)
Red Algae			
Peyssonnelia squamaria	0.49	1.4	(Squadrone et al., 2018)
Peyssonnelia squamaria	0.2-1.02	0.1-0.45	(Squadrone et al., 2017)
Dudresnaya verticillata	0.1	5.1	(Squadrone et al., 2017)
Phyllophora crispa	0.48	0.92	(Squadrone et al., 2018)
Ganonema farinosum	2.0	0.84	(Squadrone et al., 2017)
Laurencia obtusa	1.33	0.61	(Squadrone et al., 2017)
Ceramium ciliatum	$3.71x10^{-1}$	1.098	(Ryabushko et al., 2024)
Porphyra sp.	0.945-2.379	-	(Liu et al., 2018)

6. REE ACCUMULATION AND CELLULAR RESPONSES IN MARINE MACROALGAE

Macroalgae take part in the accumulation and movement of REEs in marine ecosystems because of their high biomass yield. Their photosynthetic efficiency and rapid growth enable them to produce large amounts of biomass in shorter periods compared to terrestrial plants. Brown, red, and green macroalgae show different accumulation patterns, but all can absorb REEs from dissolved seawater or suspended particles, functioning as carriers in the transfer of elements through food webs (Bergsten-Torralba et al., 2020; Fu et al., 2000; Neira et al., 2022). Brown algae are reported to reflect seawater composition more consistently and are often used as biological indicators in monitoring (Fu et al., 2000). Their wide distributin and natural productivity make them a low-cost and sustainable alternative to conventional mining (Biriș-Dorhoi et al., 2020; Cunha et al., 2024; Rétif et al., 2024). Unlike terrestrial biomass, macroalgae do not require irrigation or agricultural land. Biomass accumulated along coastlines by storms or seasonal currents can also be collected and used for energy generation or elemental recovery without additional production costs (Coelho et al., 2025; Costa et al., 2020). They also concentrate REEs on their tissues and transfer them to higher trophic levels through food chains (Neira et al., 2022). Macroalgal beds provide habitats and feeding grounds for benthic communities, contributing to REE cycling in ecosystems (Bergsten-Torralba et al., 2020). Their structural properties support this capacity especially, sulfated polysaccharides and functional groups such as carboxyl and hydroxyl in cell walls show affinity for REEs and other metals (Costa et al., 2020; Trapasso et al., 2021). This enables binding of dissolved ions as well as colloidal or particulate forms. Structural and metabolic differences among species result in variation in accumulation patterns, which can limit their use in monitoring (Aharchaou et al., 2020; Cunha et al., 2024). Recent studies have examined physiological mechanisms underlying these differences. REE ions may interact with calcium transport systems in plasma membranes due to similarities with Ca^{2+}, affecting cellular homeostasis (Piarulli et al., 2021). In brown algae, polysaccharides such as alginate and guluronic acid bind REEs through carboxyl and sulfate groups. Ion-exchange processes involving Ca^{2+} substitution and sulfate complexation also contribute to this binding (He et al., 2021; Jurkowski et al., 2022; Ordóñez et al., 2023). Photosystem II can also be affected. REEs binding to the Mn-chloride complex may disrupt the electron transport chain, lowering photosynthetic efficiency and oxygen production (Figure 1). In *Ulva* and *Gracilaria* species, this has been shown to limit light-harvesting capacity (Figueiredo et al., 2022; Trapasso et al., 2021).

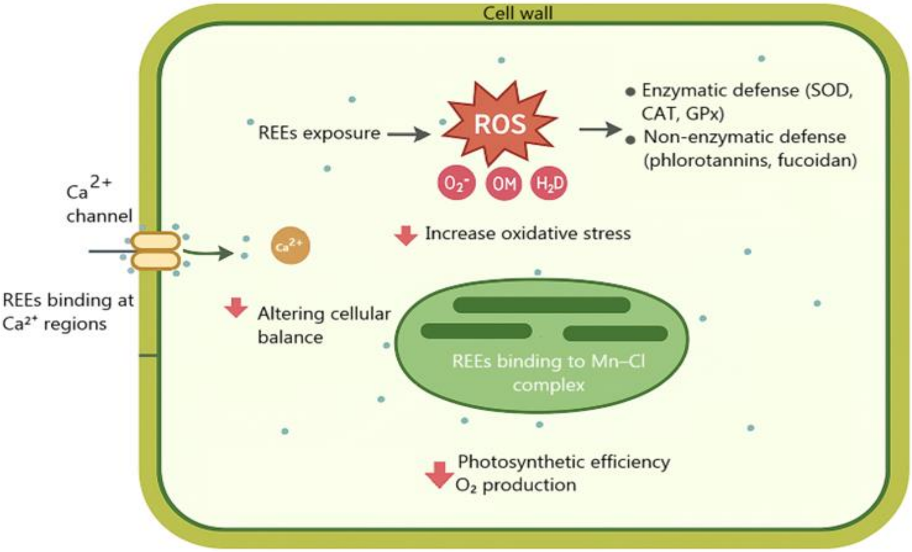

Figure 1. Schematic representation of the cellular effects of REEs in macroalgae.

REE exposure is also associated with increased oxidative stress, reflected by the accumulation of reactive oxygen species in cells. Macroalgae respond through both enzymatic and non-enzymatic defense mechanisms (Figure 1). Oxidative damage is reduced by enzymes including superoxide dismutase, catalase, and glutathione peroxidase. In brown algae, phenolic compounds like phlorotannins and fucoidan function as natural antioxidants (Gómez & Huovinen, 2020). These mechanisms contribute to stress tolerance and support the sustained potential for REE bioaccumulation. Brown algae are often better indicators of seawater trace-element chemistry, whereas red and green algae can more strongly reflect signals from suspended particulates and local sediments (Diganta et al., 2023; Morrison et al., 2008; Ryan et al., 2012). Other marine organisms, such as coralline algae and bivalves, can accumulate REEs in patterns similar to those of seawater and have therefore been proposed as useful complementary indicators (Barrat et al., 2024; Kapranov et al., 2024). Environmental conditions affect performance. Variations in salinity, shifts in temperature, and competing ions alter metal-binding capacity and cause differences in removal efficiency (Ak et al., 2022; Ciobanu et al., 2024; Costa et al., 2020). Macroalgae show high removal efficiency for some elements, such as mercury, but lower performance for others with weak binding affinity, such as cadmium (Costa et al., 2020).

7. CONCLUSION

Macroalgae have a role in the biological cycling of REEs, along with Y and Sc. The observed accumulation patterns across element groups and species show that macroalgae are not a homogeneous group, and both taxonomic differences and environmental conditions influence their uptake capacities. LREEs were found at higher levels in brown and some red algae, while green algae showed lower concentrations. MREEs and HREEs were typically recorded at lower levels overall. However, higher accumulation was observed in species from specific genera, such as *Cystoseira* and *Sargassum* among brown algae, and *Grateloupia* and *Nemalion* among red algae. Y and Sc were low in most species, with the highest values recorded in *Cystoseira* species. Macroalgae show high biomass production, easy availability in coastal areas, and cell wall functional groups that enable metal uptake. High biomass production, accessibility in coastal regions, and the presence of functional groups in cell walls support their uptake capacity. However, environmental variability, interspecies differences, and low binding affinity for certain elements remain limited factors. Rising global demand for critical components, together with the environmental impacts of conventional mining and supply security issues, has increased the strategic importance of macroalgae as renewable and locally sourced resources. High-throughput biotechnological approaches, including surface modification, biomining techniques, and omics-based analyses, may enhance the usability of this potential. Overall, macroalgae represent a unique resource as both biological reservoirs and sustainable raw material sources. Effective utilization of this potential depends on species- and habitat-specific considerations, environmental monitoring, and proper technological applications.

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CHAPTER 6

SEA LETTUCE (*Ulva* sp.): THE EMERALD OF THE SEA THAT POTENTIALITY ROLE IN THE FUTURE OF AQUACULTURE

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INTRODUCTION

Seaweeds, defined as benthic marine macroalgae visible to the naked eye, occupy diverse ecological niches along coastlines worldwide. These habitats include shallow and deep coral reefs and inter-reef zones, sandy substrates, seagrass beds, mangrove root systems, and rocky intertidal zones. Seaweeds are found in almost all aquatic environments, from marine waters to brackish and freshwater systems. They are distributed in geographic areas extending from tropical equatorial islands to polar latitudes (Diaz-Pulido and McCook, 2008; Corrigan et. al., 2025).

In the past, these marine plants, also known as weeds, were given the name "seaweed" because their uses were not fully understood. Because the historical uses of weeds (seaweed) were not fully understood, the term "seaweed" has become more common. Ancient Chinese, Greeks, and Romans were familiar with algae and cultivated some varieties for food. The scientific study of algae began in the late 18th century with Pehr Osbeck's identification and naming of *Fucus maximus* (now *Ecklonia maxima*) in 1757, followed by work from Dawson Turner and Carl Adolph Agardh. The first significant taxonomic groupings were established in the late 19th century by J.V. Lamouroux and William Henry Harvey-the latter called the "father of modern physiology" for dividing algae into four main groups based on pigmentation. Physiology emerged as a distinct field in the late 19th and early 20th centuries, with Friedrich Traugott Kützing advancing descriptive studies. Beginning in 1889, Kintarô Okamura provided detailed descriptions and distribution analyses of Japanese coastal algae. Serious study progressed with Edward Arthur Lionel Batters' 1902 British Catalogue of Marine Algae (Batters, 1902). Some of the most comprehensive systematic reviews available today, available on Algaebase and the Maryland biodiversity website, report the presence of thousands of species, representing the most comprehensive assessment to date. Macroalgae are divided into three classes: green phylum, red phylum and brown phylum of macroalgae (Chlorophyta, Rhodophyta and Phaeophyta), which they are the commercially important of the globally produced macroalga. *Ulva lactuca*, also known by the common name sea lettuce that is classified as a green seaweed within the phylum Chlorophyta, which comprises approximately 4,300 known species (Chapman, 2008). About 90% of Chlorophytes inhabit freshwater environments, with many species being unicellular. Sea lettuce refers to a group of closely related multicellular marine species belonging to the genus *Ulva* sp. (Fig. 1) and the class Ulvophyceae. In current science, *Ulva* species belong to the chlorophytes, a group comprising three traditional collections *(Ulvophyceae, Trebouxiophyceae, and Chlorophyceae*) that have not yet been fully clarified (Dominguez & Loret, 2019). A synonym is *U. fenestrata*, referring to its "windowed" or "holed" appearance (Colin, 2020). Despite its common name, it is not a lettuce. Today, it is used as a generally accepted form of taxonomic expression in nomenclature. "Phylum: Chlorophyta, Classis: Ulvophyceae, Order: Ulvales, Family: Ulvaceae, Genus: *Ulva* Species: *Ulva* spp.". The *Trebouxiophyceae* and *Chlorophyceae* diversified significantly in terrestrial and freshwater environments, while the *Ulvophyceae* dominated marine environments (Leliaert, 2012). True to its name, sea lettuce resembles leaves of green lettuce. The color is often bright green but can range from dark green to almost yellow.

Sea lettuce is not a particularly large seaweed, being no more than

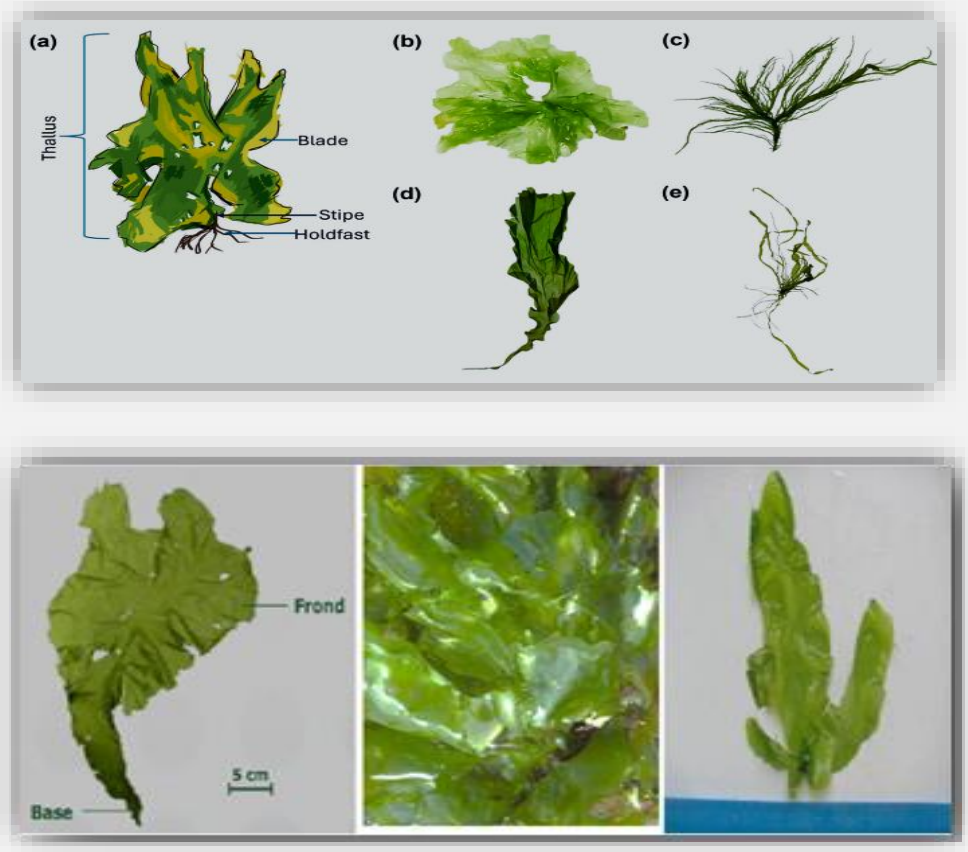

Figure 1. Illustrative representation of *Ulva* (a) and photographic representations of *Ulva* species: *U. lactuca* (b); *U. prolifera* (c); *U. linza* (d); and *U. flexuosa*. Reproduced with permission from Xia et al., 2023. This figure, copyright (2023) Elsevier, has been evaluated for scientific use with a subscription to Ege University (Pari et., al., 2025)

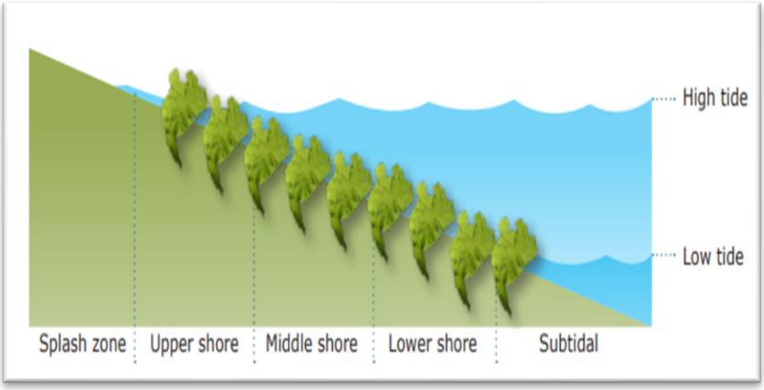

Figure 2. Distribution characteristics and habitat of *Ulva* sp. in marine ecosystems (Soler-Vila et al., 2022).

Sea lettuce is not a particularly large seaweed, being no more than 30-40 cm in size; but its bright green color and abundance make it especially noticeable in the marine environment. The species is easily recognized by tides and currents in the ecosystem and in close proximity to the coast (Fig. 2). The blade is described as being a leaf like, low thallus, and at just two cell layers it’s quite thin, with a sheetlike appearance (Fig. 1). The leaves can be narrow or broad, and single or multi-lobed. They are often orbicular or oval with wrinkle edges and riddled with holes or perforations. The blade lacks a stipe and instead emerges almost directly from the holdfast. *Ulva* species are present in the ecosystem year-round (Fig. 3). They are generally abundant in spring, summer, and autumn (Soler-Vila et al., 2022). Many *Ulva* species may be considered 'weeds' as they grow in large numbers in coastal areas of the sea, in sheltered, nitrogen-rich coastal environments such as bays. (Fig. 3). In macroalgae science, the current systematic use of *Ulva lactuca*, the genotype of *Ulva*, in its nomenclature has not yet been clarified. Genetic analyses have been performed on the With the development of phylogenies analysis methods, scientists have performed genetic analyses on the Linnaean holotype of *Ulva lactuca*, the *U. fenestrata* holotype, the *Ulva fasciata* epitype, the *Ulva lobata* lectotype and the *Ulva stipitata* lectotype. The *U. lactuca* holotype is nearly identical in its *rbcL* sequence to the epitype of *U. fasciata*, a warm-temperate to tropical species rather than the cool-temperate species to which the name *U. lactuca* is usually applied. The *U. lactuca* holotype is assumed to be of Indo-Pacific rather than Northern European origin. Analyses indicate that *U. lobata*

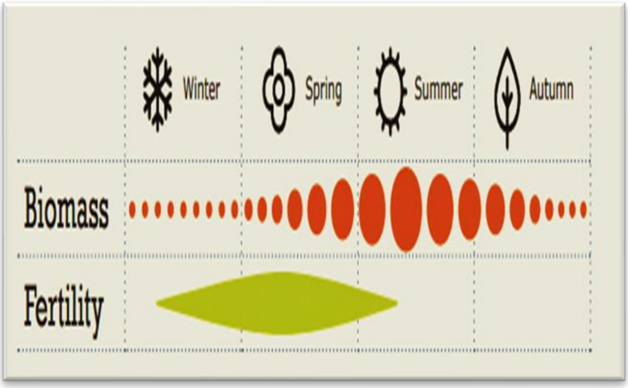

Figure 3. Note: The seasonal characteristics can vary slightly from year to year depending on climate and ecological conditions. (Soler-Vila et al., 2022).

and *U. fasciata* are heterotypic synonyms of *U. lactuca. Ulva fenestrata* is the oldest name used in the Northern Hemisphere for the cool-temperate Atlantic and Pacific species; *U. stipitata* is a novel synonym. DNA sequencing of type specimens provides a definitive method for naming *Ulva* species. (Hughey et al., 2019). Despite this, the organism's diverse adaptations allow it to have a very wide distribution from warm seas to temperate-tropical marine regions. Nowadays, numerous studies are being conducted on the benefits of seaweed, and the data found in these studies is being integrated into daily life. Because the term "seaweed" is common among scientists and the public, this term continues to be used, although the term "plants of the sea" is occasionally used in the scientific literature. More important than the name is their use and wide applications. Seaweeds grow naturally in seawater and thrive where nutrients and sunlight are sufficient. Earth is 71% covered by seawater and hosts abundant marine plants including phytoplankton, cyanobacteria, seaweeds, and seagrasses; seaweeds notably support coastal livelihoods (Amir et.al., 2025). They flourish in shallow or nearshore waters, estuaries, and brackish environments with suitable substrates such as dead corals, rocks, stones, and gravel for attachment. They are renewable and economically valuable marine resources for humanity (Misurcova, 2012; Pal et.al., 2014). Macroalgae perform critical ecological functions in marine ecosystems (Corrigan et. al., 2025). They contribute significantly to oxygen production and serve as reservoirs for atmospheric carbon dioxide (Roleda and Hurd, 2019). Additionally, numerous species contribute to the stability of coastal ecosystems by providing protective habitats that support a wide variety of flora and fauna (Rahikainen et al., 2021; Taniguchi et.al., 2022). Seaweeds also serve as an important food source for a variety of marine organisms Given their ecological importance, macroalgae are essential biotic components in the protection and conservation of coastal environments (Unsworth et. al., 2014). Throughout history, seaweeds have been used as a food source in human nutrition (McHugh et.al., 2003). Today, thanks to advances in technology, they are also used in medical research, pharmaceuticals, cosmetics, paper production, aquaculture sectors, animal nutrition, wastewater treatment, and fertilizer sectors. Furthermore, seaweed provides a potentially economically sustainable biomass feedstock for the energy and biotechnology industries, including future biofuel production systems, bioremediation, and integrated aquaculture systems (Bolton et.al., 2016; El-Beltagi et. al., 2022; Abo-Raya et.al., 2023). Scientific records indicate that seaweeds have been used as a source of nutrients in human nutrition throughout history. Nowadays, thanks to advances in technology, they are also used in medical research, pharmaceuticals, cosmetics, paper production, animal nutrition, wastewater treatment, and fertilizer (Campoy, 2023). Furthermore, seaweed provides a potentially economically sustainable biomass feedstock for the energy and biotechnology industries, including future biofuel production systems, bioremediation, and integrated aquaculture systems. Today, the benefits of marine plants (seaweeds) have been well studied in various ways and are incorporated into people's daily lives (Fig. 4). Because the term "seaweed" is already popular among scientists and the public, it is difficult to change it. However, although some scientists occasionally use the term "marine plants," "seaweed" remains widely accepted in scientific literature. More important than the name itself is the use of these seaweeds and their wide range of applications. In summary, seaweeds have always been one of the important life resources that nature has offered to us humans throughout history and have always been one of the treasures of social, commercial and environmental importance (Fig 4-5). Seaweeds naturally grow in seawater and grow faster where nutrients and sunlight are sufficient. The Earth is 71% covered by seawater and hosts a large number of marine plants. Marine plants include phytoplankton, cyanobacteria, seaweeds, and seagrasses, with seaweeds known to provide better livelihoods for coastal communities (Subba Rao, et.al., 2024). Seaweeds thrive in shallow or nearshore marine waters, estuaries, and brackish water environments where dead corals, rocks, stones, gravel, and other suitable substrates are available for attachment (Fig. 2). They are renewable and economically valuable marine resources for humankind (Lopez-Rivas and Cardenas, 2024). Seaweed cultivation is predominantly conducted on rope rafts deployed at sea. However, the cultivation of algae intended for high-value applications; such as cosmetics, bioactive metabolites, and human consumption, necessitates propagation from spores, which requires the maintenance of the microscopic phase within land-based infrastructure. The marine deployment of rope rafts (Fig. 7) presents two notable limitations. Firstly, it fails to provide the controlled growth conditions

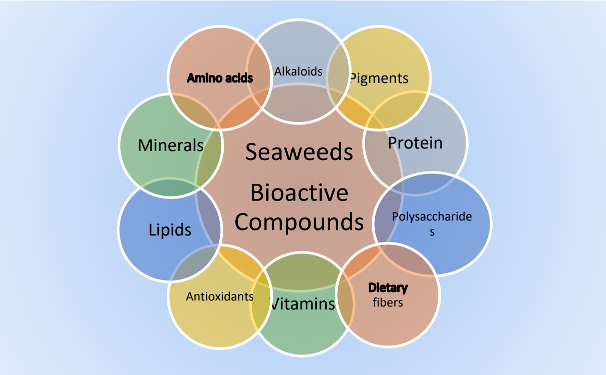

Figure 4. Main bioactive compounds from marine seaweeds.

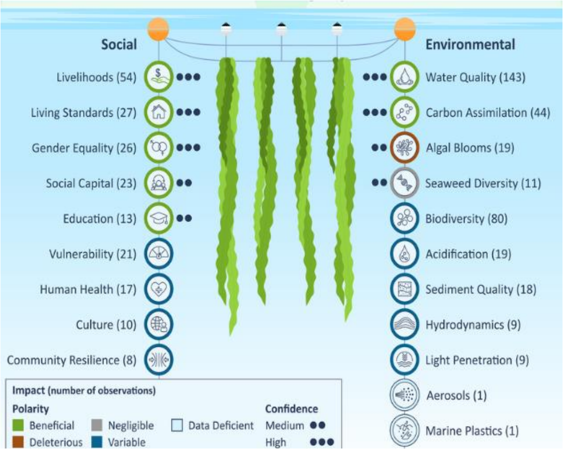

Figure 5. Social and environmental impact categories of seaweeds (Spillias et.al., 2023).

deemed essential by Hafting et al., (2015) for the consistent production of high-value seaweed products characterized by stable properties and chemical composition. Secondly, although integrated multi-trophic aquaculture (IMTA) at sea is acknowledged as a viable and promising approach effective management and control are more readily achievable in land-based systems (Fig 8). These terrestrial systems facilitate cultivation through either complete, albeit costly, or partial recirculation which are increasingly recognized as critical to the sustainable advancement of aquaculture in the future (Bolton et.al., 2016). Harvesting *Ulva* species is much simpler than harvesting microalgae because the culture system can be washed, and the biomass can be easily collected using a net or similar apparatus. *Ulva* species selected from specific habitats or cultivated in aquaculture systems can remain permanently vegetative when maintained at high densities, eliminating the problem of fragmentation during propagation. Furthermore, due to the influence of sea currents and the spatial arrangement of the culture, *Ulva* experiences significantly less self-shading than microalgae. During cultivation, intermittent light penetrates the bottom of shallow commercial pond waterways, even at high densities, just prior to harvest (Bolton et. al., 2016; BIM, 2023).

Traditional annual production of *Ulva* culture in water systems worldwide ranges from approximately 20 to 26 g of dry weight per square meter per day. This value in cultivation is consistent with typical annual microalgal biomass production in outdoor systems, depending on climatic and physicochemical conditions. It is noteworthy that year-round *Ulva* production is similar to microalgae cultivation in large-scale, open-air commercial systems, but microalgae can achieve significantly higher production rates in shorter periods in costly bioreactor setups.

2.COSMOPOLITAN SEA LETTUCE CULTIVATION.

The green pigmentation in seaweeds is primarily attributed to the presence of chlorophylls a and b, beta-carotene (a yellow-orange pigment), and various characteristic xanthophylls exhibiting yellowish or brownish hues. These pigments are present in proportions similar to those found in higher land plants and contribute to the distinct green color observed in these marine macroalgae. Similar to that of higher land plants, the algae's nutritional composition consists of starch and certain lipid fractions, which contribute to energy storage and metabolic functions (Bolton et.al., 2016).

Among green seaweeds, *Ulva* species, often referred to as the "emerald of the sea," have attracted significant attention due to their ease of cultivation and harvesting. Therefore, they have significant potential to compete with microalgae production in terms of biomass yield and utilization. While some *Ulva* spp. were considered harmful or opportunistic "weeds" that thrive in nutrient-rich, sheltered coastal waters and are often associated with eutrophication, advances in algal biology and aquaculture technology have enabled the controlled cultivation of these species. This exploitation is shifting the paradigm from viewing *Ulva* as merely a problematic bloom-producing species to a valuable resource for commercial and environmental applications.

Ulva cultivation methodologies vary globally based on the biological characteristics of the local species, prevailing climatic conditions, logistical constraints, and economic factors, and encompass a variety of approaches, from offshore methods to land-based integrated multi-trophic aquaculture systems (Fig.6).

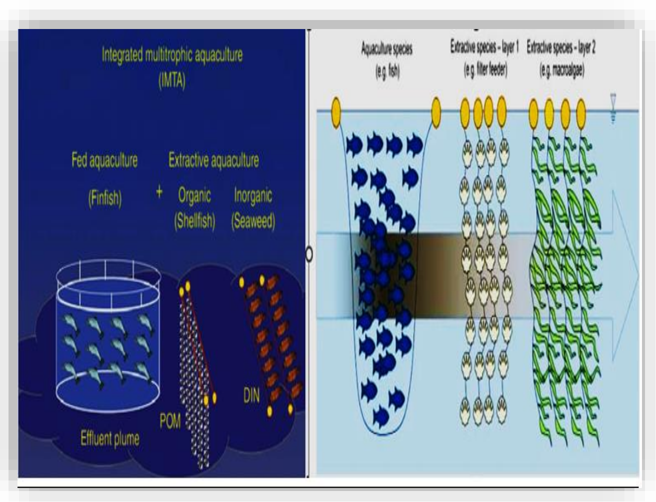

Figure 6. Integrated multi-trophic aquaculture (IMTA) in offshore sea (Chopin et.al., 2008; Lothman & Sewilam, 2023).

Optimization of these methods reflects the regional adaptations necessary to maximize yield, minimize costs, and ensure sustainability. Ultimately, *Ulva* cultivation represents a viable and scalable marine biomass production system that supports food security, bioenergy, and bioremediation goals within the framework of the expanding blue economy. Most seaweed cultivation is carried out in marine environments using rope rafts and cages.

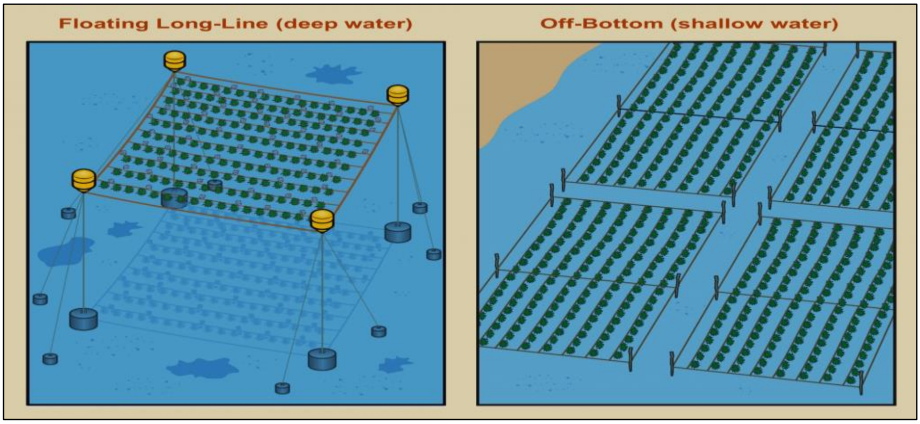

Figure 7. Examples of seaweed production methods. Photo © Colin Hayes/TNC by Reef Resilience Network, 2025.

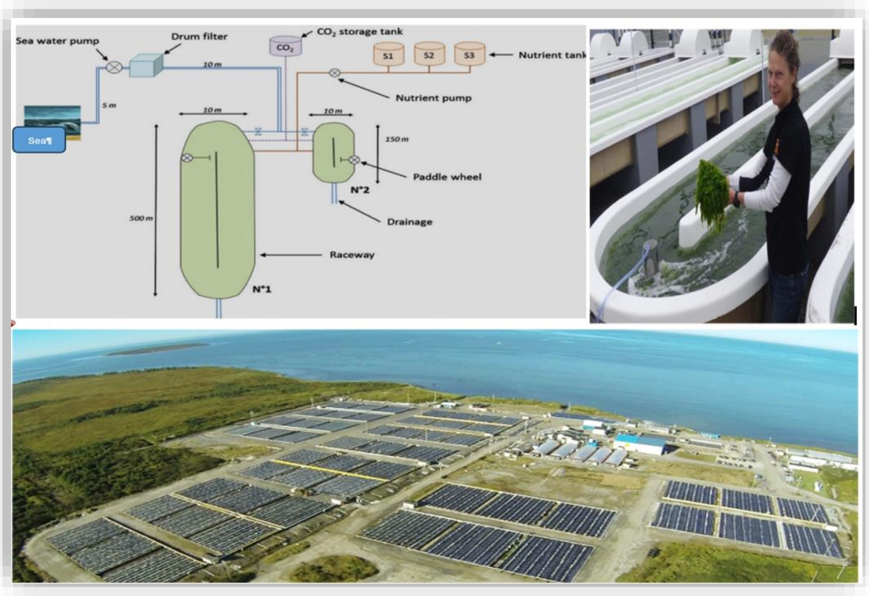

Figure 8. Seaweed cultivation system in coastal land areas (Brockmann et. al., 2015; Rennie, 2020; AS, 2022).

Seaweeds cultivated for high-value human food products are typically propagated from spores, which requires land-based infrastructures such as laboratories and closed production systems to maintain microscopic life stages. However, deploying rafts (Fig. 7) in estuarine or marine environments in open-system production presents two key limitations. First, these natural environments offer limited control over growth conditions, which Hafting et al., (2015) identified as essential for the production of future high-value seaweed products with consistent biochemical and structural properties. Second, while integrated multi-trophic aquaculture (IMTA) can be effectively implemented in open-sea environments (Troell et al., 2009), land-based systems offer greater controllability and operational simplicity. Land-based aquaculture allows the implementation of full or partial recirculation systems, which are increasingly recognized as an important element for the future development of sustainable and efficient aquaculture practices (Schuenhof et. al., 2003; Nobre et al., 2010). These controlled conditions support optimized growth parameters, biosecurity, and resource recycling, improving product quality and environmental sustainability. Current monoculture practices and perceptions within the aquaculture sector have shifted toward the expansion of carnivorous species containing lower-trophic organisms in ecologically balanced aquaculture farms (Chopin et.al., 2008). In this context, various production approaches should be implemented to ensure the diversity and sustainability of the aquaculture sector.

3.THE IMPORTANCE OF IMTA IN SEA LETTUCE CULTIVATION

Integrated Multi-Trophic Aquaculture (IMTA) has significant potential to increase sustainability in *Ulva* (sea lettuce) production. IMTA systems involve the co-cultivation of species from different trophic levels, creating synergistic relationships where byproducts and waste from one species are used as inputs or nutrients for others (Hossain, et.al., 2022), (Fig. 9). This circular nutrient recycling reduces environmental impacts such as eutrophication and improves water quality, making aquaculture more sustainable. With rapidly growing human populations and increasing environmental pollution, the co-cultivation of algae such as *Ulva* with various aquaculture species has become essential within Integrated Multi-Trophic Aquaculture (IMTA) systems.

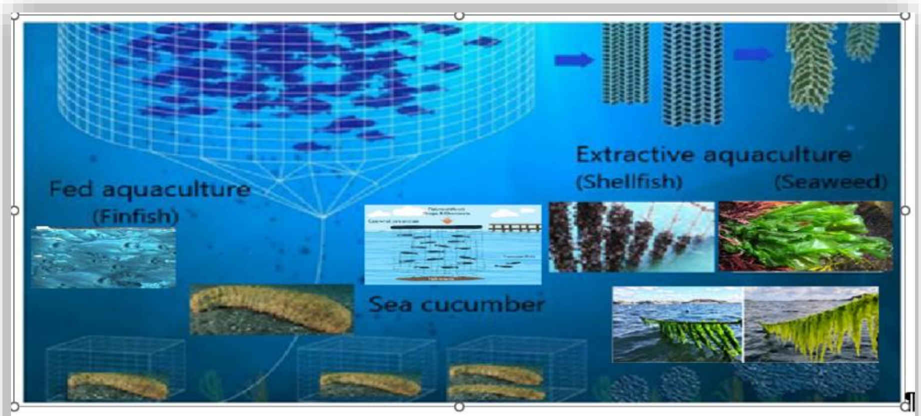

Figure 10. In general terms, an integrated multi-trophic aquaculture (IMTA) facility is a combination of fed-batch aquaculture (e.g. finfish) and organic extractive fish farming (e.g. shellfish-seaweed), utilizing particulate organic matter through extractive varieties (e.g. seaweed) that absorb the benefits of dissolved inorganic nutrient enrichment (redrawn from IMPAQT H2020 Project).¶

With rapidly growing human populations and increasing environmental pollution, the co-cultivation of algae such as *Ulva* with various aquaculture species has become essential within Integrated Multi-Trophic Aquaculture (IMTA) systems. IMTA, in particular, facilitates the growth of *Ulva* by utilizing nutrient-rich wastewater (such as nitrogen compounds) from other aquatic species, such as various fish species or sea urchins. *Ulva* acts as a biofilter, reducing nutrient pollution while producing valuable biomass. Furthermore, integrating *Ulva* sp. with other aquaculture species can increase overall productivity, resource efficiency, and economic viability compared to monocultures. However, successful implementation of IMTA with *Ulva* species requires careful system design, such as managing nitrogen fluxes through feed and sludge mineralization to avoid nutrient limitations for Ulva growth. Despite challenges in social acceptability and scalability, IMTA offers a promising sustainable production model by aligning ecological function with aquaculture profitability and circular economy principles. *Ulva* algae have the potential to support profitable multi-species production by effectively biofiltering nitrogenous waste from sea urchin cultures in recirculating IMTA systems. IMTA reduces the ecological footprint of aquaculture, ensuring the well-being of the produced species. It also enhances environmental sustainability by improving product quality and contributing to ecosystem health, including mitigating ocean acidification. The principle of circular nutrient utilization in IMTA systems converts waste streams into valuable biomass through interspecies interactions, resulting in higher yields and ecosystem balance than monoculture production. Integrating IMTA into the ecosystem plays a key role in unlocking *Ulva'* s potential for sustainability and benefits in aquaculture by optimizing nutrient cycles, reducing waste, and producing diverse, high-value outputs in environmentally responsible ways. Today, due to rapidly developing technology, the increasing demand for food and various metabolites due to the growing human population, and the pollution of nature, the production of many similar algae, such as *Ulva* in IMTA systems, along with other aquaculture species, has become a necessity.

4.GLOBAL *ULVA* SPECIES MARKET SIZE AND FORCAST: 2025-2032

The global market for *Ulva* species is projected to be valued at approximately USD 1.46 billion in 2025. It is anticipated to grow significantly, reaching an estimated value of USD 2.73 billion by 2032. This growth corresponds to a compound annual growth rate (CAGR) of 9.4% over the forecast period from 2025 to 2032. Asia Pacific is set to lead the global *Ulva lactuca* market in 2025 with a 31.6% share, driven by strong consumer demand for sustainable and nutritious food products. North America, with a 10.5% share in 2025, will be the fastest-growing region, fueled by the rising health awereness and the growing trend towords plant-based diets are driving the demand for *Ulva lactuca* in the region.

5.KEY TAKEAWAYS OF THE SEA LETTUCE MARKET

The fresh *Ulva lactuca* production sector is expected to account for 41.6% of the aquaculture market in 2025, with the food industry segment expected to hold 38.9% of the sector's market share in 2025, and e-commerce retail is expected to capture 47.8% of the market in 2025. All of these results reflect an ongoing shift in the sector toward fresh produce offerings, food applications, and digital commerce channels, within the broader trend toward sustainability and plant-based ingredients.

6.CONCLUSION

Seaweeds have long been vital natural resources that have added tremendous value to human societies throughout history. These marine plants support a variety of social, commercial, and environmental functions, making them treasures of nature (Fig 5-11). Since ancient times, coastal communities have relied on seaweeds not only as a food source but also for medicinal purposes, agricultural and aquaculture development, and industrial applications. Their versatility is evident in their roles in traditional cuisines, as sources of bioactive compounds for fish and other aquatic organisms, and as natural fertilizers that enhance soil health. Today, commercially available seaweeds like *Ulva* species have become a major industry fueling economies worldwide. Many seaweeds like *Ulva* have become essential products for sectors such as food production, cosmetics, pharmaceuticals, and biofuels.

Sustainability of seaweed farming offers a promising avenue for addressing global challenges such as food security, climate change mitigation, and marine ecosystem restoration. Environmentally, seaweeds contribute to biodiversity, provide habitats for marine life, and play a key role in carbon sequestration, helping to reduce greenhouse gas concentrations. Their ability to absorb excess nutrients in coastal waters also combats pollution and supports the health of marine ecosystems. In substance, seaweeds (e.g. *Ulva* sp.) represent a versatile resource of enduring social, commercial, and environmental importance (Fig 5-11).

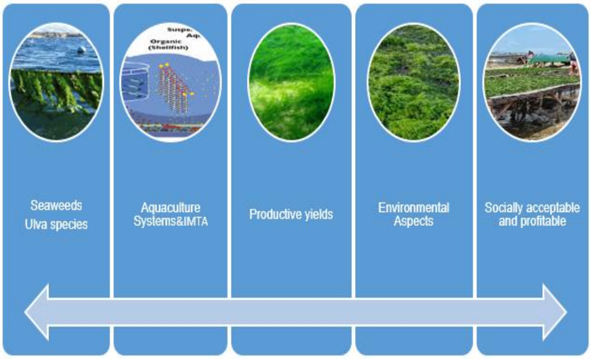

Figure 11. The importance of *Ulva* sp. cultivation in its sustainability and socio-economic context.

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CHAPTER 7

EPIGENETICS IN AQUACULTURE: MECHANISMS, APPLICATIONS, AND FUTURE DIRECTIONS

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INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors globally, accounting for a significant portion of the fish consumed worldwide today. Driven by rising demand for high-quality protein, climate-induced shifts in capture fisheries, and technological innovations, aquaculture is expected to have a significant growth by 2030. This expansion, as useful as it is for food security and economy, brings biological and environmental challenges including well recognized such as diseases outbreaks, climatic stress, feeds sustainability and genetic bottleneck.

Selective breeding has been the primary and sole method of genetic improvement in aquaculture over recent decades. Selection for growth, feed conversion efficiency, disease resistance and fillet quality have resulted in significant improvements for the main species *Salmo salar* (Atlantic salmon), *Oreochromis niloticus* (Nile tilapia) and *Penaeus vannamei* (whiteleg shrimp) since their brood can be selected on a genomic level (Gjedrem & Rye, 2018). The latter three strategies utilize the genetic programs, which are progeny test based selection, GBLUP Selection and crossbreeding. However, traditional breeding has several limitations:

- It is time-consuming, often requiring multiple generations to establish genetic gains.
- It may narrow genetic diversity, leading to inbreeding depression.
- It lacks responsiveness to rapidly changing environmental conditions.
- It does not capture non-genetic phenotypic plasticity, which plays a crucial role in early life stages.

In this context, epigenetics offers a promising layer of biological regulation that complements and improves selective breeding. Epigenetic mechanisms mediate heritable, but reversible, modifications to gene expression in response to environmental, nutritional, or stress-related stimuli, without altering the DNA sequence itself (Bird, 2002; Anastasiadi & Piferrer, 2019).

Epigenetic modifications, including DNA methylation, histone modifications, and non-coding RNA (ncRNA) regulation, offer a dynamic and reversible means by which organisms adapt to environmental changes. These modifications can be stable, particular to a certain situation, and even passed down from one generation to the next, which makes them very important for sustainable aquaculture systems (Anastasiadi & Piferrer, 2019; Piferrer, 2021;

Bird, 2002). Unlike fixed genetic mutations, epigenetic markers can be dynamic and potentially transgenerational, thus offering a way to program desirable traits more rapidly and flexibly (Piferrer, 2021).

Epigenetics, which is referring to heritable changes in gene activity that do not alter the DNA sequence, has emerged as a promising area of research in aquaculture. With the industry under pressure from disease threats, climate stress, and the push for higher efficiency, traditional breeding methods often are not enough. Epigenetic tools add a new layer of biological insight, opening up opportunities to boost fish health, growth, and adaptability in more targeted and flexible ways. As aquaculture systems become increasingly intensive and data-driven, understanding and harnessing epigenetic processes is emerging as a key strategy in sustainable fish farming. Whether used to fine-tune stress responses, improve feed utilization, modulate immune function, or precondition offspring through broodstock programming, epigenetic tools may redefine how aquaculture improves in the 21st century.

EPIGENETIC PATHWAYS IN FISH

Epigenetic regulation plays a key role in how fish adapt to changing environmental conditions. The three primary epigenetic mechanisms, DNA methylation, histone modifications, and non-coding RNA-mediated regulation, act together to modulate gene expression without changing nucleotide sequences (Figure 1).

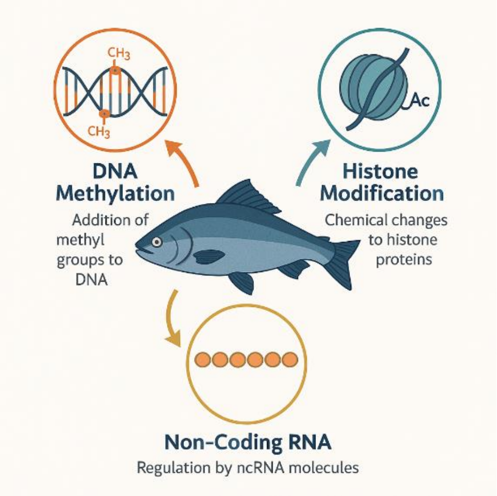

Figure 1. Epigenetic Patways in Fish.

DNA methylation involves the enzymatic addition of a methyl group to the 5’ position of cytosine rings in CpG dinucleotides. In fish, it is associated with transcriptional silencing, particularly through repression of transcription factor binding or recruitment of methyl-binding proteins that condense chromatin (Bird, 2002; Le Luyer et al., 2017). Studies in *Oreochromis niloticus* and *Dicentrarchus labrax* have linked DNA methylation to sex differentiation, growth rate modulation, and stress memory inheritance (Navarro-Martín et al., 2011; Anastasiadi et al., 2018).

Histone modifications involve chemical changes to the tails of histone proteins such as acetylation, methylation, phosphorylation, and ubiquitination that occur after protein synthesis. These modifications influence how tightly DNA is wound around histones, ultimately shaping chromatin structure and gene expression patterns. (Kouzarides, 2007). In teleosts, histone acetylation is typically associated with transcriptional activation, while methylation may exert activating or repressive effects depending on the residue and context. This has implications for immune response regulation and phenotypic plasticity (Piferrer, 2021; Cano-Rocabayera et al., 2022).

Non-coding RNAs, particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), modulate gene expression at both transcriptional and post-transcriptional levels. miRNAs bind target mRNAs to repress translation or induce degradation, while lncRNAs often interact with epigenetic complexes to regulate chromatin states (Bartel, 2004; Liu et al., 2022). In farmed species such as *Danio rerio* and *Sparus aurata*, miRNAs are involved in responses to pathogens, thermal shifts, and nutritional variation (Liu et al., 2022; Gao et al., 2020).

These mechanisms form a complex regulatory system together that helps fish adjust to environmental changes, an ability that is especially vital under the variable and often stressful conditions of intensive aquaculture.

EPIGENETICS IN FISH DEVELOPMENT AND EARLY PROGRAMMING

Fish embryos and larvae are especially sensitive to environmental cues that can leave lasting epigenetic marks. This period of developmental plasticity allows for the long-term shaping of traits through a process known as developmental programming.

- Sex Determination: One of the most studied cases of epigenetic programming in fish is temperature-dependent sex determination (TSD). In *Oreochromis niloticus*, exposure to elevated temperatures during the thermos-sensitive period leads to DNA methylation of the promoter regions of genes such as cyp19a1a and dmrt1, skewing the sex ratio toward males (Navarro-Martín et al., 2011). Similarly, in *Dicentrarchus labrax*, heat-induced masculinization is associated with methylation changes in key sex-regulating genes and a concurrent reduction in aromatase expression (Anastasiadi et al., 2018).
- Behavior and Stress Axis Regulation: Epigenetic regulation of the hypothalamic-pituitary-inter-renal (HPI) axis during early ontogeny influences stress responsiveness later in life. Methylation changes in the glucocorticoid receptor gene, nr3c1 have been documented in response to handling stress and rearing density in *Channa punctata* and *Danio rerio*, leading to altered cortisol profiles and behavioral reactivity (Bayır et al., 2025; Ali et al., 2019).
- Growth and Muscle Development: Early-life microbial exposure and immune-stimulants can prime the fish immune system via epigenetic marks on cytokine and pattern recognition receptor genes. For example, larvae of Danio rerio exposed to probiotics or β-glucans during early stages exhibit modified expression of il1β and tlr5, linked to altered methylation patterns and improved disease resistance in adulthood (Zhang et al., 2022; Cano-Rocabayera et al., 2022).
- Morphogenesis and Skeletal Development: Evidence suggests that vitamin and micronutrient availability during early ontogeny can epigenetically alter genes involved in craniofacial and skeletal formation (Morán et al., 2013).

These findings support the idea that carefully managing environmental and dietary conditions during early development can be a powerful strategy for shaping desirable traits in farmed fish using epigenetic programming to improve aquaculture performance.

NUTRITIONAL EPIGENETICS IN AQUACULTURE

Nutrients serve as both substrates and co-factors in epigenetic processes, particularly DNA methylation. Essential dietary methyl donors like methionine, folate, choline, and vitamin B12, affect methylation potential through the one-carbon cycle (Kumar et al., 2020).

- Feed that were enriched with methyl donors help to improve growth performance, stress resistance, and immune responses in species like *Danio rerio* and *Sparus aurata* (Jiang et al., 2014; Zhang et al., 2020).
- Functional feeds designed for epigenetic modulation (e.g., fortified with probiotics, antioxidants, or polyphenols) offer non-GMO strategies for phenotype optimization (Glencross et al., 2020).

Nutritional epigenetics opens the door to precision feeding strategies, where diets are:

- Stage-specific: Targeting critical windows of epigenetic imprinting
- Trait-oriented: Designed to program traits such as feed efficiency, immunity, or thermal tolerance
- Environmentally responsive: Adopted for expected farm conditions (e.g., high density, elevated temperature)

This dietary control over gene expression presents a practical avenue for trait improvement without the regulatory limits of genetic modification.

Despite promising results considering nutritional epigenetics in aquaculture, several challenges remain:

- Epigenetic responses to diet can be species-specific and dose-dependent
- Lack of standardized biomarkers for nutritional epigenetics in fish
- Long-term stability and inheritance of diet-induced epigenetic changes need further validation

Future research combining nutritional genomics, epi-genomics, and metabolomics will help building robust models for dietary programming in aquaculture species.

EPIGENETICS AND DISEASE RESISTANCE

Disease outbreaks remain one of the most pressing challenges in modern aquaculture, leading to significant economic losses and influencing animal welfare. Traditional approaches for disease control, such as vaccination, antibiotic treatment, and selective breeding, are often limited by species, resistance development, or long lead times. Epigenetic control of immune function is emerging as a promising complementary approach for developing disease resistance in aquaculture species.

- Epigenetic Control of Innate and Adaptive Immunity: Fish rely heavily on their innate immune systems, which respond rapidly to pathogens. Key components of this response, such as cytokines (il-1β, tnf-α), Toll-like receptors (tlr5, tlr9), and interferons, are known to be epigenetically regulated. DNA methylation and histone modifications at promoter regions can silence or activate these immune genes during infection (Campbell et al., 2021). For instance, histone acetylation upregulates pro-inflammatory genes in *Danio rerio* during bacterial challenge (Liu et al., 2022).
- Immune Memory and Trained Immunity: Unlike classical vertebrates, fish lack true adaptive immune memory. However, recent studies demonstrate that fish can develop a form of 'trained immunity' via epigenetic reprogramming. Exposure to immune-stimulants like β-glucans or heat-killed bacteria can cause stable epigenetic changes in hematopoietic progenitors, improving response to subsequent infections (Cano-Rocabayera et al., 2022). This opens the door for 'epigenetic vaccines' or nutritional priming strategies in hatcheries.
- Nutritional Epigenetics and Disease Resistance: Functional feeds containing probiotics, vitamins, or immune modulators alter methylation profiles of immune-related genes in species such as *Sparus aurata* and *Oncorhynchus mykiss*. For example, fish fed with selenium or nucleotides-enriched diets exhibited increased expression of il-8 and c3, correlated with hypo methylation at promoter regions (Estensoro et al., 2021).
- Pathogen-Driven Epigenetic Modulation: Some pathogens may exploit the host's epigenetic machinery to downregulate immune responses. Viral infections in fish have been associated with increased methylation of interferon-related genes, resulting in immune evasion. Understanding such host-pathogen-epi-genome interactions could guide novel antiviral strategies (Chi et al., 2023). In summary, applying epigenetic insights to disease management adds a valuable

layer of precision in improving tolerance especially during early developmental stages. When combined with genomic selection, epigenetic markers hold strong potential to improve the prediction of disease resistance traits and accelerate the breeding of more robust aquaculture lines.

Epigenetic regulation is deeply involved in the modulation of the immune system. Histone acetylation and promoter methylation of cytokine genes (e.g., IL-1β, TNF-α) determine the strength and duration of immune responses in fish exposed to pathogens or vaccines.

Prolonged exposure to stressors or specific pathogens can epigenetically prime immune cells, resulting in improved immune memory, a phenomenon known as “trained immunity” (Liu et al., 2022).

Studies in *Oreochromis niloticus* demonstrated that pre-conditioning with probiotics and immune-stimulants could alter gene expression via histone modifications and improve resistance to *Streptococcus* infections (Zahan Etti, 2025).

The fish immune system, while lacking adaptive memory in the classical vertebrate sense, can be “trained” epigenetically through environmental exposures or nutritional modifications.

- Histone acetylation and DNA methylation at cytokine gene loci (e.g., IL-1β, TNF-α) determine the scale and duration of immune responses (Liu et al., 2022; Cano-Rocabayera et al., 2022).
- This form of trained immunity has been observed in *Oreochromis niloticus*, where probiotic supplementation or exposure to sub-lethal pathogens led to improved gene expression and tolerance against *Streptococcus* infections (Zahan Etti, 2025; Zhang et al., 2022).

These findings indicate that epigenetic memory may serve as a valuable tool for improving immune tolerance in aquaculture species, potentially reducing dependence on antibiotics and improving the long-term effectiveness of vaccination strategies.

Table 1. Comparative Epigenetic Immune Responses in Aquaculture Species

Species	**Target Genes**	**Epigenetic Mechanism**	**Immune Outcome**	**Reference**
Danio rerio (Zebrafish)	il-1β, tnf-α, tlr5	Histone acetylation, DNA methylation	İmproved inflammation and rapid pathogen response	Liu et al., 2022
Sparus aurata (Gilthead seabream)	il-8, tnfα, c3	Promoter methylation	Upregulated immune genes and pathogen resistance	Estensoro et al., 2021
Oncorhynchus mykiss (Rainbow trout)	il-6, mxa, ifnγ	Histone methylation	Improved antiviral response	Morán et al., 2013
Dicentrarchus labrax (European sea bass)	cyp19a1a, dmrt1	DNA methylation of sex and immune genes	Temperature-dependent modulation of immunity	Anastasiadi et al., 2018
Oreochromis niloticus (Nile tilapia)	tlr2, il-10, tnfα	MicroRNA regulation, DNA methylation	Immune tolerance and pathogen clearance	Ali et al., 2019

Table 1 summarizes how epigenetic mechanisms influence immune gene regulation across aquaculture species, with the key findings from recent studies. It highlights the specific genes involved, the type of epigenetic regulation observed, and the resulting immunological outcomes, providing a comparative perspective on species-specific responses.

ENVIRONMENTAL STRESS AND EPIGENETIC TOLERANCE

Environmental stressors such as temperature fluctuations, hypoxia, salinity changes, handling, and crowding are common in intensive aquaculture systems and can negatively influence fish welfare, growth, reproduction, and survival. Traditionally, the response of aquatic organisms to such stressors was considered largely plastic and reversible. However, emerging research demonstrates that epigenetic modifications can mediate more stable, and sometimes heritable, responses to environmental stress. This is offering new insights into stress tolerance and adaptation in aquaculture.

One of the most powerful applications of environmental epigenetics in aquaculture lies in early-life conditioning or epigenetic imprinting. Exposing larvae or juveniles to mild, sub-lethal stressors during critical windows of development can program improved tolerance later in life. For instance, zebrafish exposed to moderate hypoxia during early development showed hypomethylation of genes related to angiogenesis (vegfa) and oxygen transport, leading to improved tolerance in adulthood (Tudorache et al., 2020). Similarly, heat conditioning in juvenile sea bass has been associated with persistent transcriptional changes linked to epigenetic modifications in stress- and metabolism-related genes (Anastasiadi & Piferrer, 2019).

This process parallels the concept of "stress preconditioning," where controlled exposure to mild stressors improves tolerance. If effectively applied, such strategies could be integrated into hatchery protocols to strengthen robustness in farmed fish without any genetic modification.

- Altered DNA methylation in stress-responsive genes such as nr3c1 (glucocorticoid receptor) and hsp70 has been recorded in *Channa punctata* and *Danio rerio* exposed to pollutants or fluctuating temperatures (Bayır et al., 2025; Ali et al., 2019).
- HPI axis plasticity may be epigenetically modulated, influencing cortisol release and behavioral stress responses (Anastasiadi & Piferrer, 2019).

Mapping such changes enables the selection of stress-tolerant broodstock and the design of conditioning protocols that pre-adapt fish to culture conditions. Epigenetic markers can be integrated into selective breeding and welfare monitoring programs, allowing producers to screen for tolerant phenotypes without full genomic sequencing.

In future breeding strategies, these markers may complement traditional quantitative trait loci (QTLs) or genomic selection models, particularly for traits difficult to phenotype directly (e.g., chronic stress sensitivity, behavior under confinement).

TRANSGENERATIONAL EPIGENETICS AND BROODSTOCK CONDITIONING

Epigenetic modifications are not always limited to individual life spans; mounting evidence suggests that epigenetic changes can be inherited, especially through the germline (gametes), influencing offspring phenotype. This phenomenon is referred to as transgenerational epigenetic inheritance, and in aquaculture, it has significant implications for broodstock management and the design of breeding programs.

- Broodstock subjected to chronic stress, suboptimal diets, or toxicants can pass on altered epigenetic marks to offspring, affecting their development, immunity, and growth (Le Luyer et al., 2017; Soliman et al., 2022).
- Conversely, broodstock conditioning through optimal nutrition or environmental enrichment may yield larvae with improved performance traits.

Broodstock diets influence the epi-genome of gametes, which can transmit altered gene expression profiles to offspring:

- In *Oncorhynchus mykiss* (rainbow trout), parental methionine supplementation increased methylation in muscle growth genes in larvae, enhancing early-stage performance (Morán et al., 2013).
- In tilapia, vitamin E and selenium-enriched broodstock diets modulated offspring antioxidant gene expression via histone modification patterns (Ribas et al., 2017).

Broodstock nutrition, environmental exposure, and handling can epigenetically program the next generation, improving or compromising performance:

- Thermal conditioning of broodstock can induce methylation changes in offspring that affect growth and sex differentiation (Anastasiadi et al., 2018).
- Dietary manipulation (e.g., methyl donors like folate, betaine, or selenium) in parents can prime stress response and immune gene expression in larvae (Ribas et al., 2017).
- Social or density stress in broodstock affects neural gene expression and cortisol sensitivity in progeny (Le Luyer et al., 2017).

These effects can be adaptive in matching offspring to expected environments, but may also carry risks of maladaptation if environmental conditions change.

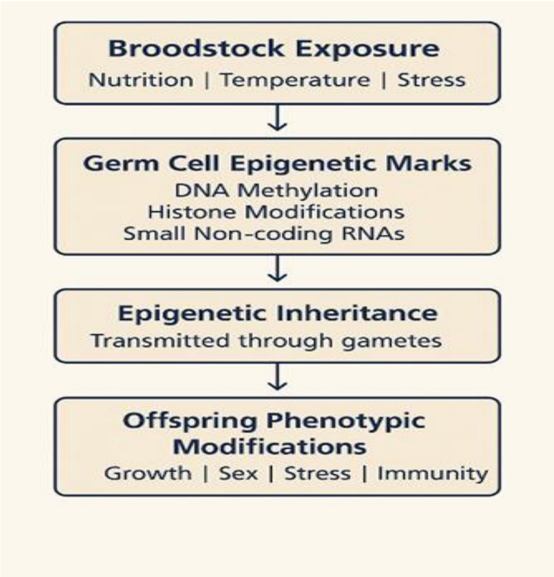

Figure 2. Epigenetic pathways linking broodstock environmental exposure to offspring phenotype in aquaculture species.

Environmental exposures experienced by broodstock, such as nutritional changes, temperature stress, or handling pressure can induce stable epigenetic modifications in germ cells. These include DNA methylation, histone modifications, and non-coding RNA expression, which may be transmitted through gametes. Once inherited, these epigenetic marks can influence gene expression during embryogenesis and early development, ultimately shaping phenotypes such as growth, sex differentiation, stress responsiveness, and immune capacity in the offspring (Figure 2). This mechanism provides a novel opportunity to strategically managing broodstock to improve larval and juvenile performance in specific farming environments.

Integrating epigenetic screening into broodstock selection could revolutionize how future generations of aquaculture species are managed.

Table 2. presents key examples of how broodstock conditioning can influence offspring traits of interest. These studies show that environmental and dietary factors can modify epigenetic markers like DNA methylation or histone structure in gametes or early embryos. These changes can then carry over to the

next generation, affecting traits such as growth, immunity, stress tolerance, and even sex ratios. Thus, they highlight how adjusting the broodstock environment could be a powerful way to guide performance in future stocks.

Table 2. Examples of Transgenerational Epigenetic Effects in Aquaculture

Species	**Broodstock Factor**	**Epigenetic Markers Affected**	**Offspring Outcome**	**Reference**
Oncorhynchus mykiss	Methyl-rich diet	DNA methylation of *myod1*, *igf1*	İmproved muscle growth	Morán et al., 2013
Dicentrarchus labrax	Thermal exposure	Methylation of *cyp19a1a* promoter	Skewed sex ratios, masculinization	Anastasiadi et al., 2018
Oreochromis niloticus	Antioxidant-enriched diet	Histone acetylation in immune genes	Improved immune capacity in larvae	Ribas et al., 2017
Salmo salar	Chronic stress	Methylation at *nr3c1* promoter	Altered stress axis activity in progeny	Le Luyer et al., 2017
Danio rerio	Hypoxia exposure	Hypomethylation of *vegfa*	İmproved hypoxia tolerance	Tudorache et al., 2020

Transgenerational epigenetics allows aquaculture producers to pre-program offspring performance based on broodstock exposure in the commercial point of view. Conditioning can be strategic, aligning offspring traits with farming environments but also risky if unintended traits persist. There is growing potential to integrate epigenetic biomarkers into selective breeding pipelines for tolerance, sex control, and performance improvement.

FUTURE PERSPECTIVES AND CHALLENGES

The integration of epigenetics into aquaculture holds transformative potential, yet its practical deployment remains limited by a number of scientific, technical, and regulatory challenges. These challenges must be carefully managed to ensure that epigenetic approaches can safely and effectively complement traditional breeding and modern biotechnologies without introducing unintended consequences.

Standardization of Biomarkers

A foundational step toward the routine application of epigenetics is the standardization and validation of epigenetic biomarkers. While promising candidates—such as DNA methylation at promoters of growth (igf1), sex differentiation (cyp19a1a), or immune genes (il1β) have been identified, they often show species-, tissue-, and context-specific variability.

Moreover, reliable epigenetic markers must be reproducible across laboratories, stable over time, and detectable in a non-invasive manner (e.g., from blood, mucus, or fin clips). High-throughput sequencing platforms (e.g., WGBS, RRBS) and methylation arrays are improving detection, but cost and accessibility remain barriers to widespread adoption in hatcheries (Imsland et al., 2021).

Causality vs. Correlation

One of the central challenges in epigenetic research is separating cause from correlation. While many studies identify differences in DNA methylation or histone marks between high and low-performing fish, it is often unclear whether these epigenetic signatures are actively shaping the traits or simply reflecting environmental influences or underlying genetic variation.

Demonstrating causality requires robust experimental approaches such as targeted epigenetic editing using tools like dCas9-TET or dCas9-DNMT, gene knockdown models, or carefully designed transgenerational studies that track trait inheritance independent of genotype. Without this level of evidence, the application of epigenetic biomarkers in selection programs remains largely theoretical.

Ethical, Regulatory, and Perceptual Barriers

Although epigenetic manipulation is non-GMO by nature, it still raises important ethical and regulatory questions:

- What are the long-term ecological consequences of releasing epigenetically programmed or stress-conditioned fish into open systems?
- Can epigenetic reprogramming result in unintended traits or intergenerational effects that are difficult to reverse?
- How will public perception differ between genetic modification and epigenetic programming, especially for food species?

Currently, regulatory frameworks often lag behind technological progress. While epigenetic tools are not subject to the same restrictions as gene editing or transgenesis, there is still a need for risk assessment protocols, particularly for applications with persistent or heritable changes.

Integrative Multi-Omics and Predictive Breeding

Looking forward, multi-omics integration will be key to unlocking the full potential of epigenetics. Combining epi-genomics, transcriptomics, proteomics, metabolomics, and microbiome profiles allows for a more holistic understanding of fish biology and trait improvements.

These datasets can be integrated into machine learning algorithms or genomic selection frameworks to enhance the accuracy of trait prediction and improve breeding outcomes. For instance, epigenetic QTLs (epiQTLs) may help explain missing heritability in complex traits such as disease resistance or stress tolerance. In this way, epigenetic data can help fine-tune breeding value estimates, track how environmental factors influence performance, and tailor nutritional strategies laying the foundation for precision aquaculture.

CONCLUSION

While scientific and logistical barriers remain, the trajectory of epigenetic research in aquaculture is clear. With responsible implementation, epigenetics offers a non-invasive, reversible, and potentially heritable layer of biological regulation that complements existing tools in genetics and biotechnology.

As climate variability, disease emergence, and sustainability pressures continue to shape global aquaculture, epigenetic tolerance may become a cornerstone of breeding and management strategies that are both efficient and ethically sound.

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CHAPTER 8

TIME SERIES MODELING APPROACHES IN AQUACULTURE

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INTRODUCTION

Time series analysis is a distinct branch of statistical data analysis that focuses on evaluating observations recorded at regular time intervals, using mathematical and statistical tools to uncover patterns and temporal structures. This analytical approach enables forecasting future outcomes from historical data, facilitates understanding of system dynamics, and supports decision-making processes, especially in contexts where temporal factors play a critical role.

Unlike conventional cross-sectional datasets, time series data consist of observations sequenced according to the progression of time. While cross-sectional data contain observations obtained sourced from different units at a defined point in at a particular point in time data encompass sequential observations obtained from a single unit (e.g., a production facility, a fish species, a meteorological station) over time. This structure necessitates the use of specialized methods in the analysis process, as features such as time dependence, autocorrelation, seasonal effects, and structural breaks cannot be directly addressed by traditional statistical methods.

Time series analysis goes beyond merely reviewing past data; it provides insights into the system's dynamic behavior, enables modeling of its reactions to external disturbances. It significantly contributes to the formulation of effective policies. In this context, time series analysis serves as both a descriptive and predictive instrument, making it a valuable component of scientific investigations and practical applications. The application areas of time series analysis are quite broad. Among its applications is the analysis and forecasting of trends in macroeconomic indicators such as GDP, inflation, and unemployment rates over time. It is widely used to predict financial variables, including stock prices, currency rates, and interest levels. Time series analysis is applied to study changes in environmental factors over time, including weather data and water quality metrics like pH and dissolved oxygen. It helps determine variations in fish growth rates, feed consumption, production efficiency, and environmental parameters over time. It also provides important information on disease spread, the time course of case numbers, and the dynamics of healthcare demand.

To summarize, time series analysis is a robust analytical framework that incorporates the temporal dimension, utilizes specialized modeling techniques tailored to time-dependent data, and supports decision-making across diverse fields. Comprehensive understanding of both theoretical principles and practical applications is essential for the effective use of this method.

2. TIME SERIES COMPONENTS

For effective modeling and interpretation, time series data are typically broken down into four key components: trend, seasonal variation, irregular fluctuations, and stationarity. Separately analyzing these components uncovers the structural characteristics of the data, enabling the development of more accurate and reliable forecasting models. Especially in fields like aquaculture, where environmental and production variables fluctuate significantly over time, a solid grasp of these elements strengthens the scientific basis for informed decision-making. The time series components are trend, seasonality, irregularity (residual), and stationarity (Montgomery et al.2015).

2.1. Trend

The trend component captures persistent directional changes in the series, reflecting its underlying long-term behavior. Typically seen as a gradual increase or decrease, it captures the overall direction of the series, distinct from transient variations such as seasonal cycles or unpredictable noise. Thus, identifying and defining the trend component is among the first tasks in decomposing time series data.

Analyzing trends is critical for interpreting changes in natural, economic, and biological systems that exhibit temporal dynamics. In this context, the trend component acts as a key indicator of structural shifts, highlighting persistent patterns and long-term changes within the system. For example, a steady rise in water temperature over several years may signal climate change. Accurate knowledge of trend direction and strength is essential for guiding production planning, resource distribution, and sustainable environmental strategies. For forecasting model construction, the trend component should be modeled separately and separated from other components to increase the accuracy of forecasting models.

2.2. Seasonality

It describes systematic, repeating patterns in data that occur over fixed periods, such as months or years. Environmental factors, biological cycles, production dynamics, and human actions are common drivers of these patterns. This component reflects cyclical patterns in the data, distinct from the overall trend.

The characteristics of seasonality are: periodic recurrences, can remain constant over time (e.g., temperatures increase every summer), or can vary (the severity of seasonal effects may increase over time), and can be predictable, playing an important role in production planning.

Seasonality can be identified through several methods: first, by visually inspecting time series plots to detect repeating patterns; second, using the autocorrelation function, which reveals seasonal patterns when correlations persist at fixed lags. Finally, through decomposition methods that break down the series into its trend, seasonality, and irregular elements. to separate time series data into its trend, Additionally, the SARIMA model incorporates seasonality directly into its structure, allowing for more accurate modeling of time-dependent patterns. Seasonality refers to regular patterns that recur during specific periods of the year. It develops as a result of environmental conditions and recurring biological processes. SARIMA incorporates seasonal structures that repeat at regular or varying periods, commonly in monthly, quarterly, or yearly cycles. When seasonal components are removed, the underlying structure of the data becomes clearer. To give an example from aquaculture: dissolved oxygen levels decrease in the summer months. feed consumption increases in the spring as fish enter the breeding season. Frequency of seasonal diseases (e.g. fungal infections in summer).

2.3. Irregularity (Residual)

Irregularity represents noise or random shocks in the data after accounting for structured elements like trend and seasonality. This component represents sudden changes, unexpected events, and statistically inexplicable deviations in the data. Irregularity is often considered an error term in the modeling process. This component represents unpredictable variations resulting from random events. In a robust model, irregularity is randomly distributed and resembles white noise. It is distinct from trend and seasonality, remaining after these systematic influences are removed. Irregularity serves as a key diagnostic for model adequacy—if this component displays any discernible structure, it suggests that the model has not fully captured the underlying dynamics (White, 1992).

Addressing the residual irregularity is a critical step; without resolving it, reliable time series modeling cannot be achieved. Therefore, the residual irregularity component should generally be decomposed using time series decomposition methods. These approaches include the Additive model ($T_t + S_t + E_t$), which assumes that the components of trend, seasonality, and error are linearly combined, and the Multiplicative model ($Y_t = T_t \times S_t \times E_t$), which assumes that these components interact proportionally.

Decomposition is typically implemented through methods available in software environments such as the statsmodels function in Python or the decompose() function in R.

Once the residual (irregular) component is extracted, the model's reliability is assessed by checking whether these residuals exhibit random behavior.

A thorough statistical assessment of the residual irregular component is critical to ensure the adequacy and precision of the time series model. The main methods used to test this component are: White Noise Test, Normal Distribution Test, Autocorrelation Test, Homoscedasticity Test, and Visual Inspection.

2.4. Stationarity

A time series is stationary when its key statistical characteristics do not change across time. These properties include mean, variance, and covariance. Stationarity assumes that the underlying behavior of a time series does not change across different time periods. For models such as ARIMA to perform effectively, the series must maintain constant statistical properties, including mean, variance, and autocovariance.

One defining feature of stationarity is that the mean of the series stays constant over time. Constant Variance means that the variability of the series does not change across time periods. Autocovariance measures how two points in a time series are related, based only on the interval separating them, not on their specific time stamps. Examples of non-stationary time series include fish growth rates, which exhibit a persistent upward trend over the years; seasonal feed consumption, which varies periodically and reflects seasonality; and rising water temperatures over time due to climate change, which demonstrate a long-term trend component.

Several techniques are available for testing stationarity. The process begins with visual analysis, where a plot of the series helps assess if its statistical properties remain unchanged over time. The second method is the Autocorrelation Function (ACF). In non-stationary series, autocorrelations decline slowly, whereas in stationary series, they diminish rapidly toward zero. The third approach is the Augmented Dickey-Fuller (ADF) test, which is among the most widely used statistical methods for assessing stationarity in time series data. Finally, the KPSS test (Kwiatkowski–Phillips–Schmidt–Shin) offers an alternative approach by testing for stationarity rather than a unit root, complementing tests like ADF for a more robust assessment.

To determine whether a time series is stationary, the ADF test is one of the most frequently applied techniques. Stationarity means that the mean and variance of a series do not change over time. Before using ARIMA, it is necessary to confirm that the time series exhibits stationarity. The primary purpose of the ADF test is to check for the presence of a unit root in the series. If a unit root is present, the series is non-stationary. If there is no unit root, the series is stationary.

Hypotheses:

H_0 (Null Hypothesis): The series has a unit root and therefore lacks stationarity.

H_A (Alternative Hypothesis): The series is stationary and does not exhibit a unit root.

The Basic Equation Form of the ADF Test, the ADF test, is based on the following regression model:

$$\Delta y_t = \alpha + \beta t + \gamma y_{t-1} + \sum_{i=1}^{p} \delta_i \Delta y_{t-i} + \varepsilon_t$$

In this formulation: Δy_t represents the first difference of the series (i.e., $y_t - y_{t-1}$); α is the constant term; β_t denotes the optional trend component; γ is the key coefficient used to test for the presence of a unit root; p indicates the number of lags included in the model; and ε_t is the error term. The key point in the test is whether $\gamma = 0$, which suggests that the time series contains a unit root and is therefore not stationary. If $\gamma<0$, the series is stationary. Accordingly, if p-value < 0.05 (H_0 is rejected), the series is stationary, if p-value ≥ 0.05 (H_0 is accepted), the series is not stationary.

3. TIME SERIES MODELS

The main objective of time series analysis is to identify and isolate structural components—such as trend, seasonality, cyclical patterns, and randomness—within the data, to understand their influence, and to forecast future values using suitable modeling techniques. Among the methods used in this context are:

AR (Autoregressive): Makes predictions based on past series values.

MA (Moving Average): Makes predictions based on past error terms.

ARMA (Autoregressive Moving Average): A combination of the AR and MA models; suitable for stationary series.

ARIMA (Autoregressive Integrated Moving Average): An ARMA model made applicable to non-stationary series by differencing.

SARIMA (Seasonal ARIMA): An ARIMA model that includes seasonal components.

VAR (Vector Autoregression): Used for multivariate time series.

GARCH (Generalized Autoregressive Conditional Heteroskedasticity): Used to model variability in financial time series.

Granger Causality Test: Tests the predictive power of one variable for another.

To effectively apply time series models, particularly ARIMA, the series must first be tested for stationarity, which means its mean, variance, and covariance do not vary with time. In non-stationary series, differentiation is usually applied to make the series stationary. Ensuring stationarity is one of the essential steps in time series analysis, particularly when implementing ARIMA models.

3.1. Autoregressive (AR) Model: A Time Series Forecasting Technique Based on Historical Data

Among the fundamental models in time series analysis, the autoregressive (AR) model stands out for its simplicity and effectiveness. It is based on the principle that past observations can explain current values. This approach is especially useful for stationary time series and short-term predictions (Box et al., 2015). The autoregressive model forecasts the current observation based on its lagged values. The general equation is:

$$X_t = c + \sum_{i=1}^{p} \phi_i X_{t-i} + \varepsilon_t$$

Here:

X_t: alue of the series at time t,

c: constant term,

ϕ_i: autoregressive coefficient at lag level i,

p: model order (number of lags),

ε_t: white noise error term.

The AR(1) model is the simplest autoregressive model and is expressed as follows:

$$X_t = c + \phi X_{t-1} + \varepsilon_t$$

The simplest autoregressive model relies solely on the previous value of the series for prediction. In contrast, higher-order AR(p) models utilize several lagged values to capture deeper patterns in the data. Before applying AR models, it is essential to ensure that the time series is stationary, meaning its statistical properties do not change over time. For example, if the condition $|\phi|<1$ is not met in the AR(1) model, the model is non-stationary and exhibits random walk behavior.

Parameters of the Autoregressive (AR) model are typically estimated using either the Yule-Walker equations or the Ordinary Least Squares (OLS) method. The Yule-Walker approach is a classical and efficient technique that utilizes the autocorrelation structure of the time series to estimate model coefficients. It is particularly useful when the series is stationary and the autocorrelations are well-defined. On the other hand, the OLS method estimates parameters by minimizing the sum of squared residuals, offering flexibility and ease of implementation, especially in regression-based frameworks. These equations allow estimating the coefficients of the AR model based on the autocorrelation function of the series. In AR(p) models, such techniques are used to formally express the dependence of the current value on its previous observations and to statistically analyze this temporal relationship.

3.2. The Moving Average (MA) Model: A Forecasting Approach Based on Error Terms in Time Series

The Moving Average (MA) model is one of the fundamental stochastic models used in time series analysis. It is based on the principle that present observations depend on previous forecast errors (white noise). MA models are a powerful tool, especially for short-term forecasting and understanding the impact of random components.

The MA(q) model incorporates the effect of error terms up to a lag level of q. Its general form is as follows:

$$X_t = \mu + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \theta_2 \varepsilon_{t-2} + \cdots + \theta_q \varepsilon_{t-q}$$

Here:

X_t: observation value at time t,

μ: series mean,

ε_t: white noise error term at time t,

θ_i: MA coefficient at lag level i,

q: model order.

3.3. The ARMA (Autoregressive Moving Average) An Integrated Method for Capturing Temporal Dependencies in Stationary Data

The ARMA (Autoregressive Moving Average) Model is a stochastic time series model that combines both autoregressive (AR) and moving average (MA) components. It enables the forecasting of stationary time series by utilizing both past observations and past error terms. Defined within the Box-Jenkins methodology, developed by George Box and Gwilym Jenkins, the ARMA model is widely applied—particularly in short-term forecasting of economic, financial, and environmental data—due to its flexibility and effectiveness in capturing temporal dependencies. The ARMA(p, q) model combines autoregressive (AR) and moving average (MA) components to model stationary time series. Its general form is:

$$X_t = c + \sum_{i=1}^{p} \phi_i X_{t-i} + \sum_{j=1}^{q} \theta_j \varepsilon_{t-j} + \varepsilon_t$$

Where:

X_t: current value of the time series, c: constant term,

ϕ_i: i-order autoregressive coefficients (AR component),

θ_j: j-order moving average coefficients (MA component),

ε_t: white noise error term,

p: order of the AR component,

q: order of the MA component.

The ARMA model is only suitable for stationary time series. If the series is non-stationary, differencing is applied to the ARIMA model.

Establishing an ARMA model generally involves the following steps: Regarding the importance of the ARMA model in the aquaculture sector, production processes and environmental variables in aquaculture are subject to both systematic and random changes over time. The ARMA model allows for more accurate predictions by simultaneously modeling these two types of changes.

3.4. The ARIMA Model: A Unified Framework for Forecasting Non-Stationary Time Series

ARIMA (AutoRegressive Integrated Moving Average) is a robust and widely used forecasting model in time series analysis. It predicts future values by incorporating both past observations and previous error terms, making it suitable for modeling non-stationary data that has been transformed to achieve stationarity. The ARIMA model is particularly effective for analyzing and forecasting non-stationary time series with underlying trends.

As with the ARMA model, the "autoregressive" (AR) component of ARIMA indicates that the current value of the series is regressed on its previous values. The "moving average" (MA) component reflects that the model incorporates a linear combination of past error terms, including those occurring simultaneously and at various time lags. This structure allows ARIMA to effectively capture both the momentum and noise in non-stationary time series data (Box et al., 2015). The "integrated" (I) portion indicates that data values are modified by the difference between each value and the previous value. The ARIMA (Autoregressive Integrated Moving Average) model is one of the most widely used parametric approaches in time series analysis.

It combines three key components: Autoregressive (AR): captures the influence of past values, Integrated (I): accounts for differencing to achieve stationarity,

Moving Average (MA): models the impact of past forecast errors.

This integrated structure makes ARIMA a powerful tool for analyzing and forecasting non-stationary time series, particularly those exhibiting trends or other forms of temporal dependence. As a core component of the Box-Jenkins approach, ARIMA is widely utilized for analyzing and predicting patterns in various domains, including economics, finance, environmental systems, and industrial production. According to Wold's decomposition theorem (Hamilton, 2020; Hamilton, 2020; Papoulis, 1965), the ARMA model is sufficient to describe a regular (also known as a completely uncertain (Maharaj, 1996)) stationary time series. This motivates us to transform such a non-stationary time series into a stationary one, for example, using differencing before using ARMA.

If the time series contains a predictable sub-process, the predictable component is treated as a non-zero-mean but periodic (i.e., seasonal) component that is eliminated by seasonal differencing within the ARIMA framework.

The ARIMA model consists of a combination of three components: AR (AutoRegressive) – Autoregressive: The current value is estimated using previous values of the series. For example, today's feed consumption may depend on the consumption amounts of previous days. I (Integrated) – Differencing: Differencing is applied to eliminate the trend in the seriesIn this approach, forecasting is based on past error terms in the series. The MA model assumes that the current value of the series is influenced by a linear combination of previous random shocks (errors), allowing the model to capture short-term fluctuations and noise. For example: Today's feed consumption may depend on forecast errors from previous days.

The ARIMA(p, d, q) model is expressed as follows:

$$\Delta^d X_t = c + \sum_{i=1}^{p} \phi_i \Delta^d X_{t-i} + \sum_{j=1}^{q} \theta_j \varepsilon_{t-j} + \varepsilon_t$$

Here:

p: AR component degree,

d: number of differencing operations (for stationarity),

q: MA component degree,

ε_t: white noise error term.

The differencing process (Δ^d) helps transform a non-stationary series into a stationary one by eliminating trends and stabilizing the mean and variance across time. This process is especially necessary to achieve stationarity in trending series.

3.4.2. Automated Model Selection and Validation in Time Series Analysis

In Python, the optimal values for the ARIMA model parameters—p (autoregressive order), d (degree of differencing), and q (moving average order)—can be automatically selected using the auto_arima function from the pmdarima library.

This function evaluates multiple model configurations and selects the best-fitting one based on statistical criteria such as the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), which help balance model complexity and goodness of fit.

3.4.2.1. Using AIC and BIC in the ARIMA Model

Selecting the correct parameters (p, d, q) when building an ARIMA model is critical to the model's success. During this selection process, information criteria such as AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) help determine the most appropriate model by comparing different model alternatives.

AIC is a model selection metric that balances goodness of fit with model complexity. It aims to identify the model that offers the most accurate predictions while minimizing information loss. Lower AIC values indicate a better trade-off between simplicity and explanatory power, making it a key criterion in automatic model selection processes.

$$AIC = -2 \cdot \ln(L) + 2k$$

In the formula, L is the maximum likelihood value, and k is the number of parameters in the model. Interpretation: A lower AIC value indicates a better model. AIC is more flexible with adding more parameters to the model (it tolerates complexity a bit better).

Similar in purpose to AIC, the BIC also evaluates model fit while penalizing complexity. However, BIC applies a stricter penalty for the number of parameters, making it more conservative in model selection—particularly when working with large datasets. This helps prevent overfitting and supports the identification of more parsimonious models.

$$BIC = -2 \cdot \ln(L) + k \cdot \ln(n)$$

In the formula, n is the number of observations, and k is the number of parameters. A lower BIC value indicates a more suitable model, as it applies a stronger penalty for unnecessary parameters. This stricter approach helps minimize the risk of overfitting and promotes the selection of simpler, more reliable models.

3.5. The SARIMA Model: An Advanced ARIMA Approach for Forecasting Time Series with a Seasonal Structure

The SARIMA (Seasonal Autoregressive Integrated Moving Average) model is an advanced extension of the ARIMA model that incorporates seasonal components into time series analysis.

By integrating seasonal autoregressive, seasonal differencing, and seasonal moving average terms, SARIMA effectively captures both short-term and seasonal patterns in non-stationary time series data. This makes it particularly useful for forecasting datasets with recurring seasonal fluctuations, such as monthly fish production, feed consumption cycles, or temperature variations across seasons. In addition to the autoregressive (AR), integrated (I), and moving average (MA) components of the ARIMA model, the SARIMA model also includes seasonal autoregressive, seasonal differencing, and seasonal moving average components. This structure allows for more accurate modeling and forecasting of time series with both short-term and seasonal fluctuations.

The SARIMA model is typically expressed as follows:

SARIMA(p,d,q)(P,D,Q)_s

Where:

p,d,q: ARIMA model parameters (including differencing for stationarity),

P,D,Q: seasonal AR, seasonal differencing, and seasonal MA parameters, s: seasonal period (e.g., s = 12 for monthly data).

The general form of the model is:

$$\Phi_P(B^s)\phi_p(B)\nabla^d\nabla_s^D X_t = \Theta_Q(B^s)\theta_q(B)\varepsilon_t$$

This formula provides a framework that incorporates both seasonal and non-seasonal components. The SARIMA model is particularly preferred for economic, environmental, and production time series where seasonal effects are particularly pronounced.

3.6. VAR (Vector Autoregression) Model: Dynamic Analysis of Multivariate Time Series

The VAR (Vector Autoregression) model is a powerful and flexible econometric technique used in multivariate time series analysis.

Unlike univariate models, VAR captures the dynamic interdependencies among multiple time series variables by modeling each variable as a linear function of its own past values and the past values of all other variables in the system. This makes VAR particularly useful for analyzing complex systems where variables influence each other over time, such as in economic, financial, and environmental datasetsFirst introduced by Christopher Sims in 1980, the VAR (Vector Autoregression) model enables the analysis of dynamic relationships among multiple time series variables by considering their historical values.

Each variable in the system is modeled as a function of its own past values as well as the lagged values of other variables, allowing researchers to capture complex interdependencies and feedback effects within multivariate datasets. The VAR model is particularly preferred in systems where interdependencies and causal relationships exist between variables. The general form of a VAR(p) model is typically represented as follows.

$$Y_t = A_1 Y_{t-1} + A_2 Y_{t-2} + \cdots + A_p Y_{t-p} + u_t$$

Here:

Y_t: Variable vector at time t (e.g., [water temperature, pH, oxygen level]),

A_i: Coefficient matrices at lag level i,

u_t: Error term (white noise).

Each variable is explained by both its own past values and the past values of other variables. This structure distinguishes the VAR model from simultaneous equation systems because all variables are modeled endogenously.

3.7. GARCH Model: Modeling Time-Varying Variance in Financial Time Series

The GARCH model is a stochastic process developed to model situations where variance changes over time in time series analysis. First introduced as the ARCH model by Engle (1982), it was generalized by Bollerslev (1986) to the GARCH (Generalized ARCH) form. GARCH models are particularly effective in explaining features such as volatility clustering observed in financial time series (Şıklar, 2019).

The GARCH(p, q) model is a widely used econometric approach for modeling time-varying volatility in time series data.

It estimates conditional variance by incorporating both the squared past error terms (ARCH effect) and the past conditional variances (GARCH effect). The most commonly applied form is the GARCH(1,1) model, which is expressed as:

The average equation is: $y_t = \mu + \varepsilon_t$

Variance equation: $\sigma_t^2 = \alpha_0 + \alpha_1 \varepsilon_{t-1}^2 + \beta_1 \sigma_{t-1}^2$

Here:

σ_t^2: conditional variance at time t,

ε_{t-1}^2: squared error term from the previous period,

σ_{t-1}^2: conditional variance of the previous period in a GARCH model,

α_1: coefficient for the lagged squared error (ARCH term),

β_1: coefficient for the lagged conditional variance (GARCH term).

This structure yields quite successful results in financial data where the variance is not constant and changes over time (Yılmaz, 2021).

3.8. Granger Causality Test: Statistical Analysis of Causal Relationships in Time Series

The Granger Causality Test is a statistical method used in time series analysis to determine whether a causal relationship exists between two or more variables and the direction of this relationship. It was first developed by Clive W. J. Granger in 1969 and introduced to the econometric modeling literature. This test is used specifically to analyze the existence of time-lagged effects between variables (Granger, 1969).

Granger causality, unlike the classical concept of causality, defines causality based on predictive power. If past values of one variable can statistically significantly predict future values of another variable, that variable is considered a Granger cause.

The general form of a linear time series model with autoregressive and exogenous components can be expressed as:

$$Y_t = \sum_{i=1}^{p} \alpha_i Y_{t-i} + \sum_{j=1}^{q} \beta_j X_{t-j} + \varepsilon_t$$

Where:

Y_t: the dependent variable at time t

α_i: coefficients for the autoregressive terms (past values of Y)

X_{t-j}: exogenous (independent) variables at lag j

β_j: coefficients for the exogenous variables

ε_t: error term (white noise)

p: number of autoregressive lags

q: number of lags for the exogenous variables

This structure is commonly used in **ARX (Autoregressive with Exogenous inputs)** models, which are useful when modeling a time series influenced by both its own past values and external factors.

If the β_j coefficients are found to be statistically significant, the variable X is considered to Granger-cause Y, as defined by Granger (1969).

This implies that past values of X contain useful information for predicting future values of Y, beyond what is already captured by Y's own past values.

In statistical and econometric time series analysis, the Autoregressive Integrated Moving Average (ARIMA) and Seasonal ARIMA (SARIMA) models are extensions of the Autoregressive Moving Average (ARMA) model. ARIMA generalizes ARMA to handle non-stationary time series by incorporating differencing, while SARIMA further extends ARIMA by integrating seasonal components to account for periodic fluctuations. These models are widely used for forecasting and analyzing time-dependent data with trend and seasonality. These models are utilized in time series analysis to uncover underlying structures and to support reliable prediction of future observations. The goal of these generalizations is to fit the data as well as possible. Specifically, ARMA assumes that the series is stationary, meaning that its expected value is constant over timeWhen a series shows a persistent trend while its variance and autocovariance remain stable, applying differencing transforms it into a stationary series suitable for further analysis. This process generalizes ARMA and corresponds to the integrated part of ARIMA. Similarly, periodic variation is removed by seasonal differencing (Shumway and Stoffer, 2017).

4. THE IMPORTANCE OF TIME SERIES ANALYSIS IN THE AQUACULTURE SECTOR

Time series analysis is a powerful statistical tool used to understand, monitor, and predict the dynamics of environmental, biological, and economic variables over time in the aquaculture sector. These analyses have a wide range of applications, from production planning and environmental monitoring to disease management and economic decision support systems (Table 1).

An in-depth comparison of time series modeling approaches in aquaculture is illustrated in Table 2.

Table 1. The Importance of Time Series Analysis in the Aquaculture Sector

Application Areas	Contributions Provided by Time Series Analysis	Component/Model Used	Impact on the Decision Process
Feed Consumption Monitoring	The weekly trend confirms the overall growth pattern, while the weekly seasonality highlights periodic fluctuations at the weekly level.	ARIMA, STL Decomposition	Optimization of feeding programs, cost control
Fish Growth Dynamics	Both height and weight show a gradual upward trend over time, indicating consistent growth.	Polynomial Regression, ARMA	Harvest timing, species selection, production planning
Water Quality Monitoring	Change in parameters such as temperature, pH, and dissolved oxygen over time	ARIMA, Classical Decomposition	Environmental sustainability, early warning systems
Disease Prevention	Current course of case numbers and detection of sudden increases	MA, ARIMA, Seasonal Decomposition	Biosecurity measures, intervention timing
Production Efficiency Analysis	Analysis of production quantities with trend and irregularity components	ARIMA, ARMA	Performance evaluation, capacity planning
Seasonal Planning	Integration of seasonal effects into production processes	SARIMA, STL	Inventory management, workforce planning, logistics decisions
Economic Evaluation	Change in input costs and sales prices over time	ARIMA, Regression Models	Pricing strategies, investment decisions

Tablo 2. Zaman Serisi Modellerinin Karşılaştırmalı Uygulama Tablosu (Su Ürünleri Odaklı)

Model	**Definition**	**Intended Use**	**General Application Areas**	**Aquaculture Application Areas**	**Data Characteristics**	**Advantages**	**Limitations**
AR (AutoRegressive	Predictions are derived from past values of the series.	Short-term forecasting, trend analysis	Economy, finance, environment	Daily feed consumption, fish weight gain	Stationary	Simple and fast forecasting, interpretable	Not applicable to non-stationary series
MA (Moving Average)	It generates forecasts by using past error terms from the time series.	Modeling the impact of random shocks	Financial fluctuations, environmental changes	Disease spread, sudden temperature changes	Stationary	Captures the impact of sudden changes	Error terms cannot be observed, estimation is complex
ARMA	It combines both autoregressive (AR) and moving average (MA) components into a single model.	Joint modeling of systematic and random effects	Economic indicators, production data	Fish growth rates, production efficiency	Stationary	Provides comprehensive modeling	Not applicable to non-stationary series
ARIMA	It is an ARMA model adapted for non-stationary time series through differencing.	Joint analysis of trend and irregularity components	Macroeconomic data, environmental indicators	Water temperature, dissolved oxygen, feed consumption trends	Non-stationary	Robust forecasting on trending data	SARIMA may be required for seasonal structure
SARIMA	Seasonal fluctuation forecasting and planning	Mevsimsel dalgalanmaların tahmini ve planlama	Seasonal sales, climatic data	Seasonal feed consumption, fish growth cycles	Seasonal and non-stationary	Directly models seasonal effects	Parameter selection is complex

VAR (Vector Autoregression)	Modeling multiple variables interacting together.	Multivariate system analysis	Economic systems, environmental interactions	Water temperature shows a slight negative correlation with growth rate, suggesting that higher temperatures may slightly reduce growth.	Multivariate and stationary	Analyzes causal relationships	Requires stationarity, interpretation may be difficult
GARCH	A model with AR components that models the time-varying variance structure.	Uncertainty and variance estimation	Financial volatility, environmental risk analysis	Volatility in production data, environmental risks	Stationary, variable	Accurately captures volatility	Complex structure, interpretation is difficult
Granger Causality Test	It evaluates whether one variable contains predictive information about another.	Causality analysis, policy development	Relationships between economic indicators	Water temperature → feed consumption, pH → growth rate	Multivariate	Provides causal direction determination	Causality can be confused with correlation

4. FUTURE RESEARCH AREAS: TIME SERIES ANALYSIS PERSPECTIVE IN AQUACULTURE

Aquaculture is a strategic production area in terms of global food security and sustainable development goals. Time series analysis is a critical tool for understanding and managing the dynamics of environmental variables, production parameters, and biological processes in this sector. Recent research highlights that time series analysis is applied not only for forecasting but also in areas such as decision-support system integration, sustainability evaluation, and system optimization (Tucciarone et al., 2024).

Integrated Time Series and Artificial Intelligence Models: Hybridization of classical models such as ARIMA and SARIMA with deep learning algorithms such as LSTM (Long Short-Term Memory) and GRU (Gated Recurrent Unit). Tucciarone et al. (2024) proposed the use of data mining and topic analysis methods for the optimization of sustainable aquaculture systems. This will enable more precise capture of seasonal patterns and better modeling of sudden changes.

Spatio-Temporal Modeling: Integrating time series analysis with geographic information systems (GIS) to account for spatial variations. Studies on the applicability of IMTA (Integrated Multi-Trophic Aquaculture) systems across different geographies highlight the need for spatio-temporal analysis (Tucciarone et al., 2024). This will enable regional production forecasts, habitat change monitoring, and environmental risk maps.

Real-Time Data Integration and IoT-Based Time Series Analysis: Developing instantaneous decision-making systems by integrating data from IoT sensors into time series models. The need for continuous monitoring of water quality parameters in closed systems such as RAS (Recirculating Aquaculture Systems) is emphasized (Tucciarone et al., 2024). This will enable instantaneous feeding optimization, environmental stress monitoring, and disease early warning systems.

Sustainability and Circular Economy-Based Time Series Applications: Using time series analysis to reduce environmental impacts and optimize resource use in aquaculture production. The potential of biofloc and aquaponics systems to optimize water and nutrient cycling can be better assessed through time-series analyses (Tucciarone et al., 2024). This allows for long-term monitoring of parameters such as water consumption, nutrient recycling, and energy efficiency.

Policy Development and Social Acceptance Analysis: Linking time-series data to social perception, consumer behavior, and policy impacts. Tucciarone et al. (2024) emphasize that the level of social acceptance of sustainable aquaculture systems changes over time and that this change should be integrated into management strategies. This allows for time-series modeling of data such as social media analytics, consumer trends, and local community responses.

In conclusion, time-series analyses are a versatile tool that can be used not only for production forecasting in aquaculture but also for system transformation, sustainability, and social impact assessment. The literature indicates that future research in this area should be enriched with interdisciplinary approaches. The integration of artificial intelligence, The integration of IoT, environmental modeling, and social sciences will enable more strategic and effective applications of time series analysis in aquaculture management.

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CHAPTER 9

PHYSIOLOGICAL RESPONSES OF FISH TO THE ADVERSE EFFECTS OF GLOBAL WARMING AND CLIMATE CHANGE

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INTRODUCTION

Nowadays, global climate change has emerged as one of the major environmental problems that profoundly impacts the structure and functioning of aquatic ecosystems. Increasing atmospheric carbon dioxide concentrations, rising global temperatures, sea level rise due to glacier volume loss, decreases in dissolved oxygen levels, and increasing ocean acidification are leading to extensive and significant changes in the physicochemical properties of aquatic ecosystems (Pörtner and Farrell, 2008; Heuer and Grosell, 2014). These abiotic changes directly affect the metabolic processes, homeostatic balance, and life cycles of ectothermic organisms, especially fish, whose body temperatures are largely dependent on environmental temperature.

In recent years, the negative effects of global warming have had a profound impact on natural waters and all biotopes. Increases in seasonal average temperatures are also leading to higher water temperatures, resulting in faster-than-normal evaporation and water loss, particularly in lotic biotopes, which are closed basins (Fig. 1). The decrease in water volume, in turn, leads to an increase in salt concentration per unit volume in saline and brackish wetlands (Alkan et al., 2025).

The negative effects of global warming directly affect all planktonic organisms that use the entire water column in aquatic ecosystems and all pelagic and demersal fish, eggs, larvae and juveniles. Fish are aquatic creatures highly sensitive to environmental stressors and exhibit a variety of physiological, biochemical, and behavioral responses in response to these changes. Increases in seasonal average temperatures lead to increased metabolic rates, while decreased oxygen solubility under the same conditions significantly reduces respiratory efficiency. This process causes imbalances in the organism's energy budget, leading to significant disruptions in growth, reproduction and immune system functions (Somero, 2010; Iftikar and Hickey, 2013).

Fish can adapt to these environmental stresses to some extent by developing various physiological mechanisms, such as heat shock proteins, antioxidant enzyme systems, metabolic reorganization, and endocrine responses. However, this adaptive capacity varies significantly among species, significantly increasing the risk of population decline or local extinction, particularly in species with limited habitats or narrow environmental tolerance ranges (Ficke et al., 2007).

A change in any of the physico-chemical properties of water affects other parameters, leading to a holistic transformation in the ecosystem. For example, changes in salinity and pH significantly challenge fish's osmoregulation processes and ion balance; this leads to structural and functional changes, particularly in organs responsible for osmoregulation, such as the gills and kidneys (Evans et al., 2005). Therefore, these abiotic factors do not act alone, but rather interact, often producing complex and potentially synergistic effects. For example, increasing water temperatures reduce dissolved oxygen, which can lead to higher temperatures exacerbating the negative effects of low oxygen levels (Jiang et al., 2021). The effects of global warming are leading to gradual changes in many interconnected water quality parameters. One of the most obvious consequences of global climate change is rising temperatures, which leads to increased evaporation, a decrease in the water budget, and an increase in organism density per unit volume. This, in turn, leads to an increase in the free ammonia concentration in water proportional to volume due to metabolic activity, a decrease in dissolved oxygen saturation, and changes in chemical indicators of water, such as pH and electrical conductivity. All of these cascades of events can increase physiological stress in fish, significantly limiting their tolerance to hypoxic conditions. Similarly, increased salinity can intensify the physiological stress caused by high temperatures or low oxygen levels, pushing organisms beyond their tolerance limits.

It is very important to determine and simulate the negative effects that global climate change may have on fish and other aquatic organisms in the near future and to develop measures that can be taken. Therefore, studying the effects of global warming and climate change on fish physiology is critical not only for understanding the individual adaptation potential of species, but also for aquaculture management, biodiversity conservation and sustainability of ecosystems.

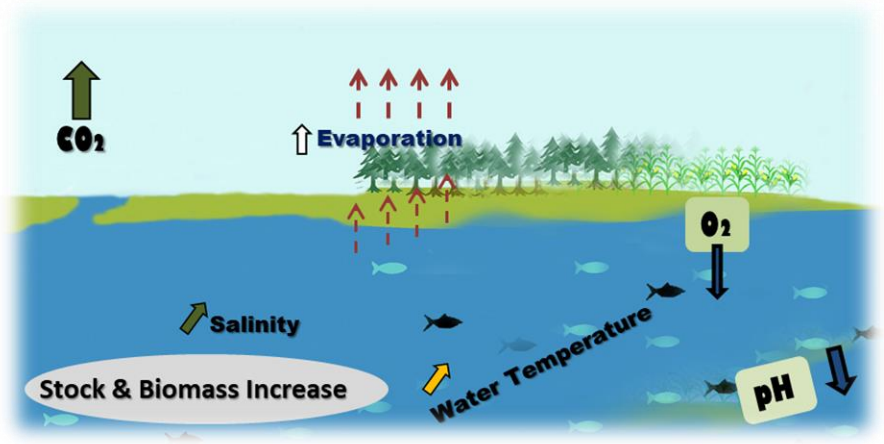

Figure 1. Schematic representation of the main effects of global climate change and temperature increase on the aquatic environment.

2. CHANGES IN WATER QUALITY PARAMETERS & PHYSIOLOGICAL REACTIONS

2.1. Water Temperature and Thermal Stress

Global temperature increases stand out as one of the key environmental factors directly determining the metabolic performance of fish. Each 10°C increase in water temperature results in an approximately two-fold increase in metabolic rates, a phenomenon known as the Q10 effect (Clarke and Johnston, 1999). Increased metabolic demand leads to a significant increase in energy requirements, while a concurrent decrease in water oxygen solubility leads to imbalances in cellular energy production and potential physiological stress (Pörtner, 2010). These mechanisms can directly affect the growth, reproduction, and overall physiological performance of fish, leading to significant shifts in ecosystem dynamics.

Temperature change is a determining factor on metabolic rate, growth performance, reproductive capacity, and general physiological functions (Pankhurst, 1997; Alfonso et al., 2021). It can restrict feeding behavior in fish and lead to physiological stress, which increases their susceptibility to diseases (Bly et al., 1997; Volkoff and Rønnestad, 2020).Coldwater fish (like salmonids) are particularly vulnerable to temperature increases, a direct consequence of global climate change. Rising water temperatures, combined with increased evaporation and decreased water volume, lead to adverse conditions such as increased fish densities, elevated ammonia concentrations, and decreased dissolved oxygen levels. These factors can lead to more severe ecological and physiological problems in species with high oxygen requirements.

However, extreme temperatures trigger oxidative stress by increasing lipid peroxidation in cell membranes. Fish respond to thermal stress by activating defense mechanisms, including heat shock proteins (HSP70, HSP90) and antioxidant enzymes (Iwama et al., 1998). HSPs contribute to maintaining cellular integrity by preventing protein misfolding and play a critical role in enhancing thermal tolerance.

2.2. Dissolved Oxygen Level

If fish density remains constant due to evaporation, particularly in closed-basin aquatic environments, stock density will increase with decreasing water volume, leading to an increase in the number of fish per volume. As a result of metabolic activities, the dissolved oxygen budget in the water will gradually decrease.

Global warming exacerbates hypoxic conditions by decreasing dissolved oxygen levels in aquatic biotopes. This significantly impacts hemoglobin-oxygen affinity, gill ventilation rate, and cardiovascular function in fish. Fish develop a series of physiological responses to this physiological stressor, including increasing erythrocyte count, increasing hemoglobin concentration, and enhancing anaerobic metabolic activities (Nilsson & Östlund-Nilsson, 2008).

Furthermore, in some species, the hypoxia-responsive transcription factor HIF-1α is activated in response to hypoxic conditions. This factor initiates a cascade of cellular regulations to maintain energy production under low oxygen conditions, increasing the synthesis of glycolytic enzymes and thus supporting metabolic adaptation at the cellular level (Richards et al., 2009). Considering all these factors, the decrease in dissolved oxygen levels will most negatively affect species that require high oxygen levels, such as salmonids.

2.3. pH and Carbon Dioxide Concentration Changes

Increasing temperature has an indirect effect on pH levels in aquatic environments. Indeed, the correlation between pH and temperature in water has been reported to be -0.097, and the correlation between pH and salinity is -

0.054 (Rugebregt and Nurhati, 2020). These values indicate a relatively weak relationship between pH, temperature, and salinity.

Furthermore, an increase in water pH shifts the balance between ammonium (NH_4^+) and ammonia (NH_3) in favor of ammonia. Because ammonia has higher toxicity than ammonium, the risk to aquatic organisms increases as pH increases. In particular, NH_3 concentrations exceeding 0.01 mg/L in water are lethal for many marine organisms, including fish (Ölmez and Saraç, 2009).

Increasing atmospheric CO_2 concentrations lead to ocean and freshwater acidification in aquatic ecosystems, leading to a decrease in environmental pH. This decrease in pH disrupts ion exchange across the gill epithelium of fish, making it difficult to maintain acid-base balance (Heuer and Grosell, 2014). To compensate, fish attempt to restore acid-base regulation by increasing the activities of the Na^+/H^+ exchanger, the Cl^-/HCO_3^- transporter, and carbonic anhydrase (CA) enzymes. Increased acidity also negatively impacts notochord mineralization in fish and can lead to decreased growth rates, particularly in the larval stage (Munday et al., 2009). In the long term, elevated CO_2 concentrations can lead to behavioral dysfunction, decreased navigational ability, and functional changes in neurotransmitter systems, leading to significant neurophysiological effects.

2.4. Salinity Change and Ion Balance

The body fluids of fish contain dissolved salts, ions, and various organic compounds, the total concentration of which is defined as osmotic density. Osmoregulation and maintenance of salt balance are achieved through a number of physiological mechanisms, including the organism's regulation of intracellular organic compound concentrations and adjustment of plasma inorganic ion levels in response to osmotic stress (Ballantyne et al., 1987).

Salinity changes in water resources resulting from climate change directly affect fish osmoregulation systems. Organs such as the gills, kidneys, and intestines, which play a key role in maintaining water and ion balance, are the first physiological structures to respond to fluctuations in environmental salinity (Evans et al., 2005). Increasing salinity increases the energy requirements of fish by making ion transport and maintaining water balance more costly, resulting in increased metabolic energy required for osmoregulation.

Fish regulate ion transport by increasing Na^+/K^+-ATPase activity to counteract the osmotic stress created by increased salinity, thus maintaining plasma osmolarity. Furthermore, the hormones prolactin and cortisol play critical roles in the physiological management of osmotic stress and contribute significantly to the maintenance of water-ion balance (McCormick, 2001).

Gills are extremely sensitive to environmental stress factors due to their direct contact with ions in the water and their ability to conduct ion and gas exchange. Therefore, as a key component of the osmoregulation mechanism, they are among the first organs affected by environmental changes (Nascimento et al., 2012). Various histopathological damages, such as epithelial cell proliferation, epithelial detachment, secondary lamella fusion, telangiectasia, hypertrophy, and necrosis, have been reported in fish exposed to increased salinity (Tavares-Dias, 2021; Hossain et al., 2022). Additionally, as a physiological response, an increase in chloride cells and mucus cells occurs in the gill tissue. Mucus secretion acts as a barrier to protect the gills from adverse effects in the aquatic environment. This increase in mucus cell density, in particular, restricts ion and gas exchange in the gill lamellae, impeding oxygen uptake and negatively impacting respiratory efficiency.

3. BEHAVIORAL AND ENDOCRINE SYSTEM CHANGES

In addition to physiological stress, climate change also causes significant behavioral changes in fish. Increasing temperatures and CO_2 concentrations can negatively impact foraging behavior, predator avoidance responses, and reproductive behavior, weakening species' ecological adaptability (Doney et al., 2012).

The endocrine system plays a central regulatory role in this process. Increased cortisol levels, in particular, are a biochemical indicator of chronic stress, leading to a restructuring of energy allocation, which can lead to decreased growth performance and reproductive success in the long term (Barton, 2002).

4. CONCLUSION

The impacts of global climate change on aquatic biotopes occur in a cascading domino effect. Therefore, creating scenarios by simulating changes in multiple interacting parameters, rather than just one parameter of water's physicochemical properties, will enable future projections of the negative impacts of global warming, which is increasingly becoming a serious global problem, to be developed, realistic predictions to be made, and measures to be taken for more effective aquatic ecosystem management.

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CHAPTER 10

THE EFFECTS OF CLIMATE CHANGE ON FISH PHYSIOLOGY AND NUTRITION

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INTRODUCTION

Aquaculture is the world's fastest growing food production sector, currently providing more than half of the fish consumed by humans and playing a significant role in global food security and nutrition (FAO, 2022). However, the future growth, productivity, and sustainability of this sector, which is so important for food production, are threatened by climate change caused by human activities. Rising sea temperatures and levels, ocean acidification, changes in rainfall patterns, and the frequency of extreme weather events are altering water quality, which in turn affects the metabolic rates and feeding behavior of farmed organisms. All these changes in water create physiological stress in aquatic organisms, leading to suppression of the immune system, slower growth rates, and increased susceptibility to disease (Maulu et al., 2021); (De Silva and Soto, 2009); (Combe et al., 2023). Changes water conditions due to climate change lead to metabolic changes, which directly affect nutritional requirements, feed composition and the rate of metabolic waste. Nutrient-derived waste outputs, such as nitrogen and phosphorus, which can contribute to eutrophication, are a factor that directly affects the environment and also has a direct impact on the sustainability of aquaculture (Bureau and Hua, 2010).

Feed is the largest production cost in intensive aquaculture and generally accounts for 50-60% of total expenses. Feed is a key determinant of both economic profitability and environmental impact (Macusi et al., 2023). In traditional aquaculture, feeds produced by commercial companies with a fixed formulation suitable for the species are generally used. These feeds are given to fish according to predetermined feeding tables. However, this is only applicable under stable environmental conditions. Changes in the water and the produced organisms resulting from climate change can render traditional feeding practices ineffective or even harmful to production. For example, under stressful conditions caused by environmental changes, overfeeding can cause significant damage not only economically but also in terms of fish health and the environment. Therefore, for the sustainability of aquaculture and global food security, it is imperative to develop smarter and more responsive feeding strategies that are adapted to the environmental changes that will occur as a result of climate change (Fawole and Nazeemashahul, 2022).

THE PHYSICOCHEMICAL EFFECTS OF CLIMATE CHANGE ON THE AQUACULTURE ENVIRONMENT

Aquaculture's need for stable water environments makes the sector vulnerable to global climate change. These impacts are already being observed and will negatively impact sustainable aquaculture if solutions are not developed urgently. The effects of climate change on aquaculture are evaluated under several key headings.

Climate change is altering some fundamental characteristics of the aquatic environment in which aquaculture takes place. While varying by species, changes in water conditions can lead to suboptimal or, in extreme cases, lethal outcomes.

Rising Water Temperatures: Fish are organisms dependent on the external environment temperature (ectotherms) and their metabolic rates are directly related to water temperature. Temperature increases within the tolerance range of fish generally accelerate metabolism. Every 10°C increase in water temperature doubles the metabolic rate. High temperatures theoretically require higher energy intake. In such cases, if sufficient feed is not available in the environment, fish growth slows down or stops. This is because fish expend energy on cellular repair and basic maintenance, reducing the energy allocated to growth and reproduction. This situation leads to low feed conversion ratios (FCR), directly increasing production costs. Above the species-specific thermal optimum temperature, metabolic rates become unsustainable, appetite loss begins, feed intake ceases, and even death can occur (Mugwanya et al., 2022); (Shadhana et al., 2025); (Volkoff and Rønnestad, 2020).

Thermal Stratification and Low Oxygen Levels: Thermal stratification in water bodies intensifies with rising air temperatures, particularly under Mediterranean climate conditions. A warm and less dense layer of water (epilimnion) sits on top of a colder and denser layer (hypolimnion), preventing oxygen exchange. The lack of mixing and insufficient light reaching the hypolimnion can lead to hypoxic (low oxygen) or anoxic (oxygen-depleted) conditions in the lower layers. This situation, particularly common in ponds and lakes, poses a significant risk to aquaculture activities in these environments. Sudden weather changes, such as storms, in these areas can cause oxygen-depleted water to rise to the surface, leading to mortality (Madyouni et al. 2025).

Expansion of Pathogen Range and Virulence: Warming waters increase the spread and impact of pathogens that are particularly prevalent in aquaculture. Vibrio species, in particular, are among the leading pathogens and have recently been spreading rapidly in the Mediterranean region, affecting the aquaculture sector. In addition to bacterial diseases, parasitic diseases are also becoming more widespread and threatening with rising temperatures. In particular, white spot disease caused by *I. multifiliis* protozoan parasites is increasingly common in aquaculture facilities. Increases in fungal diseases are also observed with rising temperatures. Saprolengia fungi, in particular, have become very common in aquaculture operations. Furthermore, high temperatures can increase the reproduction rate of pathogens, weakening the host fish's immune response and leading to more frequent and severe disease outbreaks. This situation increases the need for treatment and antibiotics, contrary to efforts to reduce chemical use in aquaculture. The development of pathogen resistance to antibiotics used in treatment is another significant problem and the most important factor threatening the future of aquaculture (Jeyachandran, 2025).

Ocean Acidification: Ocean acidification occurs when increased carbon dioxide in the atmosphere is absorbed by ocean waters and reacts with seawater to form carbonic acid. Oceans absorb one-third of human-generated carbon dioxide. This causes the pH of seawater to decrease and carbonate ions (CO_3^{2-}) in the water to decrease. This situation causes problems especially for shellfish (oysters, mussels, clams) and crustaceans (shrimp, lobsters) whose shells are made of calcium carbonate. This situation negatively affects shell formation and thickness, leading to deformities and mortality, especially during the sensitive larval and post-larval stages (Clements and Chopin, 2017). Therefore, ocean acidification directly threatens the sustainability, productivity, and economic viability of shellfish aquaculture.

The effects of ocean acidification on fish are lower than on shellfish. Apart from the otolith, which is formed from calcium carbonate and helps fish maintain balance and find direction, there is no other structure in the fish's body. Some studies have reported that otoliths change in response to increased acidification. However, the general consensus is that there is no change in otolith size. Although fish are reported to be more resistant to acidification, some studies show that acidification can impair sensory abilities such as smell and orientation. This can disrupt critical migratory behaviors such as escaping predators, selecting suitable habitats, and, particularly for salmon species, returning to their birthplace. This could particularly affect the production method used for Pacific salmon, which focuses on catching juveniles migrating to the sea when they return (Clements and Chopin, 2017); (Heuer and Grosell, 2014).

Rising Sea Levels and Increasing Frequency of Extreme Weather Events, Infrastructure Damage: Among the adverse changes associated with seasonal change facing the world are rising sea levels due to warming and sudden violent storms caused by abrupt weather changes. When such adverse conditions occur, aquaculture facilities located on water and in coastal areas are particularly affected. Storm surges and floods, whose effects are increasing day by day, physically destroy infrastructure, causing farmed species to escape into the wild. Escaped fish can have genetic and ecological impacts on natural stocks (Verdegem et al., 2023).

Salinization: The rise in sea level due to climate change causes saltwater to mix with the world's limited underground and surface water resources (Zamrsky et al., 2024). This situation, which is particularly problematic for businesses producing in freshwater near the coast, leads to the replacement of farmed species or the relocation of businesses to other areas.

Drought and Water Scarcity: One of the most significant effects of climate change is drought. Inland aquaculture, which requires water bodies such as flowing or large-volume reservoirs, is the production method most affected by drought. In flowing inland water fish farming, drought-related decreases in water volume can cause the temperature of excess water remaining in the system to rise and, due to low water inflow, the concentration of metabolic waste products such as carbon dioxide and ammonia to exceed tolerable levels. In ponds, increased water temperatures can lead to a decrease in oxygen capacity, and declining water levels can damage the farming system. Even rainfall during hot weather can carry suspended solids from the area surrounding the farming system into the farming area, leading to a deterioration in water quality (Wind et al., 2024).

Floods and Surface Runoff: Unlike drought, sudden heavy rainfall events can cause water floods carrying pollutants, pesticides, and suspended solids from agricultural fields surrounding lakes and ponds where aquaculture is practiced into aquaculture systems. Pesticides, in particular, can cause imbalances in the hematological system of fish, irregularities in the liver and metabolism, oxidative stress, and weakened immunity. In addition, pesticides can cause a decrease in total protein levels in internal organs (liver, gills, and kidneys) and muscle tissues, as well as a decrease in polyunsaturated fatty acids due to disturbances in lipid metabolism (Kim et al., 2023); (Burch et al., 2025).

DIRECT AND INDIRECT EFFECTS OF CLIMATE CHANGE ON FISH PHYSIOLOGY AND NUTRITION

Fish are poikilothermic (ectothermic) animals that are integrated with their aquatic environment. Their body temperatures and, consequently, all their biochemical and physiological conditions are determined by the water in which they live. When climate change alters the physical and chemical properties of water, this directly affects fish physiology. As a result of this changing physiological structure, nutritional needs and metabolic processes also change. Understanding this interaction between seasonal changes and fish physiology is crucial for developing effective adaptive feeding methods and plans (Volkoff and Ronnestad, 2020).

Thermal Stress and Metabolic Change: The most significant impact of climate change on aquatic environments is the increase in water temperature. Since temperature change directly affects metabolic rate, it causes changes in all metabolic activities (Fawole and Nazeemashahul, 2022).

Increased Standard Metabolic Rate (SMR): SMR represents the amount of energy required to maintain basic bodily functions in fish at a certain temperature, at rest, and without stress. As temperature increases, the SMR of fish increases exponentially (Volkoff and Rønnestad, 2020). Therefore, a larger portion of the energy consumed must be allocated solely to survival, leaving less energy for growth, reproduction, and activity. This situation can lead to a stagnation or decline in growth rates and a deterioration in the feed conversion ratio (FCR) despite maintaining or even increasing feed intake (Holker et al., 2003; Kumar and Banik, 2022).

Changing Macronutrient Utilization: Increased metabolic demand under thermal stress alters how fish utilize macronutrients.

a) Protein: High temperatures can increase protein conversion and breakdown rates. If energy from feed is insufficient, proteins are catabolized for energy, leading to reduced growth and increased ammonia production, which is more toxic at higher temperatures. This situation not only leads to energy waste, but also causes water quality problems (Wilson, 2003); (Jana et al., 2006); (Lu et al., 2021); (Lu et al., 2023).

b) Lipids and Carbohydrates: Fish lipid metabolism is directly affected by water temperature (Fang et al., 2010); (Kumar et al., 2022). Carbohydrate metabolism is also under thermal stress. Therefore, metabolic and physiological adjustments are necessary (Mahanty et al., 2022). To conserve protein, the nutritional requirement for energy readily obtainable from lipids and digestible carbohydrates may increase. However, the capacity to digest and metabolize these energy sources, especially carbohydrates, may be impaired at excessive temperatures, leading to a complex nutritional dilemma (Amin et al., 2014).

Oxygen Limitation: Warmer water holds less dissolved oxygen (DO). At the same time, the fish's metabolic rate and, consequently, its oxygen demand increase (Jana et al., 2022). This creates an "oxygen squeeze," where the difference between oxygen supply and demand narrows dangerously (Kumar et al., 2022). At critically low DO levels, feeding and digestion may cease entirely because they are energy-intensive processes. Chronic, non-lethal oxygen deficiency can lead to reduced feed intake, poor growth, and increased susceptibility to disease (Jiang et al., 2021).

Oxidative Stress, The Cellular Consequence of Environmental Stress: One of the key indirect effects of climate change (high temperature, hypoxia, and fluctuating salinity) is the triggering of oxidative stress at the cellular level. Temperature changes can cause alterations in the food chain of fish. For example, they can inhibit the growth of natural food types such as plankton, causing starvation in species that feed on them. These stresses disrupt fish antioxidant systems by creating oxidative stress at the cellular level. Oxidative stress is defined as damage to cells or tissues caused by excessive oxidative chemicals, particularly free radicals (Kumar and Banik, 2022). Environmental stress factors increase the production of reactive molecules called Reactive Oxygen Species (ROS), such as superoxide anions and hydrogen peroxide, and when these ROS accumulate, an imbalance between ROS production and the antioxidant enzyme system can trigger cell damage (Fawole and Nazeemashahul, 2022). Immune cells are particularly vulnerable to oxidative damage, leading to a weakened immune response and higher susceptibility to bacterial, viral, and parasitic infections; the virulence of these infections may also be increased by warmer waters (Dawood et al., 2020).

Gastrointestinal and Digestive Disorders: The digestive system of fish is the primary interface between the environment (via feed) and the organism and is highly sensitive to climatic stress factors.

Intestinal Microbiota Dysbiosis: The community of microorganisms (microbiome) in the fish intestine is vital for digestion, nutrient absorption, and immune homeostasis. Thermal stress causes significant changes in the composition and diversity of the gut microbiota. This can reduce the microbiome's ability to break down complex food components, thereby decreasing nutrient digestibility and utilization (Kumari and Nair 2022).

Changing Digestive Enzyme Activity: The activity of key digestive enzymes (proteases, lipases, carbohydrases) is temperature-dependent (Fang et al., 2010); (Liu et al. 2019). While activity may increase up to an optimum temperature, enzyme denaturation may occur beyond this point, disrupting the delicate synchronization of different enzymatic processes. This situation leads to incomplete digestion and greater loss of nutrients to the environment via feces (Lingaraju et al., 2023); (Sinha, 2022); (Chakravarty et al., 2022).

Intestinal Morphology and Integrity: Chronic stress can damage the intestinal mucosa, reducing the surface area available for nutrient absorption and compromising intestinal barrier function (Cantas et al. 2012). This can trigger systemic inflammation, which is both energetically costly and health-risky, by allowing pathogens or toxins to enter the bloodstream (Sekirov and Finlay, 2009); (Kumari and Nair, 2022).

Behavioral and Appetite Changes: Climate change is a factor that affects fish ecological roles, distribution, and survival. Climate-induced environmental changes directly affect fish feeding behavior, leading to certain consequences for feeding management (Maheshwari and Singh, 2025). Loss of appetite is at the forefront of these consequences. During periods of excessive heat or low oxygen, fish generally experience loss of appetite or reduced feed intake. This is a physiological adaptation to reduce metabolic heat (heat produced during digestion) and oxygen demand (Pickering and Stewart 1984).

ADAPTABLE AQUATIC FEED FORMULATION UNDER CLIMATE STRESS

The effects of the physiological and behavioral disturbances mentioned above will lead to significant changes in the nutritional requirements of fish. A feed formula designed for stable and balanced conditions will be inadequate under climate stress. This creates a new target for nutritionists and necessitates a shift from static feed formulas to dynamic, adaptable formulas that can respond to the physiological state of the fish, which is a reflection of the changing environment.

Adaptable feed formulation involves the strategic design of diets containing specific ingredients and additives that can enhance an animal's innate resilience to environmental stress factors. The goal is to go beyond merely meeting basic nutritional requirements and formulate protective and supportive diets under suboptimal conditions (Lingaraju et al., 2023). Furthermore, adaptive feed formulation enables the dynamic adjustment of feed composition based on real-time data related to environmental conditions, animal physiology, health status, and growth performance (Pailan and Biswas, 2022).

Functional Feed Additives

Functional feed additives provide powerful tools to enhance climate resilience by regulating physiological responses to environmental stress factors. These bioactive compounds, present at relatively low concentrations (typically 0.01-2% of the diet), target specific molecular pathways involved in stress adaptation, immune function, and metabolic efficiency. Some functional feed additives commonly used in aquaculture include prebiotics, probiotics, immune stimulants, enzymes, antioxidants, vitamins, plant extracts, and organic acids (Nakagawa et al. 2007).

Antioxidants: Diets can be fortified with both enzymatic and non-enzymatic antioxidants to counteract oxidative stress caused by hypoxia and high temperatures (Hoseinifar et al. 2021). These include synthetic compounds such as ethoxyquin (although its use is declining) and natural alternatives such as vitamin E (α-tocopherol), vitamin C (ascorbic acid), and carotenoids (e.g., astaxanthin) (Mishra et al. 2015). Astaxanthin, a carotenoid pigment found in microalgae, has shown superior antioxidant activity compared to other carotenoids and vitamins, effectively reducing oxidative damage (Kumar, et al., 2021).

Trace minerals such as selenium (a cofactor for glutathione peroxidase) and zinc (for superoxide dismutase) are essential for the body's endogenous antioxidant enzyme systems (Wołonciej, et al., 2016).

Natural extracts from plants rich in polyphenols are also of interest. Plant-derived polyphenols from sources such as green tea, grape seed, and rosemary extract are also of interest due to their ability to regulate antioxidant enzyme pathways and increase stress tolerance (Onomu and Okuthe, 2024). Phytochemicals, including polyphenols, carotenoids, and essential oils, provide potent antioxidant capacities that scavenge ROS produced during climatic stress. (Fawole Nazeemashahul, 2022)

Immunostimulants: Climate stressors suppress the immune system, making animals more vulnerable to disease outbreaks. These compounds provide broad-spectrum defense during vulnerable periods following stressful events by strengthening the non-specific (innate) immune system. They aim to improve fish growth, health status, stress tolerance, and disease resistance (Oliva-Teles, 2012); (Chakravarty et al 2022). Adding immunostimulants to the diet strengthens non-specific immunity, enabling fish to gain resistance against opportunistic pathogens that develop under adverse conditions (Wang et al. 2017); (Pailan and Biswas, 2022). Since these components generally have a protective (prophylactic) rather than therapeutic effect, they should be included in feed prior to periods of stress. Examples include β-glucans, mannan oligosaccharides (MOS), lipopolysaccharides, and nucleotides derived from yeast, seaweed, or fungi (Vijayaram et al., 2024).

Probiotics and Prebiotics: Probiotics are live microorganisms that provide health benefits to the host. Among microorganisms, gram-negative bacteria, gram-positive bacteria, bacteriophages, and yeasts can be used as probiotics (Akhter et al., 2015).

A healthy gut microbiome is crucial for digestion, nutrient absorption, and immune function. The gut microbiome is extremely sensitive to environmental stress. Heat and hypoxia can lead to a decrease in beneficial bacteria and a weakening of the gut barrier, causing dysbiosis, inflammation, and reduced nutrient absorption (Kumari and Nair, 2022). Supplementing with host-specific, heat-resistant probiotics (e.g., *Bacillus subtilis, Lactobacillus plantarum*) and prebiotics (e.g., fructooligosaccharides, mannan oligosaccharides) helps maintain a healthy and balanced gut microbiota under climatic stress (Ringø et al., 2016). This is also critical for robust digestion and overall health (Merrifield et al., 2010); (Kumari and Nair, 2022).

Osmolytes: To support the energy expenditure required for increased osmoregulation due to acidification and high temperatures, diets are supplemented with osmolyte compounds such as dietary salt, inorganic potassium, metals, inositol, and monosaccharides. These compounds ensure that protein structures and cells remain stable under osmotic stress without disrupting cellular function; thereby reducing the metabolic cost of maintaining ionic balance. The energy saved can then be directed toward growth and repair processes (Cao, et al., 2025).

Plant Extracts and Phytogenics: Many plants and spices, such as garlic (*Allium sativum*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*), contain bioactive compounds (e.g., allicin, gingerols, curcumin) with proven anti-inflammatory, antioxidant, and antimicrobial properties. Including them in aquatic animal feeds may serve as a natural strategy to enhance overall resilience (Serradell et al., 2020).

Adjusting Macronutrient Ratios

The dynamic nutrient profile is the key innovation in adaptive feed formulation. It addresses these limitations by providing real-time nutritional assessments that adapt to changing physiological states and health goals (Molooy et al., 2025). Here, dietary specifications are continuously adjusted based on environmental conditions, the animal's physiological state, and production goals, rather than following predefined formulas. This approach uses bioenergetic models that integrate temperature-dependent metabolic rates, stress-induced protein turnover, and activity levels to calculate real-time nutritional requirements (Molooy et al., 2025).

Highly Digestible Proteins and Amino Acids: High-quality proteins are easily digestible and contain sufficient amounts of essential amino acids

(EAAs) to meet the needs of fish (Mahanty et al. 2022). High-protein diets produce more metabolic ammonia, which is more toxic at higher temperatures and lower pH values. Therefore, during thermal stress, it may be beneficial to slightly reduce crude protein levels (by 2-4%) while providing an optimal and highly digestible amino acid profile. This reduces the heat of digestion (metabolic heat produced during digestion) and minimizes ammonia excretion (Wu, 2013). Certain amino acids have functional roles: Arginine also helps prevent heat-related mortality (Costa et al. 2014), Histidine is a precursor to histamine and plays many roles in protein interactions (Liao et al. 2013), L-Tryptophan is a precursor to serotonin, a neurotransmitter that regulates stress responses, and L-Methionine is crucial for the synthesis of the antioxidant glutathione (Chakravarty et al., 2022).

Optimized Protein/Energy (P:E) Ratios: An imbalanced P:E ratio may lead to the breakdown of protein for energy and increased ammonia excretion. Under hypoxic conditions, diets that are slightly lower in protein but supplemented with energy from highly digestible fats (e.g., fish oil) may be more efficient, as lipid metabolism requires less oxygen compared to protein metabolism (Kamalam and Pandey, 2022).

Lipids and Fatty Acids: Increasing digestible energy from lipids can prevent protein from being used for energy, allowing it to be used for growth and repair; this concept is known as the protein-sparing effect (Mock et al., 2019). Additionally, the fatty acid composition of dietary lipids is incorporated into cell membranes. The role of fatty acids extends beyond energy. Adequate levels of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (DHA), maintain membrane fluidity at varying temperatures and provide precursors for anti-inflammatory eicosanoids that can alleviate stress-induced inflammation (Sinha, 2022).

Carbohydrate Utilization: The ability to utilize carbohydrates varies greatly among species. For omnivorous species such as tilapia and carp, well-cooked, digestible carbohydrates can be a cost-effective energy source. However, under hypoxic conditions, high carbohydrate levels can be detrimental due to dependence on anaerobic metabolism. The type of carbohydrate (e.g., complex or simple) also affects water stability and digestibility (Zhou et al., 2013).

Under stressful conditions, the balance between dietary protein, lipids, and carbohydrates should be reevaluated.

New and Alternative Ingredients

In adaptable aquatic feed formulations, dependence on climate-sensitive ingredients should decrease, while maximizing formulation flexibility is necessary to ensure the sustainability of feed supply chains. The historical dependence on fish meal and fish oil from capture fisheries, which are subject to climate-induced yield variations, necessitates diversification toward alternative protein and lipid sources with stable supply chains (Tacon et al., 2011).

Hermetia illucens (black soldier fly) and *Tenebrio molitor* (mealworm beetle) insect meal provide complete amino acid profiles with high digestibility (85-92% crude protein digestibility) and can be produced year-round in climate-controlled environments (Gautam et al., 2025). Microbial proteins derived from single-celled organisms (e.g., *Candida utilis, Spirulina platensis*) provide additional formulation flexibility with protein content exceeding 60% and production capacity utilizing agricultural waste streams, thereby creating circular economy benefits (Li et al., 2024). Algal oils rich in omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs) can replace fish oil, and Schizochytrium sp. provides docosahexaenoic acid (DHA) levels exceeding 40% of total lipids without the climate sensitivity of marine fisheries (Neylan et al., 2024). The strategic inclusion of these components should consider their functional properties under climate stress; for example, insect meal contains antimicrobial peptides and chitin, which can enhance disease resistance during heat stress (Alhasyani et al., 2025).

PRECISION FEEDING TECHNOLOGIES IN AQUACULTURE

Adaptable feed production in response to the effects of climate change can be successful with the effective use of these feeds. Adaptable formulation ensures that the feed is of the correct content and suitable for the species, while precision feeding ensures that the feed reaches the fish in the right amount and at the right time. Variable factors resulting from climate change alter fish appetite and feeding intervals, leading to a decrease in feed efficiency. Precision feeding involves methods that dynamically respond to changes in both the fish and their environment, maximizing efficiency and resilience. The concept of precision feeding emerged with the idea of climate-smart agriculture (CSA). Climate-smart agriculture (CSA) is a concept introduced by the Food and Agriculture Organization of the United Nations (FAO) in 2009 to mitigate the effects of climate change and improve agricultural production (FAO, 2013). Fish feeding and management is one of the thematic areas of CSA. In addition to managing the effects of climate change, feeding management also contributes to the prevention of climate change by reducing the environmental impacts (eutrophication, carbon emissions, etc.) resulting from fish feeding activities.

Monitoring the variables resulting from climate change is the first important issue for responding to them and finding solutions. The basis of precision feeding is the collection of reliable, real-time data on fish behavior and their environment. Therefore, the first important step for good feeding management is the ability to monitor these variables, and many different technologies are used for this purpose.

Monitoring and Sensor Systems: The "Eyes and Ears" of the Farm

Computerized imaging and image analysis technology is a technology that provides direct insight into feeding activity. In this application, underwater cameras are placed near feeding sites and at strategic points at different depths. Equipped with biologically resistant lenses developed in recent years, these water-resistant camera systems with wide viewing angles typically use additional artificial lighting in spectrums invisible to fish (e.g., infrared) to enable 24/7 monitoring of aquaculture systems without altering the natural behavior of fish. The images captured by these cameras are processed using advanced machine learning algorithms. These systems are used for the following detection and analysis tasks.

Detecting Uneaten Feed: Algorithms can detect pellets falling from the water column by processing images from cameras (Fore et al., 2017). When the amount of feed not consumed by fish exceeds a certain ratio, the system stops the feeding system, preventing waste.

Analysis of Fish School Behavior: Software that processes images from monitoring systems can also track the behavior of fish schools. Healthy and hungry fish move quickly to the feeding point at feeding time. As the fish become full, the school movement disperses and their movements slow down. This change in behavior is analyzed by algorithms, which automatically stop feeding.

Estimating Fish Weight: Fish weight is crucial for determining feed content, feed size, and feeding rates. Systems that calculate fish size and weight by comparing them to known reference sizes via cameras enable appropriate feeding based on these estimates.

The basis of fish feeding varies depending on the metabolic needs and rates of the fish. The most influential external factors on metabolism are water temperature and oxygen content in the water. Continuous monitoring of these factors will ensure appropriate feeding. Again, many different types of mechanization are used for this monitoring.

Multi-Parameter Probes: These devices, placed at various locations and depths within the farming systems, continuously measure the desired critical water parameters. These are:

Dissolved Oxygen (DO): This is the most critical parameter. When DO falls below species-specific threshold values, feeding is significantly reduced or stopped to prevent stress and feed wastage.

Temperature: Data from measurement probes and computer support are used to adjust feeding programs to match the fish's temperature-dependent metabolic rate. Feeding can be shifted to cooler times of the day when oxygen levels are higher.

pH, Salinity, and Ammonia: These parameters provide a comprehensive view of water quality; all of them can affect feeding activity and should be considered in feeding decisions.

Data Analysis and Decision Support Systems: The "Brain" of the Operation

Raw data obtained from camera or water criterion measurement systems is analyzed using advanced software to provide producers with predictive information. This enables the identification of problems, the determination of solutions, and the creation of forecasts.

Appetite and Satiety Models: These models compare real-time data (fish activity captured by cameras, water temperature) with historical data (previous day's feeding, growth curve) to predict the optimal daily feeding rate. They learn the unique feeding patterns of a specific fish stock over time and become more accurate with continuous use.

Growth Projection Models: Programs that predict future growth exist through the integration of systems that detect fish size with systems that track temperature and feed consumption. These enable planning and adjusting feeding practices for purposes such as harvest planning and timing to reach the target weight.

Systems that support the use of data obtained from the aforementioned monitoring and data analysis systems in fish feeding are automatic feeding systems.

Automatic Feeding Systems: The "Hands" That Implement

Automatic feeding systems process data and carry out the task of providing fish with appropriate feed in the required proportions. These include

Pneumatic Feeders: These systems use pressurized air to deliver feed over long distances through pipes. They are effective and widely used for large sea cages.

Subsea Feeding: Used for species that prefer feeding below the surface, these systems deliver feed directly to specific depths, minimizing surface drift loss. Feeding is typically done using a pipe extending from the surface to the desired depth.

On-Demand Feeders: The most modern feeding technology, this system begins feeding when data from monitoring systems indicates conditions are suitable (sufficient DO, correct temperature) and when cameras detect that fish are actively requesting feed.

The integration of adaptive feed formulation and precision feeding is not merely a technical application. When combined, these applications become a strategic transformation that delivers powerful and mutually reinforcing economic and environmental benefits. While each approach provides benefits on its own, the real benefits emerge when they work together, enabling climate-resilient, efficient, and sustainable aquaculture.

CONCLUSION

The effects of climate change on aquaculture are greater than on many other farming systems. Minimizing these effects requires a shift from static to dynamic feeding management. Adaptable feed formulation ensures that the feed itself becomes a tool for health and resilience, while precision feeding ensures that this resource is used with maximum efficiency.

Formulating diets that increase the physiological resilience of farmed species and using these feeds with high efficiency with the help of technology can increase the resilience of the aquaculture sector to climate variability.

Adaptable feed formulation and precision feeding offer significant environmental and economic advantages, particularly in the context of climate change mitigation and adaptation. By aligning feed distribution with real-time biological and environmental demands, it is possible to reduce nutrient flows and eutrophication, which are environmentally critical. Lower organic loading leads to a reduced risk of hypoxia, improved benthic habitat quality, and fewer algal blooms. In addition, improved feed efficiency reduces the carbon footprint of aquaculture. Feed production accounts for 70-80% of total greenhouse gas emissions in fish farming. Replacing highly effective components used in feed, such as fish meal and oil, with sustainable alternatives can reduce the carbon intensity per kilogram of harvested product by up to 35%.

Adaptable formulations also increase protein utilization efficiency. By matching amino acid profiles to actual metabolic needs, nitrogen discharged into the water can be reduced, thereby improving water quality.

Adaptable feed formulation and precision feeding are also highly beneficial economically. Reducing feed loss and waste directly lowers operating costs. Considering that feed accounts for 50-70% of total production costs in aquaculture, even the smallest reduction has a significant impact on efficiency.

Positive changes in feed conversion ratio (FCR) and growth rates increase harvest yields and shorten production time. In addition, optimized nutrition improves fish health and immunity, leading to increased survival rates and profitability.

Market access and market share for the produced product are economically important factors. In recent years, certification bodies such as ASC and BAP now give priority to production systems and producers that demonstrate advanced feeding practices. Producers who adopt adaptive feeding gain market share and preference, while also securing priority in benefiting from support programs.

Consequently, the integration of adaptive feed formulation and precision feeding has become not just an option but a necessity for the future of aquaculture. In today's world, where the effects of climate change are intensely felt, adaptive feed formulation and precision feeding must be implemented without delay to ensure the sector's long-term productivity, profitability, and sustainability. Aquaculture can secure its role as a vital protein source for the growing global population not only through capacity expansion but also through smarter and more planned applications.

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CHAPTER 11

ACOUSTIC ENVIRONMENTAL ENRICHMENT IN AQUACULTURE: THE ROLE OF MUSIC IN FISH WELFARE AND PERFORMANCE

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INTRODUCTION

Global aquaculture has become the primary source of aquatic animals for human consumption. Farmed aquatic production reached 94.4 million tonnes in 2022, surpassing capture fisheries in volume and continuing to expand as a critical pillar of global food security (FAO, 2024). As production intensifies, fish welfare and environmental enrichment are increasingly recognised not only as ethical obligations but also as strategic factors that support sustainable productivity, animal health, and product quality (Arechavala-Lopez et al., 2022). Conventional environmental enrichment strategies in aquaculture have largely focused on structural complexity, substrate provision, dietary enhancement, and social enrichment, all of which have been shown to reduce stress, improve growth, and enhance cognitive performance across a variety of cultured species (Näslund & Johnsson, 2016; Arechavala-Lopez et al., 2022).

However, the sensory environment of farmed fish is not solely visual or structural; it is fundamentally acoustic. Modern aquaculture systems generate persistent underwater noise from mechanical equipment such as pumps, aerators, blowers, feeders, and inflowing water turbulence, resulting in soundscapes that differ markedly from natural habitats. Experimental evidence demonstrates that chronic anthropogenic noise can impair hearing acuity, reduce growth, and compromise disease resistance in farmed rainbow trout (*Oncorhynchus mykiss*), indicating that sound constitutes a critical welfare and performance-related environmental variable (Wysocki et al., 2007; Davidson et al., 2009). More recent work in largemouth bass (*Micropterus salmoides*) shows that production-related soundscapes influence behaviour, stress physiology, and somatic growth, reinforcing the need to actively manage acoustic conditions in farming environments (Zhang et al., 2023).

Many teleosts are sensitive to both particle motion and, in some species, sound pressure (Popper et al., 2014). “Hearing specialists” such as common carp (*Cyprinus carpio*) detect fine-scale acoustic cues via Weberian ossicles, which connect the swim bladder to the inner ear, enhancing sensitivity at low sound intensities (Popper et al., 2003). This sensory capacity highlights sound as a powerful yet frequently overlooked environmental stimulus in aquaculture. Although much of the literature emphasises the detrimental impacts of human-generated noise (e.g., shipping, pile-driving, mechanical pumps), increasing evidence indicates that structured sound, including music, may function as an intentional form of acoustic environmental enrichment.

A series of pioneering studies by Papoutsoglou and colleagues examined the effects of classical music, particularly Mozart's Eine kleine Nachtmusik (K.525) and the "Romanza" Andante, on farmed fish. In carp, exposure to Mozart under varying illumination enhanced growth performance, altered brain monoaminergic profiles, and modified carcass and hepatic fatty-acid composition, suggesting that music may act as a stress-modulating factor with implications for welfare and fillet quality (Papoutsoglou et al., 2007). Comparable trials in gilthead seabream (*Sparus aurata*) reported improved welfare indicators, growth, and product traits following exposure to classical compositions in recirculating aquaculture systems (Papoutsoglou et al., 2008; 2015). Additionally, species-specific responses have been documented: in rainbow trout reared under recirculating systems, music and white noise differentially modulated growth and physiological variables (Papoutsoglou et al., 2013).

Evidence from ornamental fish corroborates this concept. In koi carp maintained for 90 days in recirculating systems, exposure to Silk Road-style compositions, Sufi ney music, and Qur'anic recitation significantly increased specific growth rate and improved feed conversion ratio compared with silent controls, whereas urban noise caused substantial performance deterioration (Kusku et al., 2019). Furthermore, behavioural and endocrine data from zebrafish (*Danio rerio*) and guppies (*Poecilia reticulata*) suggest that slower, harmonic music, such as traditional guzheng, reduces anxiety and whole-body cortisol, indicating that well-designed music protocols may enhance welfare even in barren laboratory aquaria (Niu et al., 2025). Early laboratory studies also demonstrated that carp can discriminate among musical pieces and sound patterns, implying meaningful auditory perceptual ability applicable to enrichment (Chase, 2001).

Parallel conceptual and review articles argue that acoustic enrichment, including controlled music exposure, should be integrated into welfare-oriented aquaculture alongside structural and occupational enrichment (Arechavala-Lopez et al., 2022; Kleiber et al., 2023). A recent synthesis specifically addressing music and noise in teleosts concluded that selected musical stimuli may reduce stress and enhance growth, whereas inappropriate sound types or intensities may be aversive or harmful, emphasising species-specific and volume controlled applications (Tóth et al., 2025).

Collectively, available evidence indicates that music represents a promising yet insufficiently explored environmental enrichment tool with potential to modulate growth, feeding behaviour, welfare, stress physiology, immune function, and product quality. Conversely, poorly regulated acoustic environments, whether due to industrial noise or inappropriate soundscapes, may compromise welfare and negate potential benefits. Therefore, this chapter critically reviews current evidence regarding the role of music as an acoustic enrichment strategy for fish welfare and performance in aquaculture.

FUNDAMENTALS OF HEARING AND ACOUSTIC PERCEPTION IN FISH AND CRUSTACEANS

Fish and many crustaceans possess functional auditory systems capable of detecting sound pressure, particle motion, or both. Considerable interspecific variation exists in auditory thresholds, frequency sensitivity, and behavioural responsiveness; thus, acoustic enrichment must be tailored to species-specific hearing capabilities, frequency ranges, and ecological relevance. Failure to align stimuli with perceptual biology risks producing neutral or even stress-inducing outcomes.

In intensive aquaculture, particularly recirculating aquaculture systems (RAS), ambient noise generated by water movement, pumps, blowers, and mechanical aeration produces continuous acoustic fields that may induce chronic stress (Tóth et al., 2025). Given that fish perceive these sound fields, understanding auditory physiology is essential for designing safe and effective enrichment protocols.

Teleost inner ears comprise semicircular canals and otolithic end organs (saccule, lagena, utricle), which detect particle motion and, in some taxa, sound pressure. The lateral-line system further detects low-frequency hydrodynamic cues (Popper et al., 2003). Accessory auditory structures such as swim bladders and Weberian ossicles enhance pressure sensitivity, resulting in lower thresholds and broader detectable frequencies in hearing specialists (Ladich & Schulz-Mirbach, 2016). Recent findings confirm that fish can discriminate among amplitude and frequency variants, implying that behavioural assessments are necessary to complement electrophysiological measurements (Popper & Hawkins, 2021).

Underwater sound is composed of both scalar sound pressure and vector particle motion (Davies et al., 2024). Most teleosts primarily detect particle motion, whereas species with swim-bladder attachment detect both (Hawkins & Popper, 2018; Popper 2023). Hearing ranges vary considerably: many species detect <300 Hz, whereas specialists may detect frequencies in the kilohertz range (Popper & Hawkins, 2021). Although less understood, crustaceans perceive acoustic stimuli through statocysts and mechanosensory organs, and studies show that noise can affect survival and biochemical responses (Davies et al., 2024), though music-based enrichment remains largely hypothetical for these taxa.

MUSIC TYPES, ACOUSTIC PROPERTIES, AND DIFFERENTIAL EFFECTS

Distinguishing Enrichment Soundscapes from Noise

A critical distinction must be drawn between structured acoustic stimulation (e.g., music, rhythmic auditory patterns) and unstructured industrial noise (e.g., mechanical equipment, shipping, construction). Controlled low-frequency experimental noise in European seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) increased locomotor activity and elevated blood lactate and haematocrit, indicating acute stress (Buscaino et al., 2010). Chronic exposure to farm-related noise additionally modified oxidative status and immune parameters (Filiciotto et al., 2017).

Conversely, studies incorporating music suggest that specific auditory profiles may reduce stress and improve growth when compared with silence and broadband noise (Papoutsoglou et al., 2007; 2008; 2013; 2015; Kusku et al., 2019; Niu et al., 2025). Tóth et al. (2025) propose treating music not as background sound but as a bioacoustic enrichment stimulus requiring calibrated implementation.

Classical Music (Mozart, Bach, “Romanza”)

Much of the foundational research has employed Western classical compositions, particularly:

- Mozart: Eine kleine Nachtmusik (K.525)

- “Romanza” (Andante) – Anonymous
- J.S. Bach: Violin Concerto No. 1

In carp, repeated exposure to “Romanza” improved growth and altered physiological and neurochemical parameters (Papoutsoglou et al., 2007), while in seabream, Mozart K.525 affected stress and behavioural indicators (Papoutsoglou et al., 2008). Further comparative work found that classical music generally:

- enhanced growth and feed conversion,
- improved welfare indices, and
- supported favourable product-quality attributes,

relative to white-noise exposure at equivalent sound-pressure levels (Papoutsoglou et al., 2015).

The shared acoustic features of these pieces moderate tempo, harmonic stability, low percussive energy, and primary spectral emphasis below several kilohertz likely contribute to their non-threatening and potentially calming perceptual profile (Tóth et al., 2025).

Traditional and Ethnographic Music

Music-based enrichment need not be limited to classical repertoire. Kusku et al. (2019) examined Silk Road-style music, Sufi ney melodies, and Qur’anic recitation in koi carp. Exposed fish exhibited:

- significantly higher specific growth rates,
- improved feed-conversion ratios, and
- more stable feeding behaviour,

compared with both silent controls and fish exposed to urban noise, which produced pronounced negative effects.

Tempo, Rhythm, and Temporal Structure

Tempo and rhythmic organisation modulate behavioural and neuroendocrine responses. In zebrafish and guppies, slow-tempo guzheng music reduced anxiety-like behaviour and whole-body cortisol compared with silence or irregular, fast-tempo pieces (Niu et al., 2025). In noise studies, temporal patterns (continuous, intermittent, pulsed) affected activity and recovery dynamics, with intermittent noise producing more persistent behavioural changes (Neo et al., 2014). These findings suggest that aquaculture music protocols should consider not only what is played but also when and for how long.

Sound-Pressure Level (SPL) and Frequency Bandwidth

Even acoustically pleasant music may become aversive at excessive amplitudes. Short-term low-frequency noise (~10 min) elicited acute stress responses in seabass and seabream (Buscaino et al., 2010), and similar "dose-dependent" stress effects are reported across taxa (Vazzana et al., 2017; Filiciotto et al., 2017; Davies et al., 2024). In contrast, music studies commonly employ moderate SPLs (~120–140 dB re 1 μPa) without pathological outcomes (Papoutsoglou et al., 2007; 2008; 2015; Kusku et al., 2019). According to Tóth et al. (2025), safety thresholds must be validated for each target species and production context.

High-Energy Music and Potential Risks

Although heavy metal or high-intensity dance music has not been systematically tested in controlled aquaculture, field observations near large-scale music festivals report elevated cortisol and altered behaviour in fish exposed to intense anthropogenic sound (Rosenstiel School, 2019). Analogous evidence from mammals and birds indicates that rapid, high-energy musical stimuli may increase agitation, stereotypies, and arousal, whereas slower classical music tends to reduce stress markers (Wells, 2008; Lindig et al., 2020; Khan & Wascher, 2021; Williams et al., 2017). Until species-specific data exist, high-energy music should be approached with caution.

SPECIES-SPECIFIC RESPONSES TO MUSIC IN AQUACULTURE

Influence of Hearing Specialisation and Ecology

Auditory abilities vary across taxa due to morphological and ecological adaptations (Ladich & Schulz-Mirbach, 2016; Popper & Hawkins, 2021):

- **Hearing specialists** (e.g., carp, koi) detect wider frequency ranges and lower thresholds.
- **Hearing generalists** (e.g., salmonids) rely primarily on particle-motion sensitivity and have narrower auditory bandwidths.

This variation partially explains differential responsiveness to music.

Cyprinids: Common Carp and Koi (*Cyprinus carpio*)

Carp are classical hearing specialists. Behavioural experiments show musical discrimination ability (Chase, 2001). In aquaculture:

- Mozart "Romanza" modified growth, cortisol, and monoamine profiles (Papoutsoglou et al., 2007).
- Silk-Road, Sufi ney, and Qur'anic recitation increased growth and FCR, whereas urban noise impaired performance (Kusku et al., 2019).

Thus, cyprinids appear highly responsive to acoustic enrichment.

Sparids and Moronids: Gilthead Seabream and European Seabass

Seabream and seabass are widely farmed in the Mediterranean. Both are sensitive to noise:

- low-frequency noise elicited acute stress responses (Buscaino et al., 2010),
- background farm noise impacted immune and oxidative parameters (Filiciotto et al., 2013; 2017).

However, seabream exhibited positive responses to classical music, including improved physiological stability (Papoutsoglou et al., 2008) and enhanced growth and product quality relative to white noise (Papoutsoglou et al., 2015).

Salmonids: Rainbow Trout (*Oncorhynchus mykiss*)

Rainbow trout possess generalist auditory capacity yet remain noise-sensitive (Wysocki et al., 2007). In recirculating systems:

- white noise increased stress and inhibited growth,
- music produced neutral to moderately positive effects, potentially through noise-masking rather than direct stimulation (Papoutsoglou et al., 2013).

Small Ornamental Fishes: Zebrafish and Guppies

Recent studies in zebrafish and guppies found that slow, melodic music:

- reduced anxiety-like behaviour (greater centre-zone exploration, reduced freezing),
- decreased whole-body cortisol (Niu et al., 2025).

These species provide valuable models for mechanistic investigation (e.g., neuroendocrine and transcriptomic pathways).

LIMITATIONS, RISKS, AND RESEARCH GAPS

Despite promising results, music cannot yet be regarded as an off-the-shelf aquaculture technology.

Potential for Music to Function as a Stressor

Music is not inherently beneficial. If SPL is excessive, frequency bands overlap with alarm signals, or temporal structures are erratic, music may act as chronic noise. Numerous studies report that poorly regulated acoustic exposure:

- increases cortisol and haematological stress markers (Buscaino et al., 2010; Vazzana et al., 2017),
- modifies immune and oxidative profiles (Filiciotto et al., 2017; Davies et al., 2024),
- alters behaviour and reduces growth (Wysocki et al., 2007; Zhang et al., 2023).

Thus, species- and size-appropriate calibration is essential.

Species, Life-Stage, and Context Dependency

Auditory capability, ecological background, and life-history stage influence responses (Ladich, 2016; Popper et al., 2019). Positive outcomes in carp may not translate to salmonids or tropical marine species. Early developmental stages may be especially sensitive (Ren et al., 2022). Production setting is also relevant: fish in high-noise RAS systems may respond differently than those cultured in quiet ponds or offshore cages.

Scaling from Laboratory to Commercial Systems

Most experiments have been conducted under controlled laboratory conditions, whereas commercial facilities face challenges including:

- complex and spatially heterogeneous sound propagation (Filiciotto et al., 2013),
- risk of cross-tank acoustic contamination,
- potential contribution to ocean noise pollution.

These factors must be addressed before standardisation.

CONCLUSION

Current evidence indicates that music represents a potent yet under-utilised form of acoustic environmental enrichment in aquaculture. Teleosts and possibly some invertebrates possess sophisticated auditory systems and inhabit complex acoustic environments. In intensive production settings, these soundscapes are dominated by anthropogenic noise that can induce stress, alter physiology, and compromise welfare.

Conversely, emerging experimental studies demonstrate that well-selected and carefully calibrated musical stimuli can:

- reduce stress indicators such as cortisol, lactate, and anxiety-like behaviour,
- stabilise feeding and social behaviours,
- improve growth, feed efficiency, and in some cases, product quality.

These benefits have been shown in carp, koi, seabream, rainbow trout, and ornamental species, with preliminary indications of positive impacts on larval growth and survival. However, music is not a universal therapeutic tool: responses are highly species-, life-stage-, and context-specific, and poorly designed acoustic regimes may themselves constitute chronic stressors. Major gaps remain in mechanistic understanding (neuroendocrine, transcriptomic, epigenetic pathways) and in scaling laboratory evidence to commercial settings.

Future integration of music into aquaculture will require:

- Rigorous bioacoustic engineering and monitoring to ensure species-appropriate SPL and frequency ranges;
- Multilevel biological evaluation linking behaviour, physiology, immunity, and molecular responses;
- Species- and life-stage-specific protocols embedded within broader welfare frameworks; and
- Farm-scale trials and precision aquaculture integration, enabling music to become a controllable management variable.

If these challenges are addressed, music may become a cost-effective, non-invasive, and ethically compelling enrichment tool that complements physical, social, and nutritional strategies, thereby contributing to more humane and sustainable aquaculture systems.

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CHAPTER 12

REPRODUCTIVE OUTPUT OF *Neocaridina davidi* (Decapoda) IN RESPONSE TO FUNCTIONAL FEED ADDITIVES

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INTRODUCTION

Aquaculture today provides more than half of the global supply of aquatic products, and the controlled culture of alternative species with economic value is gaining increasing attention (FAO, 2020). The ornamental branch of this sector has also expanded rapidly, driven by hobby demands and growing interest in aquatic biodiversity. Within this field, the cherry shrimp *Neocaridina davidi* has become one of the most commonly cultured species because of its colour diversity, strong environmental tolerance and simple maintenance. Native to East Asia, *N. davidi* reaches sexual maturity within a few months and produces fully developed juveniles after a short incubation period (Pantaleão et al., 2017). Several environmental factors including temperature, photoperiod and diet shape the reproductive performance of this species. For instance, high temperature stress may reduce egg quality and alter female biochemical composition (Baliña et al., 2018), while temperature shifts can also influence sex ratio patterns (Serezli et al., 2017). Owing to its physiological flexibility and ecological tolerance, *N. davidi* has been widely used as a model species for studies on feeding, reproduction and life-history traits in freshwater decapods (Tropea and Greco, 2015; Pantaleão et al., 2017; Kohal et al., 2018; Levitt-Barmats et al., 2019; Budi et al., 2020; Sganga and López Greco, 2020; Kaya et al., 2022).

Feeding quality plays a fundamental role in shaping reproductive outcomes in ornamental shrimp. Sganga and López Greco (2020) showed that differences in commercial feed formulations directly affect female reproductive performance and offspring traits. Growth and survival of *N. davidi* also respond strongly to diet composition (Bingöl et al., 2016), and spirulina- or plant-based feed ingredients have been associated with improved reproductive success over consecutive spawnings (Kohal et al., 2018). These findings highlight that targeted feeding strategies are key to maintaining stable broodstock productivity. In crustaceans, reproductive processes depend on energy allocation, immunity, gonad development and the capacity to manage stress, which explains the growing interest in prebiotic and immunomodulatory additives (Ringø et al., 2010). Nutrient requirement frameworks, such as those defined by NRC (2011), further emphasize the importance of functional ingredients in crustacean diets.

Among these additives, mannan oligosaccharides (MOS) have received particular attention. MOS supplementation has been reported to improve stress tolerance, nutrient absorption and intestinal health (Genc et al., 2007; Zhang et al., 2012). Its positive effects have been demonstrated in various shrimp species under both conventional and biofloc systems (Gainza and Romero, 2020; Chen et al., 2020; Genc et al., 2024; Dincer and Genc, 2024; Wang et al., 2021). These outcomes suggest that MOS may also support reproductive performance in *N. davidi*. Bee pollen is another natural supplement that contains essential nutrients and antioxidants. Previous studies have noted improvements in growth, health and stress recovery in aquatic organisms (Kohal et al., 2018; Asgari et al., 2020), although excessive levels may interfere with reproductive hormones in fish (Nowosad et al., 2023). Similarly, ginseng (*Panax ginseng*), known for its adaptogenic and antioxidant properties, has been linked to enhanced stress tolerance and immune function in shrimp (Hou et al., 2024; Attia et al., 2025). The ginsenosides it contains may help reduce oxidative pressure in reproductive tissues, supporting vitellogenesis, although studies in crustaceans remain limited.

Considering these points, evaluating both the single and combined effects of MOS, bee pollen and ginseng on *N. davidi* broodstock will help address an evident gap in the literature. Identifying potential synergistic or antagonistic interactions among these additives is also important for practical applications in ornamental shrimp culture. This study investigates the effects of different doses and combinations of MOS, bee pollen and ginseng on the reproductive performance of *N. davidi* across four experimental trials. The findings aim to support the development of optimized feeding protocols and contribute to sustainable production strategies in the ornamental aquaculture sector. Additionally, insights from recent work on prebiotics in penaeid shrimp (Utami et al., 2024) provide a broader context for understanding how these additives may influence reproductive physiology and overall health.

MATERIALS AND METHODS

Experimental site and design

Neocaridina davidi was obtained from a local producer in Antalya and transported to the Aquaculture Research and Application Unit of Ankara University, Faculty of Agriculture, Dep. of Fisheries and Aquaculture

Engineering. Rectangular tanks 40 L were used. Each tank was equipped with a sponge filter, heater, LED lighting (12:12 h light:dark), and continuous aeration. Water parameters were kept within optimal ranges during the experiment: temperature 22±1°C, pH 6.8–7.4, dissolved oxygen 6-7 mg/L, ammonia <0.01 mg/L, nitrite <0.1 mg/L, and total hardness GH 6-8°dH. Juvenile shrimps were stocked in the tanks at 20 individuals/tank. Shrimp were fed *ad libitum* twice a day, and all shrimp used in the study had an initial body weight of 0.018-0.020 g. Each experiment lasted 60 days. The study was designed as four separate trials: Trials I, II, and III: MOS (M), bee pollen (P), and ginseng (*Panax ginseng*) (G) were added to the diets at levels of 0, 1, 2, and 3 g/kg. Each trial included four groups with three replicates, resulting in a total of 12 tanks per trial. Trial IV: Based on the effective doses identified in the first three trials, five combination groups were prepared: MPG0 (control), M2P1 (2 g MOS + 1 g pollen/kg), G2P1 (2 g ginseng + 1 g pollen/kg), M2G2 (2 g MOS + 2 g ginseng/kg), and M2P1G2 (2 g MOS + 1 g pollen + 2 g ginseng/kg). Each group included three replicates, and 15 tanks were used in Trial IV.

Feed additives, diet formulation and reproductive assessment

Experimental diets were prepared in the laboratory of Yalova University based on recommended shrimp feed compositions and NRC (2011) nutrient requirements. The feed formulation included fish meal, Spirulina meal, soybean meal, corn gluten, wheat flour, fish oil, soy lecithin, cholesterol, vitamin–mineral premixes, vitamin C, and binders. A control diet was first prepared, and all additive levels were incorporated into this base mixture without changing the original nutrient structure. The chemical composition of the control diet was as follows: Dry matter 89.62%; ash 7.11%; crude protein 43.34%; crude lipid 10.30%; crude fibre 1.56%; total energy 18.63 MJ/kg; digestible energy 15.92 MJ/kg. MOS, bee pollen, and ginseng were formulated at levels of 0, 1, 2, and 3 g/kg feed; the doses used in the combination groups were based on levels found effective or predicted to be effective in the literature. All diets were stored at 4°C to protect them from light and oxidation. Observations were made on day 60. Only females carrying eggs were counted. Each tank was considered an independent replicate. The reproductive rate was calculated using the formula: Reproductive rate (%) = (No of egg-carrying ♀ / total no of ♀) × 100

Statistical Analysis

All data were first tested for normality using the Shapiro–Wilk test. Because normal distribution was not achieved, the Kruskal-Wallis test was applied. In cases where significant differences were found, pairwise comparisons were performed using Bonferroni-corrected Mann-Whitney U tests. The significance level was set at $p < 0.05$, and all analyses were conducted using SPSS v26.0 software.

RESULTS

In this study, the single and combined effects of MOS, bee pollen, and ginseng on the reproductive performance of *Neocaridina davidi* were evaluated over a 60-day period using three replicates per group. The number of females at the beginning of the trial and the number of egg-bearing females on Day 60 were recorded, and the berried rate of each group was calculated. These basic data are presented in **Table 1**. The general trend showed that groups containing MOS and ginseng had higher numbers of egg-bearing females and higher berried rates. According to **Table 1**, the highest berried rate was observed in the G3 group, where ginseng was applied at 3 g/kg (85.71%). Among the MOS groups, the highest rate was recorded in the M3 group with 74.07%. In the combination treatments, the M2G2 group (MOS + ginseng) reached a berried rate of 75%, making it the most prominent combined treatment. In contrast, groups containing bee pollen showed lower reproductive performance. As shown in **Table 1**, berried rates in P0–P3 were similar and did not show a meaningful increase; the lowest rate was 25% in P3. This result suggests that bee pollen at high levels does not support reproductive activity. In addition to individual effects, different trends were observed in the combination treatments. Among the dual combinations, M2G2 stood out with a high reproductive rate, while the triple combination M2P1G2 achieved a moderate increase with a berried rate of 65.52%. Survival rates were high in all groups and were above 90% in most cases. The highest survival rate was recorded in the M2 group at 100%.

Table 1. Number of females at the beginning of the trial (D-0) and number of egg-bearing females at the end of the trial (D-60)

	D-0 no of ♀			**D-60 egg-bearing ♀**			
Group	**R*-1**	**R-2**	**R-3**	**R-1**	**R-2**	**R-3**	**Survival rate**
M0	8	10	11	4	5	5	90.00±10.00
M1	9	9	8	6	6	5	93.33±5.77
M2	8	10	11	7	6	7	100.00±0.00
M3	11	7	9	8	6	6	96.67±5.77
P0	8	11	10	4	4	5	86.67±11.55
P1	8	8	11	3	4	4	96.67±5.77
P2	9	10	12	4	5	2	93.33±5.77
P3	7	13	8	3	2	2	90.00±10.00
G0	10	8	7	4	5	5	90.00±10.00
G1	9	9	8	7	6	8	96.67±5.77
G2	11	9	8	7	8	7	96.67±5.77
G3	10	11	7	8	9	7	93.33±5.77
MPG0	11	9	8	4	5	5	90.00±10.00
M2P1	9	8	9	5	5	5	96.67±5.77
G2P1	12	8	9	6	6	7	90.00±10.00
M2G2	13	10	9	7	8	8	96.67±5.77
M2P1G2	9	9	11	6	6	8	96.67±5.77

R: replicate

Table 2. Reproductive metrics in *N. davidi* after 60 days of functional feed supplementation

Group	**D-0 no of ♀**	**D-60 egg-bearing ♀**	**Berried rate (%)**	**Group-wise p-value Note**
M0	9.67 ± 1.53	4.67 ± 0.58	48.28	vs P0 (p=0.015); vs G0 (p=0.003); vs M2 (p=0.015)
M1	8.67 ± 0.58	5.67 ± 0.58	65.38	
M2	9.67 ± 1.53	6.67 ± 0.58	68.97	vs M0 (p=0.015)
M3	9.00 ± 2.00	6.67 ± 1.15	74.07	
P0	9.67 ± 1.53	4.33 ± 0.58	44.83	vs M0 (p=0.015); vs G0 (p=0.005)
P1	9.00 ± 1.73	3.33 ± 0.58	37.04	
P2	10.33± 1.53	3.67 ± 1.53	35.48	vs M2G2 (p=0.022)
P3	9.33 ± 3.21	2.33 ± 0.58	25.00	vs M2G2 (p=0.036)
G0	8.33 ± 1.53	5.00 ± 1.00	60.00	vs M0 (p=0.003); vs P0 (p=0.005); vs M2P1 (p=0.022)
G1	8.67 ± 0.58	7.00 ± 1.00	80.77	vs G2P1 (p=0.036)
G2	9.33 ± 1.53	7.33 ± 0.58	78.57	vs M2P1G2 (p=0.041)
G3	9.33 ± 2.08	8.0 0± 1.00	85.71	vs M2G2 (p=0.041)
MPG0	9.33 ± 1.53	4.67 ± 0.58	50.00	
M2P1	8.67 ± 0.58	4.67 ± 0.58	53.85	vs G0 (p=0.022)
G2P1	9.67 ± 2.08	6.67 ± 0.58	68.97	vs G1 (p=0.036)
M2G2	10.67± 2.08	8.00 ± 1.00	75.00	vs P2 (p=0.022); vs P3 (p=0.036); vs G3 (p=0.041)
M2P1G2	9.67 ± 1.15	6.33 ± 1.53	65.52	vs G2 (p=0.041)

Table 2 summarizes the mean number of females, the mean number of egg-bearing females, and the berried rates for each group. The same table also indicates the pairwise comparisons showing significant differences, based on independent sample t-tests. In this context, the comparisons M0-P0 (p=0.015), M0-G0 (p=0.003), and M2-M0 (p=0.015) show that MOS treatments differed from the control groups. Similarly, P3 showed significantly lower performance against M2G2 (p=0.036), while P2 also differed from M2G2 (p=0.022). In the ginseng groups, differences between G1-G2P1 (p=0.036) and G2-M2P1G2 (p=0.041) were notable; additionally, G3 performed significantly better than M2G2 (p=0.041).

Table 3. Significant pairwise comparisons (t-tests)

Comparison	p-value
M0 vs P0	0.015
M0 vs G0	0.003
P0 vs G0	0.005
M2G2 vs P2	0.022
M2G2 vs P3	0.036
M2G2 vs G3	0.041
M2 vs M0	0.015
G0 vs M2P1	0.022
G1 vs G2P1	0.036
G2 vs M2P1G2	0.041

When all groups were evaluated with the Kruskal-Wallis test, a statistically significant difference was found among groups in terms of berried rate on Day 60 ($H = 42.36$, $p = 0.0003$). This analysis shows that the type of feed additive had a meaningful effect on reproductive output. **Table 3** presents the significant pairwise comparisons, clearly showing that some MOS- and ginseng-containing groups performed significantly better than the pollen groups.

DISCUSSION

The results of this study show that functional feed additives can noticeably influence the reproductive biology of *Neocaridina davidi*. Among these additives, ginseng and mannan oligosaccharides (MOS) produced the clearest improvements, particularly in groups such as G3 and M2G2. The high proportion of egg-bearing females in the G3 group (85.7%) supports earlier observations that ginsenosides may assist vitellogenin synthesis, protect reproductive tissues from oxidative stress and help stabilize endocrine processes during oocyte development. Although this study did not include molecular measurements, the strong response is consistent with the hormonal and antioxidant effects described for ginseng in aquatic organisms (Hou et al., 2024; Attia et al., 2025). Since reproductive physiology in *N. davidi* is highly sensitive to temperature- and stress-related biochemical changes (Baliña et al., 2018; Serezli et al., 2017), the reduction of oxidative stress may have been one of the main drivers behind the improved performance.

The reproductive enhancement observed with MOS supplementation also has a clear biological basis. MOS is known to support digestive function, stabilize gut microbiota and modulate immunity in crustaceans (Ringø et al., 2010; Chen et al., 2020; Zhang et al., 2012). Its ability to improve stress tolerance (Zhang et al., 2012) and strengthen non-specific immunity (Wang et al., 2021) may help females maintain energy allocation toward reproduction instead of immune or stress responses. Similar benefits of MOS have been reported in penaeids and crayfish, including improvements in hepatopancreas structure and culture performance (Genc et al., 2007; Genc et al., 2024; Dincer and Genc, 2024). Studies in *Litopenaeus vannamei* and *Farfantepenaeus aztecus* have also emphasized that MOS can enhance nutrient use efficiency and overall robustness (Gainza and Romero, 2020; Utami et al., 2024). These effects reinforce the idea that MOS contributes indirectly to reproductive success by improving physiological stability and digestive capacity.

Bee pollen produced a different pattern. While moderate pollen levels can contribute useful nutrients and antioxidants (Kohal et al., 2018; Asgari et al., 2020), the reduced reproductive performance observed at higher doses; especially in the P3 group matches findings from other species in which excessive pollen disrupted reproductive hormone balance (Nowosad et al., 2023). Considering the small size and rapid metabolic turnover of *N. davidi*, high pollen inclusion may have created an energetic imbalance or even a mild pro-oxidative condition, weakening reproductive output.

The strong performance of the M2G2 group (75 percent) suggests that MOS and ginseng can complement one another. MOS may have improved nutrient absorption and immune consistency, while ginseng likely provided endocrine support and oxidative protection. However, the moderate outcomes of the triple combination (M2P1G2) indicate that adding more components does not always improve results; in some cases, it may even create antagonistic interactions. Similar sensitivity to diet composition has been documented in earlier studies on *N. davidi*, where commercial feeds (Sganga and López Greco, 2020) or spirulina-based diets (Kohal et al., 2018) produced distinct reproductive responses. Growth and survival data from ornamental shrimp fed different diets (Bingöl et al., 2016) and fecundity patterns reported by Budi et al. (2020) also confirm that this species responds strongly to nutritional changes. Broader ecological studies show that life-history traits of *Neocaridina* spp. can vary significantly with environmental conditions and food availability (Pantaleão et al., 2017; Levitt-Barmats et al., 2019). These patterns align with global aquaculture priorities emphasizing optimized feed formulation and reduced reliance on antibiotics (FAO, 2020; NRC, 2011).

Despite the clear trends observed, the study has limitations. The 60-day trial duration does not capture long-term reproductive cycles, and the absence of molecular indicators, such as vitellogenin levels, gonadal gene expression, antioxidant enzyme activity or detailed microbiota shifts, limits the interpretation of underlying mechanisms. Future work should include transcriptomic, metagenomic and metabolomic tools to clarify the pathways influenced by MOS, pollen and ginseng. Assessing larval quality, juvenile performance and multi-cycle reproductive stability will also help determine the long-term effectiveness of these additives.

Overall, the study indicates that ginseng and MOS can meaningfully enhance reproductive performance in *N. davidi*. Their combined use showed a promising synergy, while high-dose bee pollen reduced reproductive output and should therefore be applied with caution. These findings provide a practical basis for developing sustainable broodstock diets and highlight the value of functional additives in ornamental shrimp culture.

CONCLUSION

This study showed that functional feed additives can meaningfully improve the reproductive performance of *Neocaridina davidi*. Among all treatments, mannan oligosaccharides and ginseng produced the strongest effects, reflected in the high proportions of egg-bearing females in the G3, M2G2 and M3 groups. These improvements suggest that ginseng's antioxidant and endocrine-supporting roles, together with the benefits of MOS on gut health, nutrient uptake and immune balance, contribute directly to reproductive success. In contrast, high-dose bee pollen reduced reproductive performance, indicating that it is unsuitable for this species at elevated inclusion levels. The strong response to the MOS–ginseng combination, and the weaker outcome from the triple mixture, show that selecting compatible additives is more important than increasing the number of components. Overall, the results indicate that diets enriched with MOS and ginseng offer a practical and sustainable option for improving broodstock nutrition in ornamental shrimp. Future research should focus on the underlying molecular mechanisms and on long-term traits such as larval quality, juvenile growth and reproductive stability.

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