

AQUACULTURE AND MARINE RESEARCH: THEORY AND PRACTICE

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PREFACE

Aquatic ecosystems, fisheries, and aquaculture play a fundamental role in sustaining human life by providing food, supporting livelihoods, and preserving the biodiversity of our planet. Today, however, these systems are facing unprecedented pressures stemming from resource overexploitation, habitat degradation, environmental pollution, invasive species, and the accelerating impacts of climate change. These challenges clearly highlight the urgent need for more integrated, innovative, and sustainable approaches to the management and utilization of aquatic resources.

This book brings together twelve chapters authored by experts who explore a wide range of contemporary issues in aquatic and marine sciences. The volume addresses innovative aquatic food products, novel protein sources in fish nutrition, sustainable fishing practices, circular models in aquatic industries, biosecurity strategies, and the ecological implications of non-native species. By combining scientific inquiry with practical applications, the chapters collectively reflect the evolving complexity and interdisciplinary nature of modern aquatic systems.

As editors, we sincerely hope that this book will inspire researchers, practitioners, students, and policymakers to adopt more informed perspectives, foster stronger collaboration, and contribute to the development of resilient and forward-looking strategies for the sustainable stewardship of our marine and freshwater resources.

EDITORS

CHAPTER 1

DEVELOPMENT OF A READY-TO-EAT PRODUCT FORMULATED WITH COMBINED AQUATIC PROTEINS

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INTRODUCTION

The 21st century, characterized by rapid globalization, urbanization, and demanding work schedules, has transformed time into an increasingly scarce resource for consumers worldwide. This era of time scarcity and the need for speed has triggered a profound shift in dietary habits, leading to a global surge in the consumption of convenient, ready-to-eat, and easily accessible foods, particularly fast-food. However, the parallel global rise in non-communicable diseases such as obesity, cardiovascular diseases, and diabetes has directed both consumers and public health authorities toward seeking options that are not only fast but also nutritious and healthy. This paradox has positioned the concept of "healthy convenience food" as a central strategic focus for the food industry. Modern consumers are now actively seeking tasty, reliable, ready-toeat products that are high in quality protein, fiber, vitamins, and functional components like omega-3s, while avoiding the high calorie, saturated fat, and sodium content typically associated with traditional fast food. In this context, seafood, with its rich nutritional profile and documented health benefits, emerges as an ideal raw material for sustainable and healthy ready-to-eat product formulations. Consequently, innovative products like the "Eomuk" developed in this study—using valuable marine resources such as fish, cuttlefish, and shrimp—hold significant potential to bridge a critical gap in the future food market by simultaneously addressing the modern consumer's time constraints and heightened health consciousness.

Changing family structures under current conditions have led to changes in nutritional habits. The fact that both mothers and fathers work in families, along with an increase in the number of people living alone, has increased the tendency towards consuming ready-made food (Sağlıker, 2010). As consumers seek products that are easy to prepare, ready-made food technology has also gained importance (Metin, 1999). Research results show that the preference rate for quality food consumption is increasing in connection with countries' development levels. In this respect, consumers are turning to seafood products in their diets due to the proteins, essential vitamins and minerals, and unsaturated fatty acids they contain (Lövkvist, 2014; Mohanty, 2015). Of the total global seafood production, which reached approximately 187 million tons in 2022, 165 million tons were used directly for food supply, while the remaining portion was used for the production of non-food products, primarily

fish meal and fish oil (FAO, 2024). In our country, the annual per capita consumption was reported as 7.2 kg in 2023 (TEPGE, 2024). When these values are examined, it can be said that seafood consumption is linked to countries' development levels.

In recent years, the expansion of ready-made food technology has also led to an increase in product diversity. Due to consumers' increasing desire to consume healthy food, seafood can be among the primary resources to be used. One study stated that preferring seafood in the ready-made food sector would increase the consumption of seafood and further boost the contribution to the national economy compared to fresh consumption (Oğuzhan and Yangılar, 2013). Since seafood is perishable and can quickly lose its consumption value, preservation systems from catch to sale, equipment during transportation, hygiene, processing, and packaging systems play an effective role in selling the products in the desired commercial form (processed and fresh seafood) (Atılgan, 2008)

In Türkiye in 2020, a total of 961 tons of cuttlefish were obtained from the Mediterranean, Aegean Sea, and the Sea of Marmara (TUIK, 2021). The meat yield of cuttlefish ranges between 60-80%, which is higher than that of white-fleshed fish. The chemical composition of cuttlefish is also similar to lean fish. In cuttlefish, the approximate moisture content ranges from 78-84%, protein content from 12-19%, and fat content from 0.5-1.5%. However, in terms of market value, the squid species *Loligo vulgaris*, which has a production of 421.9 tons, unfortunately holds a market value only one-third of that. The nutritional content of the jumbo shrimp (*Melicertus kerathurus*), a crustacean species, is as follows: moisture content 78.4%, protein 19%, crude fat 1.05%, and carbohydrate content between 0.30-1.0%. It contains approximately 21-22% omega-3 (Dincer and Aydın, 2014).

The health benefits of seafood consumption have been known worldwide since the 1950s and are a subject upon which much scientific research has been conducted. A prominent topic in these studies has been long-chain Omega-3 polyunsaturated fatty acids. Omega-3 fatty acids, and particularly EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid), have become the most popular topics known by people all over the world and our public. Numerous studies show that Long-Chain Omega-3 Polyunsaturated Fatty Acids (PUFA) have positive effects on many diseases. However, it should not be

forgotten that the Omega-3 structure, which stands out in terms of the benefits of fish and seafood consumption, constitutes only 0.5-5% of the whole fish (Undeland et al., 2009).

Considering its many benefits, we must remember how important it is for our health to increase our per capita seafood consumption rates. It is a significant species processed through smoking for the domestic market and especially for export. When consumption habits are examined, Rainbow trout, which is the preferred choice for consumption in regions except coastal areas, does not receive the importance it deserves and is not preferred at fishmongers in coastal regions. However, it is a white-fleshed freshwater fish species suitable for emulsified products, economical, and abundantly available yearround due to aquaculture activities. Starting from this scope, for the "Eomuk (Fish Cake)" to be produced, firstly having a name with an easy pronunciation suitable for our language and a commercial name will increase its acceptability Frying processes applied to seafood cause the polyunsaturated fatty acid composition to shift rapidly towards monounsaturated fatty acids and lead to significant reductions, especially in EPA and DHA levels. Today, consumers pay attention not only to the taste of foods but also to their health benefits. Particularly in developed countries, because obesity is becoming an increasingly serious problem, the goal is to reduce the fat content of fried foods without altering their desired sensory properties (Hosomi et al., 2012).

Fish cake is a ready-to-eat food product produced from seafood, consumed in high quantities in Far Eastern countries, especially Korea and Japan (KFDA, 2012). This food product can be prepared using one or more types of seafood and is cooked by frying, boiling, or roasting. Frying has been identified as the most preferred method (MIFAFF, 2011). Accordingly, it was planned to develop the "fish cake" product for the industry as a practical, healthy, habit-forming, and beneficial product, targeting consumers aged 18-60. A project study was carried out targeting both industrial returns and an increase in seafood consumption. The product, whose R&D phase is complete, will be marketable worldwide.

The aim is to produce a ready-to-eat food product containing seafood, similar to "fish cake" found abroad, particularly in Far Eastern countries, and known as "eomuk" in the Far East, and to introduce this new product to the Turkish market by examining its shelf life in frozen storage and its textural

parameters. The study aimed to introduce the product to the domestic market. The product was planned to be a ready-to-eat food consisting of a mixture of fish, cuttlefish, and shrimp meats, which are of high nutritional and economic value.

2. MATERIAL

2.1 Trout Material

Skinless Rainbow trout (Oncorhynchus mykiss) fillets were used as the fish material in the study. After the fish were purchased from the İzmir fish market auction, they were transported to the pilot processing plant in polystyrene boxes covered with ice. Following the cleaning process, the fillets were removed by hand. After the filleting process, they were first blast-frozen at -35°C and then stored in frozen storage at -18 to -24 °C for 48 hours until the production stage.

2.2 Cuttlefish Material

The cuttlefish (Sepia officinalis) were purchased from the İzmir fish market auction and transported to the processing plant in polystyrene boxes covered with ice. After the cleaning process, they were skinned and prepared. Following the cleaning and preparation process, they were first blast-frozen at -35°C and then stored in frozen storage at -18 to -24 °C for 48 hours until the moment of production.

2.3 Jumbo Shrimp Material

The raw jumbo shrimp (Melicertus kerathurus) material was purchased from the İzmir fish market auction and transported to the processing plant in polystyrene boxes covered with ice. After the cleaning process, the shells were removed to prepare them. Following the cleaning process, they were first blast-frozen at -35°C and then stored in frozen storage at -18 to -24 °C for 48 hours until the moment of production.

2.4 Fillers and Spices

These consisted of potato starch, flour, salt, sugar, egg white, garlic powder, ground black pepper, onion powder, and sunflower oil. They were weighed according to the production formula and used in the production.

2.5 Packaging Material

During the production stage, the batter was stuffed into baton sausage casings made from synthetic or cellulose material. For the storage stage, vacuum packaging bags, produced by heat-sealing multilayer films made from EVOH, PA, and PE plastic polymers, were used.

3. METHOD

3.1 Production Method

After all the materials to be used in the batter content were weighed according to the formulation, the following procedures were carried out:

Table 1. Product Batter Formulation

Material	% Content
Fish	39.12
Cuttlefish	19.56
Shrimp	19.56
Potato Starch	7.82
Flour	7.82
Salt	0.47
Sugar	0.47
Egg White	3.13
Garlic	0.47
Onion	0.47
Sunflower Oil	0.78

3.2 Equipment Used in Production

Talsa brand Bowl Cutter (30 kg), Öztriyakiler brand Planet Mixer (20 kg), Talsa brand Stuffer/Filling Machine, Blast Freezer Unit (-35,-45 °C), Bone Saw/Band Saw, Öztriyakiler brand Industrial Oven, Cold Storage Unit (0,+4 °C).

Table 2. Product Production Flow Chart

1. RAW MATERIAL PREPARATION

- Procurement of trout, cuttlefish, jumbo shrimp
- In cleaned form: 10 kg trout, 5 kg cuttlefish, 5 kg shrimp

2. BOWL CHOPPING / MIXING PROCESS

(at -2°C)

- Chop/mix for 5 min
- Add salt + sugar \rightarrow mix for 3 min
- Add starch + oil \rightarrow emulsify
- Add egg white powder + flour → mix for 5 min at low speed
- Add spices (garlic, onion, black pepper)
 → mix for 5 min at low speed

3. STUFFING/FILLING

- Using a hydraulic stuffing machine
- Filled into casings
- •

4. FREEZING

Blast freezer at -35°C / -45°C

 → until the core temperature
 reaches -18°C

5. PORTIONING

- Casings are removed
- Band saw → cut into 125 gr pieces

6. THERMAL PROCESSING / COOKING

• At 195 °C for 20 minutes

7. COOLING & PACKAGING

- Cooling at room temperature
- Packaging into 500 gr packages



Figure 1. Production pictures

3.3 Methods for Biochemical Composition Analysis

The crude fat content was determined using the method of Bligh and Dyer (1959). Crude protein analysis was performed according to the AOAC (1984) method by measuring the total nitrogen content. Moisture and crude ash analyses were conducted following the procedures outlined by Ludorff and Meyer (1973).

3.3.1 Fatty Acid Analysis

The fatty acid composition of the products and their raw materials (trout, squid, and shrimp) was determined to observe the changes resulting from the production process. The analysis was performed by the Arge Far laboratory through a service procurement agreement. The method involved the extraction of total lipids followed by the conversion of fatty acids into their methyl esters (FAMEs) via transesterification. The resulting FAMEs were then separated, identified, and quantified using Gas Chromatography with a flame ionization detector (GC-FID).

3.3.2 Determination of Atherogenic Index and Thrombogenic Index

Following the determination of the fatty acid composition, the Atherogenic Index (AI) and Thrombogenic Index (TI) were calculated using the formulations established by Ulbricht and Southgate (1991). These indices were used to assess the potential of the products to increase the risk of coronary heart disease upon consumption.

3.3.3 Sensory Analysis

Upon completion of production, a sensory evaluation was conducted using a 9-Point Hedonic Scale test, based on the method of Peryam and Pilgrim (1957). For each product group, 50 untrained panelists were used to assess their preference or degree of liking.

3.3.4 Statistical Analyses

All analyses were performed in triplicate. Statistical analysis was conducted using the SPSS for Windows 16.0 statistics package program. The results are presented as mean \pm standard deviation (SD).For multiple comparisons, one-way analysis of variance (ANOVA) was applied to data that met parametric assumptions. Groups to be compared were evaluated against each other on the basis of the analysis day. For groups where a significant difference was found by this test, the Post-hoc Tukey HSD test and Duncan test were used to identify the source of the difference. The independent T-test was used to compare the mean values between fried and baked products resulting from the production process. For all tests, a value of P < 0.05 was considered statistically significant.

4.RESULTS

The proximate composition (moisture, protein, fat, and ash) of the baked final products formulated with trout, shrimp, and squid is presented in Table 2.

()				
Component	Trout	Shrimp	Squid	Product
Protein	$16.00 \pm 0.50^{\rm a}$	17.00 ±	14.00 ±	15.00 ± 0.55^{ab}
		0.60^{a}	0.40^{b}	
Moisture	80.00 ± 1.00^{a}	77.00 ±	84.00 ±	55.62 ± 2.50^{d}
		1.20 ^b	0.80°	
Crude Fat	$2.00\pm0.15^{\rm a}$	1.00 ± 0.10^{b}	1.00 ± 0.10^{b}	5.94 ± 0.45^{c}
Crude Ash	$1.00\pm0.08^{\rm a}$	2.00 ± 0.12^{b}	0.00 ± 0.05^{c}	1.85 ± 0.15^{b}
Carbohydrate	$1.00\pm0.20^{\rm a}$	3.00 ± 0.25^{b}	$1.00\pm0.15^{\rm a}$	$20.33 \pm 1.80^{\circ}$

Table 2. Chemical Composition of Raw Materials and Thermally Processed Product (%)

Values are presented as mean \pm standard deviation (SD) (n=3). Means within the same row with different superscript letters are significantly different (p < 0.05).

Thermal processing significantly altered the chemical profile of all products compared to their raw materials. A notable decrease in moisture content was observed across all groups, accompanied by a concurrent increase in the concentration of other components due to the loss of water. Significant differences (p < 0.05) in the final protein and fat content were evident among the three product types, reflecting the distinct compositional profiles of the raw species used.

As summerized in Table 2, the proximate composition of the final baked product differed significantly from the natural profiles of the raw ingredients (trout, shrimp, squid), underscoring the decisive impact of thermal processing and formulation strategy. Statistical analysis confirmed significant differences (p < 0.05) among the groups for all components. The most pronounced change was an approximately 30% reduction in moisture content (55.62% in the final product), a direct and expected consequence of intense water loss during baking, which aligns with findings reported by Abraha et al. (2018). While this dehydration led to a general concentration of other components, the marked and statistically significant increases in both crude fat (reaching 5.94%) and carbohydrate (reaching 20.33%) contents cannot be attributed to the concentration effect alone. Given the low inherent fat (1-2%) and carbohydrate (1-3%) levels in the raw materials, these results strongly suggest the external addition of fat and the use of binders or fillers, respectively, in the product formulation. Concurrently, the observed values for protein and ash reflect both

the concentration effect and the distinct compositional contributions of the raw ingredients, particularly the higher ash content from shrimp. In conclusion, the nutritional profile of the final product was shaped not merely by the natural constitution of the raw materials, but predominantly by the applied thermal processing and, most notably, the specific formulation design.

Table 3. Fatty Acid Composition of Raw Materials and the Final Thermally Processed Product (%)

Fatty acid composition (%)				
	Trout	Shrimp	Squid	Product
Total Saturated	24.53±0.03	38.50±0.10 ^b	50.81±0.14	19.47±0.0
Fatty Acids (ΣSFA)	a		c	2^{d}
Total	42.39±0.15	7.66±0.03 ^b	20.14±0.15	32.84±0.0
Monounsaturated	a		c	2^{d}
Fatty Acids (ΣMUFA)				
Total Polyunsaturated	33.08±0.06	53.97±0.09b	29.04±0.15	47.60±0.0
Fatty Acids (ΣPUFA)	a		c	1^{d}
PUFA/SFA Ratio	1.35	1.40	0.57	2.44
ΣOmega-6	18.51±0.72	0.72±0.01 ^b	2.79±0.0 ^{2c}	40.95±0.0
	a			1^{d}
ΣOmega-3	14.56±0.57	53.25±0.23 ^b	26.25±0.39	6.65±0.01 ^d
	a		c	
Omega-3/Omega-6	0.79	74.06	9.41	0.16
Ratio				
EPA –	2.93±0.07a	21.30±0.20 ^b	12.28±0.33	0.87 ± 0.00^{d}
C20:5n3			c	
(Eicosapentaenoic				
Acid)				
DHA - C22:6n3	7.78±0.22a	31.77±0.65 ^b	13.98±1.23	3.97±0.03 ^d
(Docosahexaenoic			c	
Acid)				
ALA-C18:3n3	3.46±0.07 ^a	0.18 ± 0.09^{b}	0.00 ± 0.00^{c}	1.64±0.00 ^d
(Alpha-Linolenic Acid)				
DHA/EPA Ratio	2.66	1.49	1.14	4.57

Values are presented as mean \pm standard deviation (SD) (n=3). Means within the same row with different superscript letters are significantly different (p < 0.05).

Statistical analysis of the fatty acid composition presented in Table 3 revealed significant differences in all parameters between the raw materials (trout, squid, shrimp) and the final product (P<0.05). The final product exhibited the statistically lowest saturated fatty acid (SFA) content (19.47%, P<0.05) and, simultaneously, the highest polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio (2.44, P<0.05) compared to all raw materials; these are positive attributes indicating high lipid quality. However, its total monounsaturated fatty acid (MUFA) content (32.84%) was statistically lower than that of trout (42.39%, P<0.05) but significantly higher than that of squid and shrimp (P<0.05). The most notable alteration in the product's fatty acid profile was observed in the omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids. The final product had the statistically highest omega-6 content (40.95%, P<0.05), replacing the omega-3 fatty acids that were predominant in all raw materials. Consequently, the omega-6/omega-3 ratio (6.25) was statistically significantly higher (P<0.05) than that of all raw materials (1.26, 0.01, and 0.11, respectively). This dramatic shift and the statistically significant reduction in valuable EPA and DHA omega-3 fatty acids (P<0.05) are likely due to the high susceptibility of long-chain polyunsaturated fatty acids to oxidation during thermal processing and/or a result of the product formulation, a challenge in preserving beneficial lipids as highlighted by Tan et al. (2023). Therefore, although the final product has a favorable profile in terms of low SFA and a high PUFA/SFA ratio, the thermal processing-induced loss of omega-3 fatty acids and the failure to maintain a balanced omega-6/omega-3 ratio represent a significant drawback for its nutritional quality.

The Atherogenic Index (AI) and Thrombogenic Index (TI) values presented in Table X are critical for assessing the product's potential effect on cardiovascular health. Low AI and TI values are associated with a lower risk of coronary heart disease and thrombosis.

Table 4. Atherogenic and Thrombogenic Index Values

	Trout	Shrimp	Squid	Product
Aterojenik index	0.36±0.02a	0.41±0.02 a	0.66±0.03b	0.39±0.00 ^a
Trombojenik indeks	0.31±0.01 a	0.21±0.02 b	0.40±0.01°	0.32±0.02 a

Values are presented as mean \pm standard deviation (SD) (n=3). Means within the same row with different superscript letters are significantly different (p < 0.05).

Squid (0.66 ± 0.03) has a statistically significantly higher AI value than all other groups (p < 0.05). Trout (0.36 ± 0.02) . shrimp (0.41 ± 0.02) . and the final product (0.39 ± 0.00) have AI values that are not statistically different from each other and are considered low. Statistically significant differences were found among all groups (p < 0.05). The lowest TI value was observed in shrimp (0.21 ± 0.02) . while the highest value was detected in squid (0.40 ± 0.01) . Trout (0.31 ± 0.01) and the final product (0.32 ± 0.02) have statistically similar TI values. at a low-to-medium level. The AI (0.39) and TI (0.32) values of the final product are consistent with raw materials like trout and shrimp and fall into the **low-risk** category. Busova et al., (2020) emphasized that an AI value below 0.5 indicates a positive effect in preventing atherosclerotic processes. Similarly. in a review. Nava et al., (2023) reported that diets with a TI value below 0.5 may be beneficial in reducing platelet aggregation and the risk of intravascular clot formation. In this context, it can be stated that the final product has a positive profile for cardiovascular health.

The relatively high AI and TI values of squid are consistent with its saturated fatty acid (SFA) content and omega-3/omega-6 ratio. Capra et al.,(2023) reported that a high SFA/LCPUFA (Long-Chain Polyunsaturated Fatty Acids) ratio in the diet could have a negative effect on vascular health by increasing AI and TI values. The fact that the final product's AI value is very close to trout's. while its TI value is slightly higher, may reflect the effect of thermal processing on the fatty acid composition. The decrease in omega-3 fatty acids during thermal processing (as shown in Table 3) may have had a particular negative effect on the TI value. This finding aligns with the work of Biandolino et al., (2023). which indicated that fish processing techniques can alter the product's thrombogenic potential. In light of statistical analysis and current literature. It can be concluded that the developed final product's Atherogenic and Thrombogenic Index values do not indicate a profile that increases cardiovascular disease risk; on the contrary, they can be categorized as low-risk foods. similar to trout and shrimp. It appears that the product formulation has created an acceptable cardiovascular risk profile in the final product by balancing the high-risk fatty acid profile of squid.

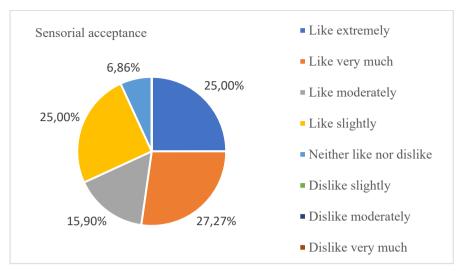


Figure 2. Sensorial panel results

Following production, a 9-point Hedonic Scale test based on the methodology of Peryam and Pilgrim (1957) was conducted with 50 untrained panelists to objectively measure sensory acceptance. The test results, where panelists rated their liking on a scale of 1-3 (Unacceptable), 4-5 (Fair), 6-7 (Good), and 8-9 (Very Good), clearly delineated the product's consumer appeal. The data revealed that approximately 68.17% of panelists (the combined percentage from the "Excellent," "Liked Very Much," and "Moderately Liked" categories) rated the product above "Fair" (i.e., with a score of 6 or higher). This finding is a strong indicator that the product is generally accepted and liked by the consumer base. Notably, the fact that 25% of panelists rated the product as "Excellent" (corresponding to the 8-9 point range) indicates a significant potential to cultivate a dedicated "fan base." However, the highest concentration of ratings in the "Moderately Liked" category (likely corresponding to the 6-7 "Good" range) suggests that while the product is perceived positively, there is an opportunity for improvement to meet the expectations of this segment more fully and elevate its status from "Good" to "Excellent." In conclusion, the product has achieved a marketable level of sensory acceptance, and it is recommended to focus on optimizations targeting the "Good" segment to enhance its overall success.

CONCLUSION

In this study, a formulation for the ready-to-eat product known as "Eomuk," widely consumed in the Far East, was developed for the Turkish market using trout, cuttlefish, and shrimp meat. Its shelf life, nutritional parameters, and sensory acceptability were evaluated.

The research results demonstrated that the chemical composition of the developed product significantly differed from the natural structure of the raw materials. A notable decrease in moisture content was observed alongside an increase in crude fat and carbohydrate content, attributable to the added oil and binders in the product formulation. Analysis of the fatty acid composition revealed that the final product possessed positive attributes such as a low saturated fatty acid (SFA) content and a high polyunsaturated to saturated fatty acid (PUFA/SFA) ratio. However, a significant reduction in valuable longchain omega-3 fatty acids (EPA and DHA) was detected due to thermal processing, leading to an increased omega-6/omega-3 ratio. In contrast, the Atherogenic Index (AI: 0.39) and Thrombogenic Index (TI: 0.32) values were found to be in the low-risk category, similar to trout and shrimp, indicating that the product has a positive profile for cardiovascular health. Sensory analysis results showed that approximately 68% of panelists rated the product as "Good" or higher, proving that the product is generally accepted by consumers and has achieved a marketable level of acceptability.

In conclusion, this study successfully developed a "Fish Cake (Eomuk)" product that is practical, healthy, well-liked by consumers, and has the potential to increase per capita seafood consumption in Türkiye. Minimizing omega-3 loss from a nutritional perspective and further improving sensory properties stand out as crucial focal points for future R&D efforts. The developed product holds high potential for commercial success in both domestic and international markets.

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CHAPTER 2

A NEW APPROACH FOR SUSHI PRODUCING: THE PRODUCTION OF ULVA SUSHI ROLLS FROM DRIED ULVA SHEETS INSTEAD OF SEAWEED NORI SHEETS

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INTRODUCTION

The importance of next-generation alternative protein sources for sustainable food futures have received increased attention recently due to their significant not only environmental, but also health impacts compared to meat consumption (Kılınç and Kılınç, 2025). Algae (seaweeds) are a promising a good source of alternative protein as they can meet nutrition, environmental, production needs as well as health (Diaz et al., 2022). They are a rich source of minerals (including calcium, iron, and iodine), dietary fiber, essential vitamins (such as C, A, and B12), bioactive compounds (fatty acids, proteins, and essential amino acids), and antioxidants. These nutrients give seaweed remarkable health benefits, including antioxidant, antimicrobial, antidiabetic, and antiinflammatory properties (Sinha et al., 2026). Actually, seaweeds are healthy and nutritious product (Losada- Lopez et al., 2021). Nutritional attributes of seaweeds make them a valuable addition to various diets, particularly for those seeking plant-based, nutrient-dense alternatives (Sinha et al., 2026). In this approach, many food products have been produced from seaweeds and/or various food products enriched with seaweeds (Kılınç et al., 2011; Turan et al., 2011; Kılınç et al., 2013a; Kılınç et al., 2013b; Kılınç and Kılınç, 2022; Kılınç et al., 2023; Kılınç and Tekoğul, 2024 a; Kılınç and Tekoğul, 2024b; Tekoğul and Kılınç, 2024a; Tekoğul and Kılınç, 2024b; Harsha Mohan et al., 2025). Actually, seaweeds offer various usages, ranging from direct consumption as food to the synthesis of industrial and functional food products. Furthermore, they have been a staple in many coastal cuisines for centuries, offering a rich source of unique flavors, and nutrients. For this reason, seaweeds are consumed in various forms, including fresh, dried as well as processed into products like, salads, soups, snacks and sushi. Moreover, they are also used in the production of functional food products like alginate, agar, and supplements, biofuels, cosmetics, and alongside applications in pharmaceuticals (Sinha et al., 2026).

Seaweeds are photosynthetic marine macroalgae, including the groups; red (*Rhodophyta*), brown (*Phaeophyceae*), and green (*Chlorophyta*). *Enteromorpha* spp., *Codium* spp., *Caulerpa* spp., *Monostroma* spp., *Ulva* spp. are mostly known green seaweeds as a very good source of food. Additionally, Gelidium, Glacilaria, Pterocladis, Eucheuma, Betaphycus species can be given an examples of red seaweeds, whereas Laminaria, Undaria, Hizikia, Sargassum

species have been located in the brown seaweeds (Kılınç et al., 2013c). Porphyra species are edible red algae that are cultivated and consumed mainly in South-East and East Asian countries such as China, Korea, and Japan. Sheetlike dried foodstuff prepared from these Porphyra species is mostly known as "nori," which is traditionally used in the Japanese cuisine sushi. Owing to the recent popularity of sushi, the market of nori is nowadays devoloping in lots of countries. They are mostly recognized for their valuable nutritional richness, being excellent sources of essential dietary components such as various of minerals, fiber, and also protein (Isaka et al., 2015). Indeed, this edible seaweed products have historically been consumed in Asian cuisine, although their consumption in North America remains primarily confined to preparations like sushi and other imported Asian dishes (Rioux et al., 2017). The commercial market for edible seaweeds is predominantly characterized by three major types: the brown seaweeds, specifically wakame (Undaria spp.), kombu (Laminaria spp.) and the red seaweed, nori (Porphyra spp.). These products are commonly imported from East Asian nations, particularly South Korea, and China in a dehydrated, ready-to-eat format. Furthermore, wakame (Undaria spp.), which is indigenous to the northwest Pacific Ocean, is highly regarded for its sweet flavor as well as distinctive texture profile. For the reason of this special characteristics, it is a frequent and popular ingredient in culinary applications such as salads, and soups (Choi et al., 2009). Seaweeds, such as kombu, wakame, and nori, have also become increasingly popular foods in Western countries (Shimshoni et al, 2025). It is consumed as dried Nori flakes and is often used to prepare soups, cakes, and also sushi and and its popularity has now spread to other parts of the world because of the sushi consumption (Bito et al., 2017). Sushi is a traditional Japanese dish consisting mainly of cooked acidified rice (the size of one bite), which is covered with raw fish or seafood (nigiri sushi). Rice can also be in the shape of a roll (maki sushi), filled by raw fish or vegetables, wrapped in a slice of seaweed (Atanassova et al., 2008; Leisner et al., 2014; Kılınç and Kılınç, 2024). Sushi is typically accompanied by soy sauce, pickled ginger, wasabi paste as table condiments. The most common ingredients of animal origin for making sushi are indicated as raw tuna, salmon, halibut, large freshwater prawns, and cooked shrimp, whereas the vegetarian versions of maki are filled mainly with raw vegetables such as spring onion, avocado or cucumber. Sushi is one of the few foods in which we can encounter the consumption of raw (fish) meat (Hoel et al., 2015). Nori is a dried edible seaweed product used in Japanese cuisine made originally from seaweed from the genus Porphyra. Porphyra usually grows in sub-tropic coastal waters and is rarely found in tropical zones. For this reason, it is important to find local raw materials to substitute Porphyra to produce nori-like products (Sinurat et al., 2022).

On the importance of subject, in terms of sustainability, dried Ulva sheets were produced from fresh Ulva lactuca, an important seaweed found in Turkish seas, to be used in sushi wrapping. In addition, Ulva sushi rolls were developed using various formulations for the purpose of producing novel functional food products. Furthermore, literature studies about 'the importance of nori and ulva seaweeds as functional food, the cultivation and harvesting of ulva and nori seaweeds, chemical contaminants of seaweeds and sushi, persistence of sanitizer-resistant bacteria in sushi production, microbiological quality, the prevelance of pathogenic bacteria and parasites in sushi, the case studies about sushi consumption as well as future directions for sushi safety and quality control' were also included in the study.

MATERIAL AND METHODS

Material Obtaining and The Preparation of Dried Ulva Sheets From Fresh Ulva lactuca

Ulva lactuca can survive in salinity ranging from 0.15 to 0.30%, pH ranging from 7.8 to 8.3, and temperatures ranging from 18°C to 24°C (Sharma et al., 2025). Light: It is cultured in tanks at a density of 1 kg/m² (fresh weight) under a 12-16 hour photoperiod. For Ulva production, 10 mL/L F2 Culture Medium is applied to seawater weekly. Vegetative propagation is done (Dinesh Kumar et al., 2023)

After being harvested freshly Ulva lactuca material (Picture 1a)., Materyal are laid out on a tray for use in sushi production. Ulva sheets were pressed three times for making them just like nori sheets at high heat for 3 seconds. Then, they were oven-dried for 5 hours at 70°C, following the method of Putri and Ningtyas. (2017). Before drying, samples of fresh, dried, and plated Ulva lactuca are shown in (Picture 1b).,. It has been reported that the highest drying rate was obtained at 70°C, and this temperature was found to be the most

suitable temperature to describe the drying properties of nori, compared to temperatures of 50°C and 60°C (Kurniawan and Bintoro, 2019).



Picture 1a. Ulva lactuca (Linnaeus, 1753) (Original).



Picture 1b. Dried and plate U. lactuca (Original).

THE PREPARATION OF ULVA SUSHİ ROLLS BY USING DRIED ULVA SHEETS

After being prepared dried ulva sheets, different sushi rolls were produced by using various seafood products, seasonings or flavourings, such as vegetables. The boiled rice is subsequently combined with a mixture of sugar, oil, salt as well as vinegar. In the preparation of Ulva sushi rolls by using dried

Ulva sheets instead of dried nori sheets, dried Ulva sheets have been used, in this study. The cooked vinegared rice, along with various seasonings or flavourings, such as vegetables, has been then placed inside the Ulva wrap (Pictures 1c). Different formulations of Ulva sushi rolls are seen in the below Pictures 2-7.



Picture 1c. The Preparation of Ulva Sushi Rolls (Original)

THE FORMULATIONS OF ULVA SUSHI ROLLS

Different seafood pruducts, vegetables, spices, ingredients were used in each formula of Ulva Sushi rolls with cooked vinegared rice, Six different formulations of Ulva Sushi Rolls were produced in this study as follows: 1: Ulva Sushi roll with cooked vinegared rice, shrimp, grilled vegetables, black cumin, susame, aragula and beet, 2: Ulva Sushi roll with cooked vinegared rice, fish cooked in lemon, pumpkin, curly dock, parsley, sauerkraut and pickled celery, 3: Ulva Sushi roll with cooked vinegared rice, surimi, scallion, pickled, sesame, black cumin, avocado and lemon, 4: Ulva Sushi roll with cooked vinegared rice, omelette, red paper, cumquat, mint, scallion, mustard, grilled vegetable ,sumac and pomegranate syrup, 5: Ulva Sushi roll with cooked vinegared rice, omelette, red paper, cumquat, mint, scallion, mustard, grilled vegetable ,sumac and pomegranate syrup, 6: Ulva Sushi roll with cooked vinegared rice, tuna, red paper, purple onion, corn, lettuce, parsley, tomato, pickled cucumber, lemon, and purple onion



Picture 2. Ulva Sushi roll with cooked vinegared rice, shrimp, grilled vegetables, black cumin, susame, aragula and beet (Original)



Picture 3. Ulva Sushi roll with cooked vinegared rice, fish cooked in lemon, pumpkin, curly dock, parsley, sauerkraut and pickled celery (Original)



Picture 4. Ulva Sushi roll with cooked vinegared rice, surimi, scallion,pickled, avocado and lemon (Original)



Picture 5. Ulva Sushi roll with cooked vinegared rice, omelette, red paper, cumquat, mint, scallion, mustard, grilled vegetable ,sumac and pomegranate syrup (Original)



Picture 6. Ulva Sushi roll with cooked vinegared rice, tuna, red paper, purple onion, corn, lettuce, parsley, tomato, pickled cucumber, lemon, and purple onion (Original)



Picture 7. Ulva Sushi roll with cooked vinegared rice, grilled fish, shrimp, sea beans, grilled onions, susame, lemon, arugula, mint, potato, garlic and avocado (Original)

THE IMPORTANCE OF NORI AND ULVA SEAWEEDS AS FUNCTIONAL FOOD

Nori is mostly recognized as a functional food due to its very good profile of biologically active components that contribute positively to human health. Its composition includes a relatively high abundance of minerals, proteins, vitamins, and also significant levels of dietary fibers. Furthermore, nori is a very rich source of specialized compounds such as polyunsaturated fatty acids (PUFAs), taurine, carotenoids, and mycosporine-like amino acids (MAAs), notably porphyra-334 (Isaka et al., 2015). Some of the studies have been summarized as follows about nori. Investigation and strengthening of the potential of R-phycocyanin obtained from Nori flakes as a food colorant were examined in one study (Veličković et al., 2023). In another study, the antiinflammatory properties of porphyrans (D1-D4) obtained from four colorless nori (Pyropia yezoensis) with different growth histories were studied to examine possible variations in their bioactivity (Yanagido et al., 2018). Hence, the inclusion of nori in the diet is associated with nutraceutical benefits derived from this dense concentration of bioactive constituents, which are very important for human health (Isaka et al., 2015). The authors found in the other study that discolored waste nori with no commercial value, contained much higher level of porphyran than normal nori that was a sheeted food stuff prepared from P. yezoensis used in sushi (Isaka et al., 2015). The incorporation of nori powder (Neopyropia yezoensis) into high-moisture meat analogues

(HMMA) has been investigated to assess its impact on the final product's physicochemical and structural properties. The study demonstrated the NP's role in modulating HMMA nutrition, and texture, providing critical insights for developing fiber-enhanced, nutrient-fortified HMMAs. (Ren et al., 2025).

The other seaweed, Ulva lactuca, a widely grown macroalgae along the Mediterranean coast, belongs to the phylum Chlorophyta and is commonly known as Sea lettuce. Specifically, this Ulva species, are commonly found along the rocky or sandy coasts of seas, and oceans. Actually, green macroalgae of the genus Ulva have also attracted interest as a protein source due to their high production potential and high proportion of essential amino acids (Juul et al., 2024). In lights of the above sentence, Marine green macroalgae represent a valuable, yet largely underexploited, natural resource for bioactive phytochemicals with potential therapeutic applications. Beyond carotenoids, Ulva is an excellent source of essential minerals (including potassium, calcium, sodium, magnesium, iron, iodine, and copper), Vitamin B, dietary fibers, and proteins (Mohan et al., 2023). The study confirmed the efficacy of the optimized green extraction (obtained from U. lactuca) approach in the preparation of safe and valuable bioactive compounds that could be used in the development of seaweed-based functional foods and nutraceuticals (El-Deeb et al., 2026).

THE CULTIVATION AND HARVESTING OF ULVA AND NORI SEAWEEDS

The green seaweed Ulva sp. is a future food candidate, but both compositional, such as sensory qualities, and protein content depend on cultivation and harvesting conditions (Wendin et al., 2024). The floating long-line system was found to be the superior cultivation method for U. lactuca, supporting its potential not only for global food security, and diverse industrial utilization, but also for sustainable farming (Chamily et al., 2025). For example, integrating Ulva cultivation into Integrated Multitrophic Aquaculture (IMTA) systems proves feasible for large-scale production and enhances environmental sustainability. Furthermore, Ulva seaweed is an easily available natural resource that has the potential to address global malnutrition without compromising the health of the food ecosystem (Khan et al., 2024).

The cultivation of nori (P. yezoensis) very often suffers from malnutrition due to deficiencies in trace elements, phosphorus, and nitrogen in the culture field, resulting in a deterioration of the quality of nori, which is correlated with its color. High-quality nori has a colour of blackish glossy, whereas discolored nori has no commercial value. For this reason, the occurrence of discoloration has become a very serious problem for nori production (Isaka et al., 2015). Nori is primarily harvested along the Chinese, and Japanese coasts, using nets suspended on the surface of the sea, and is characterized by its distinctive flavor and is often used to wrap onigiri or sushi rolls (Ferdouse et al., 2018). With their very fast growing potential and their role in sustainable food practices, seaweeds are increasingly recognized for their versatility in food product development (Sinha et al.,2026). Therefore, harvesting or cultivating seaweed from clean seas is an extremely important issue for human health. Otherwise, they may be exposed to many chemical and microbiological contaminants that threaten human health.

CHEMICAL CONTAMINANTS OF SEAWEEDS AND SUSHI

Coastal areas where seaweed cultivation and harvesting take place have been identified as being contaminated by various persistent pollutants, primarily due to the discharge of urban, agricultural as well as industrial wastes runoff into the sea (Volschenk et al., 2019). For this reason, diet is a very common source of exposure to toxic trace elements (Butts et al., 2020). Seaweeds accumulate toxic pollutants found in surrounding waters, such as ammonium, trace elements, pesticides, and dioxins (Ficheux et al., 2022). For example, one research analyzed 13 heavy metals (oid) and REE (rare earth element) in samples of Japanese edible seaweed Nori sheet (Neopyropia the concentrations reflect the geological yezoensis), showing that characteristics of the cultivation areas (Toyoda et al., 2025). In another study, enquiries were concurrently conducted at points of purchase to systematically document the types of foodstuffs available on the French market, identifyed the specific seaweed species incorporated, as well as determined the percentage of seaweed content within the products. The novel data generated through this comprehensive work were anticipated to provide a valuable resource for both regulatory safety agencies as well as safety assessors facilitating improved risk

analysis and policy development (Ficheux et al., 2022). A dietary risk assessment and classification model based on trace element analysis was studied in commercially sold dried seaweed Products (Shimshoni et al., 2025). Although sushi is considered a healthy food, it can also be a way to be exposed to chemical contaminants such as potentially toxic trace elements. Iodine levels were higher in samples containing seaweed, while Inorganic Arsenic (IAS) concentrations were higher in sushi containing rice. In contrast, the total organic mercury (Hg) and methylmercury (MeHg) were significantly higher in the tuna sushi samples (González et al., 2021). A study evaluating Hg content in sushi samples marketed in Brazil found that tuna sushi contained the highest levels of MeHg. For tuna sushi specifically, organic Hg accounted for almost 90% of the total mercury content. The consumption of just 140g of tuna sushi per week was found to exceed 100% of the provisional tolerable weekly intake (PTWI) for methylmercury recommended for children, raising significant public health concerns regarding chronic exposure (Lima de Paiva et al., 2016). The assessment of the toxicological risk for Belgian consumers from chronic exposure to heavy elements, namely As, Ni, Hg, Pb, and Cd through the intake of raw or seaweed-based food products was presented in this study. For all elements (except Hg), toxicological limits were approached in the case of maximum exposure due to consumption of certain seaweed-based food products. Furthermore, the study also showed that the introduction of innovative foods to a developing market could lead to potential health issues because of the changes in consumption patterns, with increased consumption of seaweed and its derivatives in Europe. (Babaahmadifooladi et al., 2022).

PERSISTENCE OF SANITIZER-RESISTANT BACTERIA IN SUSHI PRODUCTION, MICROBIOLOGICAL QUALITY, THE PREVELANCE OF PATHOGENIC BACTERIA AND PARASITES IN SUSHI

The global increase in the consumption of raw fish preparations, such as sushi and sashimi, is primarily driven by their recognized nutritional quality. Fish are an exceptional source of vital dietary components, including, unsaturated fatty acids (notably docosahexaenoic acid or DHA), fat-soluble vitamins as well as essential amino acids. Raw consumption is often favored to

maximize the preservation and bioavailability of these nutrients. However, this preference introduces significant food safety challenges. Due to the inherent composition of fish meat, it is highly susceptible to rapid spoilage, which subsequently creates an environment conducive to the proliferation of pathogenic bacteria and/or parasites, thereby elevating the risk of foodborne illnesses (Miyazaki et al., 2025). For example, inadequate acidification of sushi rice at the retail level is a critical factor that can promote the proliferation of pathogenic bacteria. Specifically, the growth of foodborne pathogens such as Clostridium perfringens and Bacillus cereus is enhanced when sushi rice is not properly acidified or stored. Recovery of these bacteria was confirmed when rice was maintained at an abusive temperature of 37.7 °C even with slightly acidic conditions (pH 4.2 or 4.6). Consequently, to effectively inhibit the growth of these pathogens and ensure food safety, the maintenance of proper rice acidification to a pH of 4.2, combined with appropriate storage conditions, is essential (Mohammad et al., 2020). The presence of L. monocytogenes in sushi is a concern, demonstrating that sushi consumption can be a risk for consumers because of human listeriosis (Ramires et al., 2021). Actually, sushi restaurants have become very popular in Europe, with an increase in consumption of the sashimi specialty. Pathogenic bacteria such as Listeria monocytogenes, Bacillus cereus, and Staphylococcus aureus have been reported in such foods (Miguéis et al., 2017). Additionally, the genus Aeromonas, which includes human pathogenic bacteria, is commonly isolated from seafood products. The rising consumption of ready-to-eat seafood, such as retail sushi with a shelf life of up to three days, presents significant food safety concerns regarding the potential proliferation of pathogenic Aeromonas spp. during storage prior to raw consumption (Hoel et al., 2018). One study comparing the microbiological quality of frozen supermarket sushi and fresh sushi bar sushi demonstrated a significant difference in microbial loads. The mean count of aerobic mesophilic bacteria count was substantially lower in frozen sushi than in fresh sushi. Furthermore, fresh samples showed a higher prevalence of Staphylococcus aureus and Escherichia coli. Pathogens were detected in a minority of all samples (Salmonella spp. and Listeria monocytogenes). These findings of study suggested that the microbiological quality of industrially processed sushi was determined superior to that of freshly prepared sushi, which was hypothesized to stem from the high

variability in the hygiene practices and skills of individual preparation cooks (Atanassova et al., 2008). The surfaces of the squid sushi production line were found to harbor sanitizer-resistant bacteria, with the nylon contact surface demonstrating the highest microbial load in another study. Eight bacterial isolates exhibited resistance to three different types of common sanitizers. The study identified the genera Pseudomonas, Serratia, Bacillus, and Acinetobacter, as being highly prevalent and particularly resistant to these sanitizing agents, highlighting a critical concern regarding the efficacy of current hygiene protocols and the potential for cross-contamination in ready-to-eat food preparation environments (Sinlapapanya et al., 2025). The other study aimed to assess the microbiological quality of various sushi components (wasabi paste, maki, pickled ginger, nigiri) sourced from retail outlets, kiosks, and restaurants across the Czech Republic. While vegetarian maki exhibited the highest overall microbial loads, wasabi paste displayed the most significant contamination, specifically demonstrating the highest levels of both total viable bacteria as well as lactic acid bacteria counts. These high counts for wasabi suggested inadequate hygienic preparation and/or storage practices within the restaurants. Importantly, sushi products prepared in large food processing plants showed the lowest levels of contamination across all groups of microorganisms indicating a clear distinction in safety and quality based on the production environment. Salmonella spp., and Listeria monocytogenes were not found. However, 23 samples (17%) were contaminated with Bacillus cereus (Hulankova and Furmancikova, 2022). This study investigated the prevalence and virulence of the emerging foodborne pathogen Aeromonas hydrophila, which primarily originates in the aquatic environment in various seafood and ready-to-eat (RTE) sushi products under different distribution conditions and seasons. Seasonal analysis of both sashimi and sushi revealed that the summer prevalence of A. hydrophila isolates carrying putative virulence genes was significantly lower in sashimi but the highest in sushi. These findings strongly suggested that RTE sushi was highly susceptible to contamination from multiple sources during its manufacturing or distribution stages, warranting closer scrutiny of hygiene practices (Park et al., 2021). However, in another study conducted, Anisakid parasites, Staphylococcus aureus and Bacillus cereus were examined in Sushi and Sashimi from Restaurants in the Seattle Area. Staphylococcus aureus, and Bacillus cereus and were detected in rice

from six restaurants each, but in none of the samples were these two organisms found together, and the levels were well below those of public health importance. In addition to this, all the nematodes were third-stage juveniles of the genus Anisakis. Except for two moribund nematodes, all the juveniles from the sushi had died, most likely the result of the practice of using previously frozen fish (Adams et al., 1994).

THE CASE STUDIES ABOUT SUSHI CONSUMPTION

An investigation documented a case of cholangitis accompanied by Vibrio fluvialis bacteremia in a Japanese female patient. The condition was potentially linked to the frequent consumption of supermarket sushi, which may have served as the source of infection, particularly given the patient's underlying condition of a gallbladder-duodenal fistula (Itoh et al., 2025). In one study, Enterobacteriaceae, detected in sushi, common consumer products, is increasing concern about human exposure to superbugs and is spurring the need to improve surveillance of this public health issue (Vitas et al., 2018). In the other study, a 32-year-old man was admitted to the author's hospital with a 1day history of acute onset epigastric pain after ingesting Japanese sushi (bitesized pieces of cold cooked rice stuffed with fish, eggs or vegetables and wrapped in seaweed) (Kajihara, 2018). Anasakiasis is a foodborne zoonotic disease associated with the consumption of raw or undercooked fish (Golden et al., 2022). A 15-year-old Japanese male presented to the hospital with a live worm found in his sputum, three days after consuming conveyor belt sushi (including tuna, yellowtail, and whelk). He reported experiencing throat discomfort overnight, which led to the discovery of the moving organism. The worm was subsequently confirmed to be Anisakis simplex via DNA sequencing. This case highlights the rapid manifestation of anisakiasis following the consumption of raw fish products (Hara et al., 2019). A 28-yearold male was assessed for food allergy after experiencing two episodes of allergic reaction following sushi consumption. The initial episode, which occurred upon his first exposure to sushi (containing crab, salmon, rice, seaweed, eel sauce, ginger, wasabi, and soy sauce), began approximately 20 minutes post-ingestion. Symptoms included itching of the hands, face, and ears, accompanied by significant swelling of the face and hands, and nasal congestion (Afshan and Mathur 2023). A study, titled 'Detection of Antibiotic

Resistance in Escherichia coli Strains: Can Fish Commonly Used in Raw Preparations such as Sushi and Sashimi Constitute a Public Health Problem?', investigated the prevalence of antibiotic-resistant E. coli in fish used for raw consumption. The lack of monitoring in aquatic environments and excessive use of antibiotics promotes the development and spread of antimicrobial resistance, and fish can be reservoirs of antibiotic resistance genes that can easily be transmitted to humans through raw fish consumption, creating a public health problem (Silva et al., 2019). Furthermore, long-term exposure to cadmium, arsenic, mercury, and lead can cause oxidative damage, increasing neuro and cardiac risks (Huang et al., 2025).

FUTURE DIRECTIONS FOR SUSHI SAFETY AND QUALITY CONTROL

The safety of ready-to-eat foods is an important issue. Improper transportation and storage of ready-to-eat products can lead to foodborne illness outbreaks (Liang et al., 2016). Salmonella infections associated with seafood consumption have the potential to cause serious illnesses, with the incidence of foodborne illness associated with contaminated seafood reported to have increased in the last ten years (Viazis et al., 2025). Based on the results of the one study, the authors suggested that the combination of 6 h at \leq 15 °C could be used to distribute sushi considering Salmonella contamination. However, further studies must be carried out in order to predict the multiplication of other pathogenic bacteria, such as Listeria monocytogenes, in order to determine a time-temperature that included all pathogenic bacteria that may be present in sushi (Silva et al., 2020). Frequent insanitary observations in food manufacturing operations primarily involve failures in sanitation controls, posing significant risks to food safety. The most recurrent issues identified during inspections include concerns regarding the safety of water used for food processing and ice production, as well as the cross-contaminate food and food contact surfaces. Furthermore, instances of inadequate cleaning and sanitizing procedures have been commonly noted. These sanitation controls maintain confidence in the food supply chain, recalls, protect consumers as well as prevent foodborne outbreaks (Viazis et al., 2025). To secure the sustainable future of sushi as a cherished culinary tradition, further research is imperative to develop practical solutions addressing current safety and quality issues. This

research should prioritize fundamental measures, including improving sanitation protocols, ensuring hygienic handling practices, and establishing temperature regulation. Additionally, innovative technological approaches such as utilizing non-thermal plasma and developing advanced edible coatings offer promising avenues for enhancing sushi safety and extending its quality (Kılınç and Kılınç, 2024). For example, one study examined the effect of non-thermal plasma (NTP) on the total viable count and lipid oxidation of two common sushi products: nigiri and hosomaki. Sushi samples were processed with NTP using a dielectric barrier discharge system with potential differences of 70 and 80 kV for 5 minutes and then samples were stored at 4 °C. Although the effect of NTP on total aerobic counts was not statistically significant, a trend in log reduction could be observed with a decrease of 1-1.5 log cfu/g. The protein and moisture content of the sushi, as well as the composition of fatty acids, were not affected by the treatment. Simultaneously, The TBA index of the treated samples increased significantly by 0.4-1.5 mg /kg, while hosomaki reached a higher TBA index than nigiri.Indeed innovative technological approaches have effected for enhancing safety as well as extending quality of sushi samples (Kulawik et al., 2018). In the other example, the authors investigated the effect of applying pre-designed furcellaran-gelatin edible coatings with 20% green (GT) and pu-erh (RT) tea extracts on changes in cold-stored salmon nigiri. The treatment significantly inhibited the lipid oxidation (Kulawik et al., 2019). Furthermore, one study successfully introduced a novel integrated culture-based and molecular approach designed for the detection of three distinct Arcobacter species (A. butzleri, A. cryaerophilus, and A. skirrowii) in sushi and fresh vegetables. The results demonstrated that the integrated methodology operated with high efficiency and sensitivity although it was noted that certain matrix-specific features, such as pH, could potentially influence the outcome. Nonetheless, the developed method was considered suitable for the routine analysis of various food matrices, and it held significant potential for enhancing the diagnostic capacity of laboratories involved in the monitoring of zoonotic agents within the food and veterinary sectors (Savelli et al., 2025). Moreover, the government also recommended more frequent routine checks and food hygiene training for workers in sushi shops to minimize the risk of foodborne disease outbreaks (Liang et al., 2016). In this approach, the aim of one study was to evaluate the

performance of the food safety management system (FSMS) in relation to the microbiological food safety (FS) outcome of salmon nigiri sushi in selected outlets of a Japanese chain restaurant. It turned out that the performance of FSMS that were not ISO 22000 certified with respect to the microbiological FS outputs of salmon nigiri sushi of FSMS was found better than that of ISO 22000 certified FSMS (Fathurrahman et al., 2021). In the other study, the authors described the design and development of a radio frequency identification (RFID)-based sushi management (RFSM) system in a conveyor belt sushi restaurant to improve operational efficiency. This system has been designed to help a conveyor belt sushi restaurant achieve better inventory control, rapid replenishment, and improve service quality, as well as food safety control (Ngai and Suk, 2008). Despite the increasing popularity of sushi, there is a lack of research on food safety issues related to the preparation of raw fish and sushi rice. In a study made study revealed substantial non-compliance with Food Code requirements among Minnesota restaurants, specifically concerning raw fish and sushi rice practices, thereby indicating elevated public health risks. For instance, among restaurants using Time as a Public Health Control (TPHC) for sushi rice, only 26% had the necessary written procedures, highlighting systemic operational failures. Furthermore, critical hygiene violations included observed bare hand contact during sushi preparation in 17% of establishments, collectively underscoring a significant need for improved regulatory adherence for both parasite destruction and TPHC protocols (Hedeen, 2016). Another study analyzed sixty-one samples collected from twenty-three restaurants to evaluate their microbiological quality and safety. The principal findings of this study revealed that a substantial majority, specifically 63.93%, of the analyzed samples were classified as unsatisfactory. This classification was attributed to the detection of elevated levels of various microbial indicators and pathogens, including Bacillus cereus, Enterobacteriaceae, mesophiles Staphylococcus aureus, yeasts and moulds. These results collectively indicated a pronounced need for significant improvements in existing food quality and safety systems within these establishments, particularly through enhanced implementation and adherence to HACCP (Hazard Analysis and Critical Control Points) principles (Miguéis et al., 2015). These mixed-methods used in these study highlighted significant knowledge gaps in food safety measures and their impact on consumer confidence as well as purchasing decisions. Findings indicated a

growing market demand for high-quality, convenient seafood products that feature transparent labeling addressing both sustainability concerns and food safety. The researches underscored the necessity of improved marketing strategies education to facilitate informed consumer choices. Ultimately, this study offered actionable insights for the seafood industry to support sustainable growth by effectively aligning public health priorities with current market demands (Sciortino et al., 2025). Research has been indicated a general lack of correlation between publicly accessible ranking systems provided by food inspectors and the actual microbiological content of sushi products. This discrepancy may stem from the fact that current regulatory compliance monitoring often has been neglected to routinely include two critical parameters: personal hygiene of staff and the initial microbial quality of the products utilized. Consequently, the microbiological examination of sushi has not typically constitute a routine component of hygienic monitoring programs. Furthermore, a questionnaire study revealed that this limited scope of routine microbiological oversight has generally not widely known among consumers, suggesting a gap between public perception and the reality of food safety monitoring practices (Leisner et al., 2014).

CONCLUSION

Sushi consumption has been increasing in our country, as well as all over the world, in recent years. All raw materials to be used in sushi making must be in good quality. The raw materials to be used in sushi production (seaweed, fish, aquatic products) must be harvested from clean waters or grown in clean waters. Otherwise, these raw materials may contain many contaminants that are harmful to human health. In terms of food safety and to prevent post-consumption illness risks, sushi should be prepared in accordance with hygiene and sanitation rules. In addition, it is very important to take the necessary precautions and carry out controls in sales places where sushi is sold in terms of human health. In sushi production, acidified rice, various seafood and various vegetables are added and it is wrapped with seaweed called nori. The study aimed to use Ulva lactuca, which are abundant in the sea coasts of Türkiye and also cultivated in terms of sustainability, as an alternative to nori seaweed. For this purpose, Ulva lactuca was harvested fresh, dried and pressed to produce Ulva sushi sheets. Thus, the study showed that dried Ulva sheets can be used

as an alternative or preferred instead of nori sheets in sushi wrapping. The study also included the production of not only dried Ulva sheets, but also Ulva Sushi rolls in different formulations in accordance with the taste of the Turkish people. It is thought that Ulva sushi rolls, a functional food produced in this study, will be a pioneer in sustainable and safe food production, and will increase the consumption of aquatic products. In addition, it is desired that both the Ulva Sheets produced and the Ulva Sushi Rolls be brought into the economy and that their production and consumption be increased. It is estimated that the study will be a pioneer and shed light on future studies to be carried out for the production of new functional products such as 'Ulva sushi sheet' and 'Ulva sushi rolls'. This study is a new approach for sushi producing to find local materials such as Ulva lactuca to substitute Porphyra to produce nori-like products.

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CHAPTER 3

DRIFNET TRIALS FOR SWORDFISH (Xiphias gladius L, 1758)

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INTRODUCTION

Today, a significant portion of people's protein needs are met from the marine environment. Seafood, with its richer and healthier nutritional content than land-based protein sources, supports human development and healthy living. Global aquaculture production has reached 186 million tons (FAO,2024). A large portion of this production is met by fishing. Global warming, overfishing, and pollution are the most significant negative factors affecting existing fish stocks. Of these factors, overfishing is the most controllable from a fisheries management perspective. Environmental awareness, which has been widespread since the 1950s, has increased perceptions of pollution, while overfishing, which began in the 1970s, has now reached the level of protected areas, species-specific protective approaches, and organizations

In particular, in marine structures that are inland and shared by many countries (the Mediterranean, Baltic Sea, and Caspian Sea), the management of common stocks is managed by organizations that allow for international participation. ICCAT is one of these.

Determining the species' stock within the scope, understanding biological data, implementing sustainable fishing quotas and regulations, and even controlling the sale of catches demonstrate the success of these organizations. The organization established for bluefin tuna, a large pelagic species in the Mediterranean, has now begun implementing administrative decisions on the issue, including swordfish.

Therefore, the concept of sustainable fishing should be implemented in fisheries management by aligning with these organizational policies on a national basis. Türkiye's swordfish fishing situation is currently in this position.

Swordfish, classified among large pelagic fish, derive their name from the elongated, sword-like rostrum extending from the upper jaw (Figure 1). *Xiphias gladius* (Linnaeus, 1758) are highly migratory species of significant economic importance within the fisheries sector. They are primarily distributed in temperate marine waters. Swordfish can attain lengths of up to 4.5 meters and body weights reaching 650 kilograms. Predominantly feeding on pelagic fish and cephalopods such as squid, swordfish have been recorded at depths of up to 800 meters (Froese and Pauly, 2010).



Figure 1. Swordfish (Xiphias gladius L. 1758)

Swordfish (Xiphias gladius L.) are a solitary species, meaning they live alone and do not form schools. They exhibit spawning behavior at different times depending on the region. Spawning occurs from January to October in the Eastern Atlantic, from February to April in the Western Atlantic, and from June to September in the Mediterranean Sea (Abid at. all, 2018). While swordfish prefer cooler waters for feeding, they migrate to warmer, temperate waters to spawn.

Although typically found in temperate seas, their distribution can extend as far north as Iceland during the summer months (Nakamura, 1986). A review of global swordfish catches over the past 50 years indicates a decline up to the year 2000, followed by a stabilization and a slight upward trend between 2000 and 2010. The peak in global swordfish production occurred in 2003, with an estimated 118,000 metric tons, while in the Mediterranean, production was recorded at approximately 20,400 metric tons in 1988 (Akyol at. All, 2010).

Swordfish fisheries are active worldwide, particularly in tropical and temperate waters. In the North Atlantic, the leading swordfish-harvesting countries are Spain, the United States, Canada, Portugal, and Japan. In the South Atlantic, the main harvesting nations include Brazil, Japan, Spain, Taiwan, and Uruguay (FAO,2024).

It has been reported that swordfish fishing dates back to ancient times, with the use of harpoons as early as the pre-Christian era (Ward at all, 2000), (Kahraman and Dağlı, 2008), in their work, documented that swordfish were caught in the 1630s at tuna traps (dalyans) located along the shores of Beykoz

in Istanbul. At the beginning of the 1900s, (Deveciyan, 1926) reported that swordfish were caught in the Sea of Marmara and the Bosphorus using harpoons and drifting pelagic gillnets (driftnets).

Later, Artüz, 1964, and Onat, 1970, noted that during the 1960s, swordfish in the same region were captured using gillnets, longlines, traps, and harpoons. They also observed that the harpoon fishing season typically began in April and they reported that harpoon fishing typically continued until early June, while gillnet fishing in the Bosphorus was carried out between September and November, specifically on moonless (dark) nights (Alıçlı and Oray, 1995).

Today, swordfish fishing is carried out using harpoons, longlines, pelagic gillnets, and purse seines along both the Aegean and Mediterranean coasts of Türkiye, involving approximately 150 vessels. Although swordfish fishing is a traditional practice in Türkiye, studies on the biology of the species are relatively scarce (Akyol and Ceyhan, 2010),(Deveciyan, 1926), (Demir at. All,1994),(Tokaç, at all, 1991),(Gökoğlu and Oray, 1992), (Anonim, 2006a),(Alıçlı, 2008),(Öztürk at. All, 2001), (Akyol at. All, 2005), (Erdem and Akyol, 2005), (Akyol and Ceyhan, 2007), (Ceyhan and Akyol, 2009), (Akyol at. All, 2010),(Golani at. All, 2006).

Drifting pelagic gillnet fishing is one of the oldest and simplest methods used for harvesting swordfish worldwide (Di Natale and Notarbartolo, 1994). These nets are defined as surface-set gillnets with floats along the top line and no weights on the bottom line. In the Mediterranean, drifting pelagic gillnets are commonly used for targeting tuna and especially swordfish (Tudela at all, 2005).

Following the increased availability of synthetic fibers and knotless mesh nets in the 1950s, monofilament nylon driftnets became widely used for swordfish fishing by the mid-1980s. These nets quickly gained popularity due to their higher efficiency compared to harpoons. Because harpoon fishing is dependent on specific weather conditions, requires experience, and typically yields lower catch volumes, nets have become the preferred method even among traditional harpooners. In Chile, governments actively promoted the transition to driftnets (Weidner and serrano,1997).

In the late 1980s, concern arose over high levels of bycatch (non-target species) in international waters, particularly in fisheries targeting salmon, squid, and albacore (Thunnus alalunga) by Japan and Taiwan. In response, in

1991, the United Nations imposed a ban on driftnets longer than 2.5 kilometers. Subsequently, many countries banned the use of these nets. However, some countries including Chile, Mexico, Japan, and certain Mediterranean fisheries continued swordfish fishing using driftnets shorter than 2.5 kilometers (Folsom at all, 1997).

Today, it has been reported that illegal swordfish fishing continues in the Mediterranean using modified gillnets that are submerged to avoid detection (Anonym.a,2006). It is estimated that more than 600 vessels are involved in illegal pelagic gillnet fishing in the region. Reports indicate that over 100 vessels operate in Italy, 70 to 100 in France, 150 to 300 in Morocco, and more than 110 in Türkiye (EJF, 2007),(FAO,2008).

When examining the historical development and diversity of swordfish fishing in Türkiye, it is observed that between 1930 and 1970, fishing began in the Bosphorus using trap nets (known as "dalyans") between April and February. Over time, this method evolved with the introduction of harpoons, drifting gillnets, and longlines (paraglines).

In recent years, large pelagic fish stocks shared by multiple countries have begun to be protected under quota systems enforced through international agreements. The International Commission for the Conservation of Atlantic Tunas (ICCAT), established initially for the management of Atlantic bluefin tuna, has emphasized the importance of species-specific fisheries management. Consequently, through international cooperation, various sanctions have been imposed on countries harvesting from the same stocks, particularly concerning tuna and swordfish. In the Mediterranean, efforts have focused not only on identifying biological characteristics of the species but also on promoting the use of effective fishing gear, assessing fleet capacity, estimating mortality rates, and determining whether stocks are over exploited. Additionally, identifying non-target species (bycatch) caught using swordfish gear has been an important step towards sustainable fisheries management.

Today, the most efficient swordfish fishing occurs during specific seasons, particularly on moonless nights when the fish migrate, using surface-set drifting nets carried by currents. ICCAT's regulations since the early 2000s have included restrictions on fishing quotas and the prohibition or modification of certain fishing gears (ICCAT, 2008). Türkiye has been among the countries

most adversely affected by these changes in terms of swordfish production, which has shown fluctuating trends.

As a response to ICCAT's restrictions, some fishers in Türkiye made modifications to their nets, altering them so they no longer conformed to the official definition of driftnets and continued fishing. However, according to Communiqué No. 2008/48, published on 10 July 2010, the use of driftnets was banned during the fishing season. Furthermore, the use of modified driftnets targeting highly migratory species—such as swordfish, Atlantic bluefin tuna (Thunnus thynnus), albacore (Thunnus alalunga), and little tunny (Euthynnus alletteratus) was officially prohibited as of 1 July 2011 (Anonym, 2006b).

Following the ban on gillnets from July 2011, active swordfish fishing in Türkiye was restricted to longline (paragline) fishing in the Fethiye region and harpoon fishing around Gökçeada. Although global swordfish production continued at similar levels despite the ban on drifting nets—thanks to the widespread adoption of longline fishing—Türkiye failed to transition effectively to alternative fishing methods. Since 2011, swordfish has only been caught via longlines in Türkiye. However, this method is labor-intensive and requires significant expertise. Additionally, challenges such as bait supply issues and the low economic profitability of the method have caused Türkiye's swordfish catch to decline drastically—from around 400 tons to as low as 34.9 tons, representing a 90% decrease (TUİK, 2024).

In recent years, with the reintroduction of drifting nets into fishing practices, swordfish catches in Türkiye have begun to rise again (Table 1.)

Table 1. Distribution of swordfish production in Türkiye by year (TUİK, 2024)

Yıllar	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
(ton)	34,9	76,5	441	427	414	402.4	390,4	378,7	378,7	378,7

This situation has led to significant national economic losses for Türkiye, a country located along the migratory route of swordfish in the Mediterranean, and has caused hardship for fishermen and their families who rely on this fishing method for their livelihoods. At the beginning of 2017, ICCAT's proposal to allocate swordfish fishing quotas based on each country's historical catch levels raised concerns that Türkiye would receive a very low quota due to its minimal reported catches in recent years. In response, the Ministry of

Food, Agriculture and Livestock, through the General Directorate of Fisheries and Aquaculture, aimed to increase national production by reopening swordfish fishing with drifting gillnets. As a result, the use of drifting pelagic gillnets was authorized once again.

The objective of this study is to gather data on the technical characteristics and catch composition of drifting gillnets, which were used in Türkiye until 2002 and banned until 2017. This study is based on data from project number 2017/SÜF/006, conducted under the coordination of the General Directorate of Fisheries and Aquaculture of the Ministry of Food, Agriculture and Livestock, and carried out at the Faculty of Fisheries, Ege University.

CHARACTERISTICS OF SWORDFISH DRIFTNETS Sivrice Driftnets

Swordfish fishing along the Turkish coasts is carried out using drifting gillnets between May and October. The Southern Aegean, Northern Aegean coasts, the Gulf of Saros, the Turkish Straits, and the Sea of Marmara all located along the species' migratory route are the regions where fishing activity is most intense. In these areas, nets are typically designed and named according to the localities in which they are used.

The swordfish nets used in the Sivrice region are made of polyamide (PA) material with a twine thickness of 210d/54–60 and a full mesh size of 480–490–500 mm. Nets with a full mesh size of 500 mm are most commonly used. The hanging ratio (E) is 0.66. The total length of the net is 35 meters, and the depth ranges between 15, 17, 18, and 25 meshes. The float line is equipped with 5–6 mm polypropylene (PP) rope, and No. 2 floats are attached (Akyol at. all, 2010). (Figure 2.)

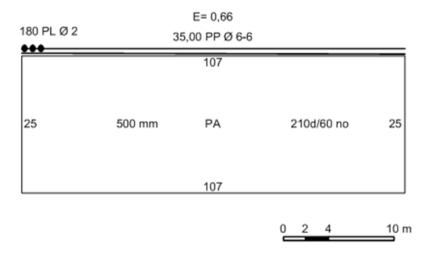


Figure 2. Sivrice Driftnets technical drawings

Fishing in this region takes place between May and October. The nets are set parallel to the coastline at depths ranging from 200 to 500 meters near the water surface. Typically, between 100 and 130 net panels are deployed after sunset, and the nets are hauled around 03:00–04:00 AM, taking into account environmental and weather conditions as well as the lunar phase. Besides swordfish, other species caught in the nets include Atlantic bluefin tuna (Thunnus thynnus), various shark species, the spearfish (Tetrapturus belone), ocean sunfish (Mola mola), among others.

Fethiye Driftnet

The swordfish nets used in the Fethiye region are made from polyamide (PA) material with twine thickness of 210d/54–60 and full mesh sizes of 440–460–500 mm. Nets with a full mesh size of 460 mm are the most preferred. The hanging ratio (E) is applied as 0.57. The length of one net panel is 115 meters, and the depth ranges between 25 and 30 meshes. The float line is equipped with 5 mm polypropylene (PP) rope and uses strip polyurethane floats (Figure 3).

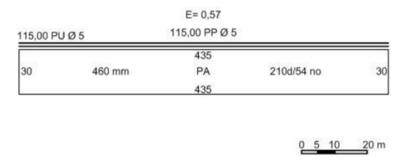


Figure 3. Fethiye Driftnets technical drawings

Fishing in this region is conducted between May and July, with nets deployed in waters located 12–13 nautical miles offshore, spanning from Finike to the eastern part of Rhodes Island. These nets, typically ranging from 40 to 60 panels in total length, are set after sunset and retrieved early in the morning. In addition to swordfish, the catch often includes Atlantic bluefin tuna (Thunnus thynnus), bullet tuna (Auxis rochei), various shark species, dolphinfish (Coryphaena hippurus), albacore (Thunnus alalunga), little tunny (Euthynnus alletteratus), Atlantic bonito (Sarda sarda), spearfish (Tetrapturus belone), and occasionally loggerhead turtles (Caretta caretta) and dolphins (Akyol at all., 2010).

Poyrazköy Driftnets

In the Istanbul region, swordfish nets used by fishermen in Poyrazköy are made of polyamide (PA) material with a twine thickness of 210d/54–60 and a full mesh size of 460 mm. The hanging ratio (E) is 0.66. One net panel measures 132 meters in length. Net depths vary between 20, 30, and 50 meshes. The float line is equipped with 6–8 mm polypropylene (PP) rope and No. 6 plastic floats (Akyol at all., 2010).

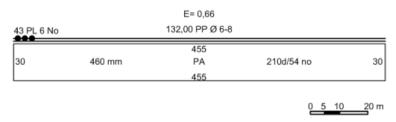


Figure 4. Poyrazköy Driftnets technical drawings

Fishing in this region is carried out between May and October in waters ranging from 300 to 800 meters in depth. Nets, typically consisting of 40 panels, are set after sunset on moonless nights and retrieved around 03:00–04:00 AM, depending on weather conditions. Besides swordfish, catches also include species such as Atlantic bluefin tuna (Thunnus thynnus), dolphinfish (Coryphaena hippurus), and ocean sunfish (Mola mola), among others.

Poyrazköy Auxiliary Driftnets

An auxiliary net with smaller mesh sizes, known locally as the "Aykırılı" net, is attached to the upper part of the main swordfish net to facilitate operations. Developed by fishermen from Poyrazköy, the main net is made of polyamide (PA) material with a twine thickness of 210d/54 and a full mesh size of 440 mm. Attached to its upper section is a 20-mesh-high net made of 210d/18 PA with an 85 mm mesh size. This auxiliary net prevents floats from becoming entangled in the meshes. The hanging ratio (E) of the net is 0.77. The total length of the net is 155 meters, and its depth is 70 meshes. The float line is equipped with 5–6 mm polypropylene (PP) rope and 30 No. 10 plastic floats (Akyol at all., 2010) (Figure 5).

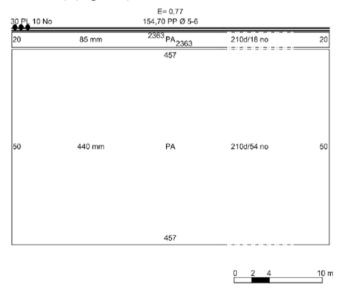


Figure 5. Poyrazköy Auxiliary Driftnets technical drawings

These nets are used in the Aegean and Mediterranean Seas between May and September in areas with strong currents and deep waters. Typically, the nets consist of 15 panels in length and are set after sunset during moonless nights. They are hauled around 03:00–04:00 AM, depending on weather conditions. In addition to the target species, swordfish, the nets also catch Atlantic bluefin tuna (Thunnus thynnus), albacore (Thunnus alalunga), dolphinfish (Coryphaena hippurus), ocean sunfish (Mola mola), and other species (Akyol at all., 2010).

MATERIAL AND METHODOLOGY

Material

These nets are used in the Aegean and Mediterranean Seas between May and September in areas with strong currents and deep waters. Typically, the nets consist of 15 panels in length and are set after sunset during moonless nights. They are hauled around 03:00–04:00 AM, depending on weather conditions. In addition to the target species, swordfish, the nets also catch Atlantic bluefin tuna (Thunnus thynnus), albacore (Thunnus alalunga), dolphinfish (Coryphaena hippurus), ocean sunfish (Mola mola), and other species (Akyol at all., 2010).

37°54'36.77"N - 27°06'58.03"E 37°50'09.66"N - 27°07'02.45"E 38°00'31.64"N - 26°35'59.66"E 37°52'43.50"N - 26°37'06.37"E



Figure 6. Study area

The fishing operations for the study were carried out using the fishing vessel SALNUR 2, which is 11 meters in length and equipped with a 350 HP engine (Figure 7).



Figure 7. Fishing vessel SALNUR 2

The Salnur 2 fishing vessel is designed for offshore fishing operations and is equipped with a hydraulic net hauler. It is also outfitted with bridge equipment essential for both navigation safety and fishing operations. Some of the onboard devices include an echo sounder, chart plotter, and surface radar (Figure 8).



Figure 8. Acoustic and navigation devices

Traditional Swordfish Net Used in the Study

Two different net models with distinct structures were used in this study. The first is defined as the traditional swordfish net. These nets are made of polyamide (PA) material, with a twine thickness of 210d/60 and a full mesh size of 500 mm (Figure 9).



Figure 9. Traditional Swordfish Drifnets

The hanging ratio (E) is 0.66. One net unit is 107 meters in length, with a depth of 25 meshes. The float line is rigged with 6 mm polypropylene (PP) rope, and No. 2 plastic floats are used (Figure 10).

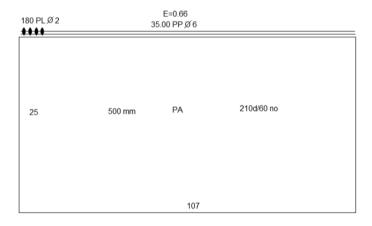


Figure 10. Traditional Swordfish Drifnets technical drawings

"Doctor Collar" Driftnet

The "Doctor Collar" refers to a net design in which, instead of using conventional plastic floats on the float line, buoyant polyurethane material is embedded directly within the collar itself. According to local accounts, during fishing operations with traditional nets, plastic floats would often become entangled in the mesh during deployment. In response, a medical doctor

involved in fishing is said to have designed this type of net by incorporating polyurethane strips into the float line, eliminating the need for external floats. As a result, this float line design came to be known as the "Doctor Collar" (Figure 11)



Figure 11. Doctor Collar" Driftnet and Swordfish

The netting section is made of polyamide (PA) material with a twine thickness of 210d/54 and a full mesh size of 460 mm. The hanging ratio (E) is 0.57. One net unit measures 115 meters in length, with a depth ranging up to 30 meshes. The float line is rigged with 5 mm polypropylene (PP) rope, and a 5 mm diameter strip of polyurethane floatation material is integrated into the float line (Figure 12).

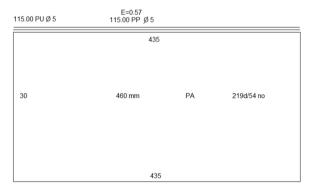


Figure 12. Doctor Collar" Driftnet Technical drawings

Methodology

The technical specifications of the nets used in the study were measured using calipers and a measuring ruler. The total lengths of the nets were determined through interviews with local fishermen. For proper deployment at sea, the nets were arranged (neta) in three main parts: the float line, the net body, and the lead line (lower edge of the net) (Figure 13). During this process, in traditional swordfish nets, the correct and orderly stacking of the floats is a critical step to prevent tangling or operational issues during deployment.



Figure 13. Settings of Driftnet

During the dark phases of the moon, the nets are deployed at sunset in a straight line across the sea surface. A lighted floating buoy is attached to each end of the net, allowing it to drift freely with the current. Throughout the night, the fishing vessel monitors the net's position and issues warnings to nearby vessels to prevent any damage to the gear. Depending on current and wind conditions, the net may drift 3 to 5 nautical miles overnight. This drifting enables the net to be effective over a broader area. At sunrise, the nets are retrieved from the sea using a hydraulic winch, and the fish are sorted by species (Figure 14). In the laboratory, biometric measurements of the fish are taken to determine the species composition.



Figure 14. Hauling nets from the sea

If a large fish is encountered in the net, the winch is stopped, the net is removed from the hauling drum, and the section containing the fish is manually transferred onto the deck. The empty portion of the net is then reattached to the winch. This procedure prevents large fish from falling into the sea or being damaged by getting crushed when passing through the bow-mounted hauling drum.

In order for swordfish gillnets to be exempt from the "driftnet" classification, certain modifications were made to their rigging: lead weights were added to the lead line, and sea anchors (paravanes) were attached to the float line. The study examined whether these gear modifications influenced the catch efficiency and how they affected the overall fishing operation.

FINDINGS

Following 24 fishing operations, catch compositions from 4 different vessels were determined. A total of 220 individuals belonging to 5 species were caught. Swordfish (Xiphias gladius) was the most abundant species with 68 individuals captured (Table 2).

Species	N	Max lenght (cm)	Min lenght (cm)	Average lenght (cm)	Max weight (kg)	Min weight (kg)	Average weight (kg)
Xiphias gladius	68	164	58	114	91	4.6	23
Coryphaena hippurus	46	92	28	54	12	0.7	3,4
Thunnus thynnus	24	86	72	80	28	22	26
Katsuwonus pelamis	54	63	56	59	7	4.5	5.8
Brama brama	28	62	54	58	5.2	4.3	4.8

Table 2. Some biometric data of species

When examining the biometric data of the species, it was determined that swordfish (Xiphias gladius) caught ranged from a minimum length of 58 cm to a maximum length of 164 cm, and from a minimum weight of 4.6 kg to a maximum weight of 91 kg. The average length was found to be 114 cm, and the average weight was 23 kg (Figure 15).



Figure 15. Swordfish

Skipjack tuna (Katsuwonus pelamis), known as Yazılı tuna in Turkish, is the second most caught species in the catch composition. The captured

individuals range in length from a minimum of 56 cm to a maximum of 63 cm, and in weight from 4.5 kg to 7 kg. The average length was determined as 59 cm, and the average weight as 5.8 kg (Figure 16).



Figure 16. Skipjack tuna (Katsuwonus pelamis)

The third most caught species in the catch composition is the dolphinfish, known as Coryphaena hippurus. The captured fish ranged from a minimum length of 28 cm to a maximum of 92 cm, and from a minimum weight of 0.7 kg to a maximum of 12 kg. The average length was determined to be 54 cm, and the average weight 3.4 kg (Figure 17).



Figure 17. Dolphinfish

Brama brama, known as the pomfret, is a species rarely encountered along the Aegean coasts. The captured fish ranged from a minimum length of 54 cm to a maximum of 62 cm, and from a minimum weight of 4.3 kg to a maximum of 5.2 kg. The average length was determined to be 58 cm, and the average weight 4.8 kg (Figure 18).



Figure 18. Atlantic pomfret fish

With 24 individuals, the Bluefin tuna (Thunnus thynnus L, 1758) ranks last in the catch composition. According to the ICCAT agreement, fishing for Bluefin tuna is allowed under a quota system with the issuance of a BCD (Catch Document) certificate. Therefore, the tuna caught are usually immediately cut and packaged as meat upon being brought out of the sea, and are not brought ashore whole. The captured fish ranged from a minimum length of 72 cm to a maximum of 86 cm, and from a minimum weight of 22 kg to a maximum of 28 kg. The average length was determined to be 80 cm, and the average weight 26 kg (Figure 19)



Figure 19 Bluefin Tuna

During the operation, it was determined that the weight attached to the lead line of the swordfish nets negatively affected the operation. The weight altered the net's posture, causing the sections of the net suspended in the water to gather around the weighted area. The use of a sea anchor (drogue) attached to the float line allowed the net to drift more slowly. However, the volumetric appearance of the sea anchor made fish near the anchor more aware of the net, which adversely affected the catch efficiency.

DISCUSSION AND CONCLUSION

This study is important in terms of developing an alternative net model to the driftnets banned by ICCAT in 2002, and determining the effects of the new model on marine mammals and sea turtles. In the survey conducted by Akyol and Ceyhan in 2010, it was reported that dolphins and sea turtles were not observed in the catch composition of the Sivrice swordfish net, Poyrazköy swordfish net, and Poyrazköy aykırılı swordfish net, whereas dolphins and sea turtles were observed in the catch composition of the Fethiye swordfish net. In our study conducted in the Gulf of Kuşadası and south of Çeşme, no mammals or sea turtles were encountered. The higher activity and density of sea turtles and mammals in the South Aegean and Mediterranean may explain their presence in the swordfish nets in the Fethiye region. The occurrence of unwanted species in that region as a negative example for driftnets used along all Turkish coasts reveals that the ban is regional in nature.

In the study by Tudela et al. (2005) conducted off the coast of Morocco, it was reported that despite the ICCAT ban, a large fleet using driftnets carried out 369 fishing operations over 12 months. They reported that the driftnets averaged 6.5–7 km in length, composed of 177 sets, and that by the end of the season, one vessel caught 2990 swordfish along with 237 dolphins, 498 blue sharks, 542 shortfin make sharks, 464 perbeagle sharks, and 46 sea turtles. The total annual bycatch was estimated at 3110–4184 dolphins and 20,262–25,610 sharks. These numbers vary regionally. The lower temperature of the Aegean Sea limits the ecological distribution of sharks. The absence of sharks or dolphins in our study indicates that ICCAT should implement different regulations for different regions. Indeed, following the decisions made in 2002, Turkey's swordfish production decreased from 400 tons to 39 tons.

During the period when driftnets were banned, Akyol and Ceyhan (2014) reported that in the catch composition of swordfish using longline fishing, the target species swordfish comprised 78.6% in number and 73.3% in weight. Of course, longline fishing targets specific species and is considered more environmentally friendly and efficient for the target species compared to driftnets. However, the scale of application remains very small within overall swordfish fisheries. This is due to difficulties in obtaining bait in the summer, the high cost of available bait, and the experience required for the fishing method.

In Italy, during the 1980s and 1990s, swordfish accounted for only 18% of the catch in driftnets used, and scientific studies revealed that many marine mammals and whales were killed by these nets. This situation led to the initiation of international sanctions. Until 1998, the Italian fleet of 600 vessels was reported to cause the death of 8000 marine mammals annually. In our study, the target species accounted for about 40% of the catch composition. The absence of mammals or turtles among the bycatch species supports the shows that in the area where our study was conducted, driftnets do not affect marine mammals and turtles.

Since 2006, Turkey has included the ICCAT-recommended ban on driftnets for swordfish fishing in the fisheries regulations (Anonymousb,2006). This ban has been met with opposition from fishermen who catch swordfish and albacore using driftnets. Fishermen have reported that these nets have been traditionally used for many years, have high selectivity for the target species,

and only rarely catch sea turtles and dolphins, which are released back into the sea alive. Legal challenges against the ban and the resulting loss of fishing rights are ongoing.

In conclusion, some rules and sanctions imposed by ICCAT on Mediterranean countries at the international level have caused hardships for fishermen engaged in swordfish fishing in Turkey. The Mediterranean is a vast basin that includes many areas with diverse hydrographic and abiotic factors. Applying sanctions uniformly across the entire Mediterranean based on problems observed in driftnet use off the coasts of Africa and assuming the same issues exist in the northern Aegean Sea negatively impacts Turkey's swordfish fishery, which has been operating without problems for many years, nearly bringing it to a halt and causing hardship for those involved in swordfish fishing. Regional-level analysis of ICCAT sanctions and the implementation of region-specific decisions and regulations would help ensure the future of fish populations as well as the continuation of small-scale traditional fisheries.

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CHAPTER 4

PRAWN FISHING WITH TRAMMEL NETS IN THE GULF OF MERSIN

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INTRODUCTION

Mersin Bay is one of the regions hosting the richest prawn populations in Turkey (Gökçe et al., 2016). All of Turkey's prawn production is achieved through fishing. A significant portion of the 724.0 tons of green tiger prawn is caught in the Mediterranean (TUIK 2024).

Bottom trawls are the most commonly used gear in prawn fishing. Trammel nets, pots and beam trawls are also widely used in various regions and conditions (Kalawar et al., 1985; Engvall, 1991; Fujimori et al., 1996; Thomas et al., 2003; Bayhan and Gökçe 2010). Prawn fishing is a highly profitable activity, with shrimp products accounting for over 15% of the international trade value (Teixeira et al., 2020). Furthermore, shrimp trawls can cause serious ecological damage to benthic habitats. Therefore, interest in small-scale fishing practices, particularly those conducted with trammel nets, has increased significantly in recent years.

This chapter presents technical information on trammel nets, widely and effectively used in the economically valuable prawn fishery in the Mersin Bay. It also examines the structural and equipment characteristics of the fishing vessels using this gear, the operational procedures of the fishery, the composition of the species caught, and the major challenges encountered during the fishing process. Solutions to these challenges are also discussed.

2. MATERIALS AND METHODS

2.1. Research Area

The data obtained as a result of face to face interviews with the captains of trammel net fishing vessels in the Çamlıbel Fishing Port (Fig. 1), Erdemli Fishing Port (Fig. 2) and Karaduvar Fishing Port (Fig. 3) located in the Mersin Gulf and direct field studies were evaluated and presented in the study.



Figure 1. Çamlıbel Fishing Port



Figure 2. Erdemli Fishing Port



Figure 3. Karaduvar Fishing Port

2.2. Technical Plan of the Trammel Net

Nets are generally divided into two categories: gill nets and trammel nets. Trammel nets are used in prawn fishing. Trammel nets, used in prawn fishing, have an inner panel of twine in the middle and outer panels on either side. These are rigged with a float line on the top and a lead line on the bottom. Although the amount and properties of the float and lead used, the rigging factor of the net, and the properties of the rope used in the net show some minor regional differences, the general technical plan of the net is as follows (Fig. 4)

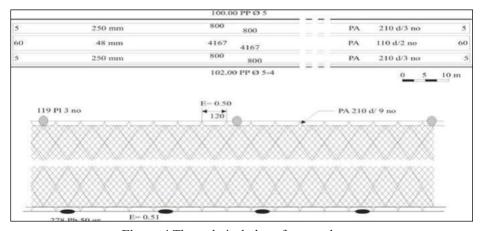


Figure 4. The technical plan of trammel nets

2.3. Fishing Boats

All trammel-net fishing vessels in the Gulf of Mersin were found to be wooden. Their average length was 8 m, and the average engine power was 75 hp (Fig. 5).



Figure 5. Trammel Net Boats

2.4. Prawn Fishing Operation

Within the scope of the current legal regulations, trammel net vessels are allowed to engage in different fishing activities at certain periods of the year in Mersin Bay. Fishermen choose their fishing method based solely on economic gain. In Mersin Bay, they primarily fish for shrimp, sole, cuttlefish, red mullet, and sardines. Longline fishing is also practiced intensively during certain periods. However, prawn fishing is one of the most popular fishing activities available year-round. Nets are generally cast in an S-shape over mud and sandy areas at depths of 5-40 fathoms. Nets are generally released into the sea around 2:00–3:00 a.m. and collected after a waiting period of approximately 2–2.5 hours during the summer periods. In the other months, nets are usually released around 4:00 p.m. and retrieved before sunrise.

2.5. Target Species

The most important target species caught in trammel nets is *Penaeus semisulcatus*, while *Melicertus kerathurus* is also caught (Fig. 6).



Figure 6. Target Catch

Among the species caught incidentally, that is, those with economic and market value, sole (*Solea solea*), black mullet (*Umbrina cirrosa*), red gurnard (*Chelidonichthys lucernus*), red mullet (*Mullus barbatus*) and species belonging to the Sparidae family are caught (Fig. 7).



Figure 7. Incidental Catch

Species that are discarded into the sea, i.e., have no economic value, include *Rissoides desmaresti*, *Charybdes longicollis*, *Goneplax rhomboides*, *Raja clavata* and *Rhinobatos rhinobatos* (Fig. 8).



Figure 8. Discards Catch

3. PROBLEMS ENCOUNTERED IN PRAWN FISHING

Illegal fishing is one of the most significant problems faced by fishermen in Mersin Bay. Especially between April 15th and September 15th, when trawling is prohibited, and within the 2-mile limit, trammel net fishing is carried out illegally. This situation damages the nets of trammel-net fishermen who operate legally. Furthermore, because such illegal fishing methods result in a large quantity of prawn, they also lower prawn prices, creating unfair competition and negatively impacting fishermen. Furthermore, this illegal fishing, which occurs during the breeding season and negatively impacts their stocks due to the capture of numerous small individuals, has led to considerable complaints from trammel net fishermen. Another significant problem encountered in prawn fishing is discarded catch. Discarded species such as crabs and mantis shrimps pose a significant problem for fishermen. These species damage the net, causing it to wear out more quickly, and also cause

additional labor loss for fishermen when removing these species from the net (Fig. 9).



Figure 9. Removing discarded species from the net

Some fishermen have recently complained that puffer fish and sea turtles are damaging the nets and eating the fish in them.

4. PROPOSING SOLUTIONS

Illegal fishing during the prohibited season has serious negative impacts on fish stocks and constitutes a fundamental issue requiring significant attention. It is crucial to increase inspections, particularly during breeding seasons when fishing is prohibited, and to implement adequate deterrent sanctions. Furthermore, organizing awareness and informational meetings for fishermen will significantly contribute to supporting sustainable fishing and mitigating this problem.

There are some studies in the literatüre have been conducted to prevent discarded species such as crabs and mantis shrimps from being caught in trammel nets. Using a guarding net between the bottom of the trammel net and the lead collar significantly reduces discards. The sardon net can be made of thicker netting, tarpaulin, greenhouse cloth, or greenhouse nylon (Gökçe, 2016; Eryaşar et al., 2020; Bozaoğlu 2023) (Fig. 10).

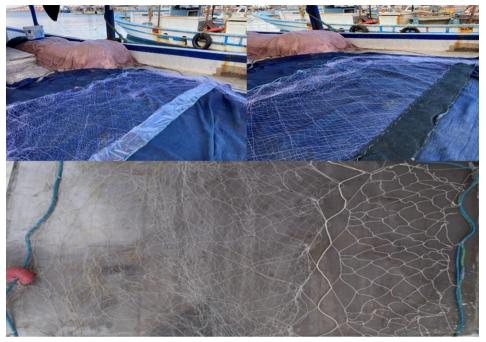


Figure 10. Use of guarding nets in trammel nets

The Ministry of Agriculture and Forestry periodically pays a per-tail fee to address the pufferfish problem. Increasing these incentives could encourage fishermen to use alternative fishing methods to catch this species. To address the sea turtle problem, these species can be removed by attaching acoustic pingers, which emit sounds at specific frequencies, to nets.

5. CONCLUSION

Mersin Bay is one of the most important fishing grounds for the region. Green tiger prawn is one of the most important species caught in this fishing ground. Therefore, a sustainable fishing policy for this species requires the optimal use of water resources. First and foremost, the stocks of this species must be protected. For a species to maintain its stock, it must be allowed to reproduce at least once. Therefore, the catchable size of that species must be determined by determining the species' initial breeding length. In addition, information should be recorded not only on fish stocks but also on all fishing gear and equipment used in fishing. Fishermen's socio-economic data must also be collected and continuously updated. Finally, scientific studies should be

conducted in collaboration with fishermen. For example, modifications to nets will be more likely to be implemented by fishermen and more quickly. This will allow the fleet to more easily adopt the data obtained from scientific studies.

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CHAPTER 5

CIRCULAR BUSINESS MODELS IN FISHING GEARS

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INTRODUCTION

Nowadays, marine pollution is one of the most significant global environmental problems, and a major component of this problem is abandoned, lost or otherwise discarded fishing gears (ALDFG). Thanks to their strength, longevity and low cost, plastic fishing equipment has replaced natural materials and become an indispensable part of the global fishing industry. However, lost fishing equipments that are not returned to the boat after operations, poses a great risk to the seas (Derraik, 2002).

According to data from the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization (FAO), 10% of the plastics in the oceans each year originated from fishing equipment, which equates to approximately 640,000 tons (Macfadyen et al., 2009) and this amount is equivalent to 64 times the weight of the Eiffel Tower. Plastic is a persistent material that has an indefinite lifespan in the marine environment, harming coral reefs and a variety of species, from all fish to large marine mammals, turtles and seabirds (figure 1) (HillNotes, 2020). Additionally, animals mistakenly feed on small pieces of plastic causing microplastics to enter the food chain of marine life and finally humans.

According to a study by Richardson, et al. (2022), approximately 2963 km² of gillnets, 75,049 km² of purse seine nets, 218 km² of trawl nets, 739,583 km of longline and more than 25 million pots and traps are lost in the oceans each year. These losses create serious socioeconomic and environmental impacts on the sustainability of fisheries, exacerbating existing pressures from overfishing, fish stock declines and climate change. Data from FAO's global ALDFG surveys with fishers (figure 2) allow for extensive analyses at national, regional and global levels (GESAMP, 2021). Most of the fishing equipment abandoned, lost or discarded in the seas or oceans do not rapidly decompose in nature, but continues fishing passively for long time. ALDFGs cause marine organisms to become entangled and die, while also causing serious damage to the marine ecosystem. By covering marine habitats, it reduces light and oxygen permeability, which threatens habitats such as seagrass meadows and coral reefs while also causing microplastic pollution (Brown et al., 2005). This situation negatively impacts marine biodiversity, as well as those who depend on fishing for their livelihoods. ALDFGs pose a broad threat to marine life, fisheries, and fish consumption (Figure 1).



Figure 1. A sea turtle caught in a plastic fishing net (BBC.com, 2018).

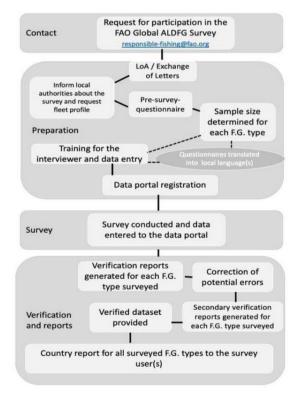


Figure 2. Process for implementation of the FAO Global ALDFG Survey (GESAMP, 2021).

According to data from the Turkish Ministry of Agriculture and Forestry dated April 24, 2023, efforts from 792 sites to clean the country's terrestrial area from ghost nets have resulted in scanning an area of 103 million m², removing approximately 800,000 m² of nets, 35,000 traps, dredges, and other

abandoned fishing gears and preventing the deaths of around 2.5 million aquatic creatures that could have been trapped in these nets.

In 2014, the project entitled "Cleaning the Seas from Abandoned Fishing" Gear" was launched by the General Directorate of Fisheries and Aquaculture under the Ministry of Agriculture and Forestry of Türkiye. The project aims to remove ALDFG and raise public awareness on the issue. In later years, the project was expanded to include inland waters as well. By consulting with fishers to locate lost nets, cleanup efforts were carried out with the participation of NGOs, fishers, several municipalities, universities, and private companies. So far, activities to remove ALDFGs have been conducted in İstanbul, Kocaeli, Tekirdağ, Yalova, Balıkesir, Çanakkale, Bursa, İzmir, Mersin, Hatay, Adana, Muğla, Sinop, Konya, Isparta, Ankara, Diyarbakır, Muş, Batman, Van, and Bitlis. According to the Ministry's reports, in rivers and lakes located in Ankara, Diyarbakır, Muş, Batman, Van, and Bitlis, an area of 20,264,000 m² was scanned, and a total of 29,290 m² of nets and 10,500 traps, pots, and similar abandoned fishing tools were removed from 36 locations. Within the scope of the Marmara Sea Action Plan, cleanup operations were conducted in Balıkesir, Bursa, Çanakkale, Tekirdağ, Kocaeli, İstanbul, and Yalova. In these areas, 1,699,068 m² were scanned, and from 85 sites, a total of 211,300 m² of nets and 16 traps, dredges, and similar abandoned fishing gears were removed from the waters. In 2022, compared to 2021, the project achieved a 254.8% increase in the removal of ghost nets and a 158.5% increase in the recovery of traps, pots, and other fishing tools (Ministry of Agriculture and Forestry, 2023).

In this context, the Circular Economy (CE) approach provides an essential framework for steering the fisheries sector away from the traditional linear "produce–use–dispose" model (figure 3). CE not only redefines waste management but is also conceptualized as an alternative economic system that seeks to integrate economic activity with environmental well-being in a sustainable manner (Díaz Enríquez, 2022; Janiszewska et al., 2025). This approach necessitates a shift from conventional disposal and storage methods in the End-of-Life (EOL) management of fishing gear toward recycling and alternative reuse strategies.

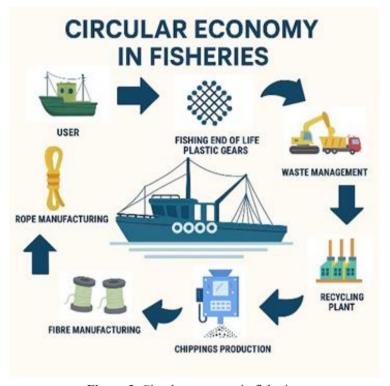


Figure 3. Circular economy in fisheries.

This study emphasises the impacts of ALDFG on marine ecosystems, explores the underlying causes of this issue, and discusses potential solutions within the framework of circular economy principles. In addition, it evaluates the technologies and practices related to the recycling and reuse of fishing gear, highlighting the challenges and opportunities in this area.

Ghost Fishing and Its Impacts on Marine Ecosystems

One of the most significant environmental impacts of ALDFG is the phenomenon known as "ghost fishing." Ghost fishing occurs when lost or abandoned fishing gear continues to catch marine organisms in the ocean (Brown et al., 2005).

According to a report by WWF (2024), ghost nets are the *most deadly* form of plastic waste for marine life and can go on killing marine creatures for decades or even centuries after entering the ocean. This issue affects 66% of marine mammal species, half of all seabird species, and all sea turtle species.

The problem is especially severe for passive fishing gears such as gillnets, trammel nets, pots, and traps. Dramatic images of fish and other marine animals entangled in lost nets highlight the wasteful and destructive nature of this problem (Brown et al., 2005).

The consequences of ghost fishing are devastating. Plastic fishing gear can smother coral reefs and entangle a wide range of species—from small fish to large marine mammals, turtles, and seabirds. Many animals mistakenly ingest small plastic fragments, leading to blockages, internal injuries, and starvation. Moreover, as plastics break down into microplastics, they attract toxins on their surfaces and eventually enter both marine and human food chains (GGGI Newsletter, 2024).

According to Richardson et al. (2022) in their study "Global Estimates of Fishing Gear Lost to the Ocean Each Year," about 2% of all fishing gear used annually is lost to the ocean. These losses correspond to approximately 2,963 km² of gillnets, 75,049 km² of purse seine nets, 218 km² of trawl nets, 739,583 km of longlines, and over 25 million pots and traps. The study also notes that bottom-contact fishing gears have higher loss rates than others—for example, an average of 3.94% of all bottom trawl nets and 0.76% of midwater trawl nets are lost each year. These figures underscore the global scale and severity of the problem.

Establishing circularity in fishing gear management requires the implementation of strong policy mechanisms and Market-Based Instruments (MBIs) (EU, 2020; Sala & Richardson, 2023) (table 1). These tools aim to promote systemic change by assigning responsibilities to manufacturers, fishers, and waste management organizations, ensuring that environmental accountability is built into every stage of the fishing gear lifecycle.

Table 1. Market-Based Instruments (MBI)

Extended Producer Responsibility (EPR) schemes

Deposit-refund schemes

Buy-back programmes / Reward schemes

Environmental taxes (to raise revenue to support recycling logistics and infrastructure) Certification schemes

Indirect (also known as 'no-cost' or 'no-special fee') port waste fees

Extended Producer Responsibility (EPR)-Based Models

Extended Producer Responsibility (EPR) is a type of Market-Based Instrument (MBI) that requires producers to take full physical and financial responsibility for the entire life cycle of the products they place on the market in this case, fishing gear and its components particularly for their EOL management. The main objective of this responsibility is to shift the cost and burden of waste management away from fishers, local authorities, or public budgets and onto the producers who design and manufacture these products (Sala & Richardson, 2023).

Mechanism and Objectives of Extended Producer Responsibility (EPR):

- 1. Transfer of Responsibility: EPR assigns financial and physical responsibility to fishing gear manufacturers for the collection and recycling of their products. This often requires producers to establish and operate a collective recycling program through a Producer Responsibility Organization (PRO).
- 2. Encouraging Circular Design: Since producers are responsible for the end-of-life costs (collection, transport, and processing), they are financially motivated to design more durable, recyclable, and resource-efficient fishing gear.
- 3. Generating Funding: The revenue collected through EPR schemes (in the form of producer fees) is used to support the infrastructure for collection, pre-treatment, transportation, and final recycling, particularly at ports and landing sites.

When EPR systems are implemented collectively through a PRO, the roles and responsibilities of relevant stakeholders are typically outlined as shown in table 2.

Table 2. Key stakeholder roles and responsibilities of EPR.

Stakeholder	Primary roles and responsibilities				
	Assume physical and financial responsibility for				
Producers/Manufacturers	EOL management of their products (fishing gear).				
	Pay PRO fees. Design more recyclable products.				
	To collectively establish and operate a scheme for				
Producer Responsibility	the collection, transportation, and processing of EOL				
Organization (PRO)	equipment on behalf of producers.				

Government/Regulators	To develop, implement, and monitor EPR regulations.				
Port Authorities/Local	To provide collection points (facilities) for collecting				
Governments	and pre-processing EOL equipment from fishers.				
Fishers/Users	To responsibly return EOL and collected ALDFG to designated collection points.				
Recycling Businesses	To process the collected fishing gear and produce clean raw materials (recycled pellets) for new products.				

EPR policies and schemes provide governments and policymakers with a range of financial instruments to enhance a country's or region's recycling and waste management standards. They also ensure that producers assume full physical and financial responsibility for the EOL management of their products including all parts and components of fishing gear (Sala & Richardson, 2023). The key advantage of this responsibility is that it offers a strong incentive for producers to design fishing gear that is easier to recycle, more resource-efficient, and has improved functionality.

A concrete example of how EPR can be applied to fishing gear at a regional scale is the European Union's Single-Use Plastics Directive (EU) 2019/904 (2019). Under this directive, manufacturers of fishing gear containing plastic are required to cover the costs of collection, transportation, processing, and awareness-raising activities (EU, 2020). The funds collected through EPR schemes provide governments and policymakers with important financial resources to raise recycling and waste management standards, supporting collection, pre-treatment, transportation, and final recycling infrastructure especially at ports and landing sites.

The IUCN and related stakeholders have offered several key recommendations to ensure the effective establishment of EPR systems. These include:

• adopting a phased approach,

- clarifying legal terminology related to EPR, and
- designing a coordinated implementation framework at national, local, and international levels (Sala & Richardson, 2023).

The success of such initiatives depends on developing EPR models tailored to local conditions and to the unique life cycle of fishing gear.

Deposit-Return and Buy-Back Programs

Deposit-return systems are a type of MBI that require consumers to pay a deposit when purchasing a product, which is refunded when the product is returned at the end of its life cycle. These mechanisms have shown high success rates in the collection of EOL plastic bottles as seen in countries such as Denmark and Germany (Balcers et al., 2019). According to Brodbeck (2016), deposit-return schemes can also encourage the recycling of EOL fishing nets that might otherwise be incinerated, dumped into the sea, or poorly managed. For these systems to be effective, operational flexibility is crucial. For example, fishers should be allowed to return repaired or combined fishing gear, and there must be a sufficient number of return points available. Additionally, reward or buy-back programs such as those implemented in the Republic of Korea, where fishers receive financial compensation for bringing recovered lost nets back to port directly incentivize efforts to retrieve ALDFG (Sala & Richardson, 2023). However, these economic incentives must be implemented with caution due to potential risks associated with the Moral Hazard theory. Deposit or buy-back actions should be carefully monitored to prevent intentional loss or abandonment of fishing gear by individuals seeking financial rewards. This potential for misuse highlights the need for transparent and traceable monitoring systems (such as tracking and record-keeping mechanisms) to ensure the effectiveness and integrity of these incentive-based programs.

Plastic Pollution and Microplastics

Since a large proportion of fishing gear is made from plastic materials, ALDFG significantly contributes to the global plastic pollution problem. Plastics can persist in the marine environment for decades without degrading and gradually break down into microplastics. These microplastics can be easily ingested by marine organisms and eventually enter the human food chain, posing serious risks to both marine ecosystems and human health (Cole et al.,

2011; Rochman et al., 2015; Kühn & Van Francker, 2020; Ocean Conservancy, 2022; Richardson et al., 2022).

According to studies conducted by the European Chemicals Agency (ECHA), microplastics intentionally added to products such as cosmetics, detergents, and paints, as well as those unintentionally released from tires and textile fibers, represent a significant environmental threat (Hill, 2023).

Reducing microplastic pollution in the oceans is therefore closely linked to the circular management of fishing gear, which emphasizes reuse, recycling, and responsible end-of-life handling within the framework of CE principles.

CIRCULAR ECONOMY APPROACH AND FISHING GEAR

The traditional linear economy model is based on the principle of extracting natural resources, producing goods, consuming them, and then discarding the waste. This approach leads to rapid resource depletion and high levels of pollution. In contrast, the CE seeks to emulate the natural cycles of ecosystems by eliminating waste, keeping products and materials in use for as long as possible, and restoring natural systems (Veronesi Burch et al., 2019; Çetin & Yılmaz, 2021).

In the fisheries and aquaculture sector, the circular economy offers a more holistic and comprehensive vision of sustainability. While it includes traditional priorities such as preventing overfishing and reducing pollution, it also considers the environmental impact of all materials in the supply chain including plastics, metals, fuels, gases, and other inputs (Cunningham et al., 2022).

Principles of the Circular Economy and implementation to the Fisheries Sector

The circular economy encompasses several key principles that promote sustainability throughout the life cycle of fishing gear:

 Eco-Design: Designing products to minimize their environmental impact during production and use. This includes material selection, product lifespan, and recyclability (Veronesi Burch et al., 2019). For fishing gear, eco-design promotes the use of durable, repairable, and recyclable materials (Ecos, 2024).

- Using Waste as a Resource: Reusing production waste and byproducts as new resources, either within the same sector or in other industries. For example, fish processing waste can be repurposed as fertilizer or animal feed (Cunningham et al., 2022).
- Circular Use and Reuse: Extending product life through sharing, repairing, and reusing. Fishing gear can be used multiple times under circular business models that encourage maintenance and refurbishment (Ecos, 2024).
- Material Recycling: When products reach the end of their life cycle, materials should be recycled and reintegrated into new production processes (Veronesi Burch et al., 2019). This principle is particularly critical for plastic-based fishing gear, given its high environmental persistence.

Preventing Gear Loss and Enhancing Circularity

The main causes of fishing gear loss include adverse weather conditions, interactions with marine life, and entanglement with seabed obstacles. In addition, operational and behavioral factors play a role such as poor maintenance and lack of crew training in gear management. Training programs and better maintenance practices have been identified as the most effective measures for preventing gear loss (Richardson et al., 2022).

The Environmental Coalition on Standards (ECOS) advocates for robust and mandatory policy solutions to improve gear design, extend product lifespan, and ensure proper end-of-life management. These initiatives contribute to reducing Abandoned, Lost, or Discarded Fishing Gear (ghost gear).

In the European Union, new standards are being developed under the EN17988:2024 series, focusing on improving the circularity of fishing gear. These standards aim to significantly reduce plastic waste and promote sustainable material use in the fisheries sector (Ecos, 2022).

CIRCULAR BUSINESS MODELS AND INNOVATIVE SOLUTIONS

The circular economy offers significant opportunities for new business models and innovative solutions in the fisheries sector. These models aim to maintain the value of fishing gear for a longer period through repair, reuse, and recycling into new products (Ecos, 2024).

One notable example is AQUAFIL's ECONYL® Regeneration System, a chemical recycling process that produces 100% nylon 6 from post-consumer waste such as fishing nets and carpet scraps made of polyamide 6. This system saves approximately 70,000 barrels of oil and generates 80% less CO₂ emissions compared to virgin nylon 6 made from petroleum-based raw materials (Compobasso, 2002). Such innovative approaches can create new and unexpected funding opportunities for the fishing industry.

In recent years, significant technological advancements have been made in the recycling and reuse of fishing gear. New methods have been developed for collecting and recycling ghost nets from the oceans, and the environmental benefits of these methods are being widely studied. At the same time, international collaborations and projects aimed at reducing marine plastic pollution are increasing. For example, the Blue Circular Economy Project seeks to transform discarded fishing gear into business opportunities, contributing directly to circular economy goals.

Recycling fishing gear helps achieve more sustainable fisheries by preserving and extending the use of valuable materials and components, encouraging the recovery of damaged or end-of-life equipment, and providing alternatives to landfilling (Sala & Richardson, 2023).

Circularity Potential of Different Fishing Gear Types

Not all fishing gears have the same recycling or circularity potential. A Multi-Criteria Decision Analysis (MCDA) study by Havas et al. (2024), which evaluated the end-of-life circularity potential of six commercial fishing gears in Norway, highlighted these differences. The study assessed environmental sustainability, economic return, and technological feasibility. Results showed that purse seines had the highest circularity potential, followed by trawl nets and Danish seines, mainly because these gear types are typically larger in volume and less contaminated, making them technically easier to recycle.

Conversely, gillnets, longlines, traps, and pots were identified as the most challenging gear types to manage under circular economy principles. These findings are essential for guiding policy and management strategies for instance, EPR policies and ALDFG recovery efforts should focus primarily on the highest-risk gear types.

A global risk assessment by Gilman et al. (2021) found that set or anchored gillnets and drifting or anchored Fish Aggregating Devices (FADs) used in tuna purse seine fisheries pose the greatest global risk of generating ALDFG.

Implementation Challenges

- <u>Data Gaps:</u> A lack of accurate data on the quantity, type, and distribution of lost, abandoned, or discarded fishing gear remains a major obstacle to building an effective circular economy system. For example, the widely cited estimate that 640,000 tons of fishing nets enter the ocean each year is considered an uncertain and difficult to track figure (Grimstad et al., 2023). Reliable data are crucial, especially for understanding material flows at local and regional scales.
- <u>Logistical Challenges:</u> Collecting and transporting end-of-life fishing gear from remote regions to recycling facilities is a significant logistical barrier. In areas such as Greenland, fishing nets often accumulate in local storage areas because there are no nearby recycling facilities, and each municipality is responsible for its own waste management. Transporting these materials to Europe carries substantial financial, carbon, and political implications (Grimstad et al., 2023).
- Recycling Infrastructure: Although most modern fishing gear is made from recyclable plastics such as polyethylene, polypropylene, and polyamide, there are still relatively few facilities capable of processing these materials effectively in their natural form. Due to their shape and texture, fishing gear can entangle processing machinery, making them incompatible with facilities designed for consumer plastics (Grimstad et al., 2023).

• <u>Legislation and Incentives</u>: The absence of clear and consistent regulations and incentives to support circular economy practices slows the sector's transition. Measures such as green public procurement and minimum recycled content requirements could play a key role in accelerating the shift toward circularity (Compobasso, 2002).

Opportunities and Proposed Solutions

Despite the challenges outlined above, there are significant opportunities for implementing circular economy practices in the management of fishing gear. These opportunities can help promote sustainable resource use, reduce pollution, and strengthen the economic and environmental resilience of the fisheries sector.

a) Local Solutions and Transnational Coordination

In remote regions, developing local solutions or ensuring transnational coordination is essential to achieve a more circular economy for fishing nets and ropes. Localized approaches can often be more sustainable than alternatives that require long distance transportation, which involves high financial costs and carbon emissions (Grimstad et al., 2023).

Programs such as Nofir-Bringing Value to Marine Waste represent effective transnational collection systems that gather fishing nets from around the world for recycling, reuse, and energy recovery (Grimstad et al., 2023).

b) Stakeholder Collaboration

The successful recycling of fishing gear depends on the active participation of a wide range of stakeholders, including policymakers, regulators, fishing gear manufacturers, distributors, fishers, port authorities, waste management agencies, and recycling enterprises. Strong collaboration ensures that these actors can support one another's efforts and share responsibilities effectively across the entire value chain (Sala & Richardson, 2023).

c) Innovative Technologies and Business Models

Several types of recycling processes can be applied to fishing gear:

- Primary recycling (direct reuse),
- Secondary recycling (mechanical recycling),
- Tertiary recycling (chemical recycling, recovery, or thermal conversion), and
- Quaternary recycling (energy recovery).

Advancing and implementing these technologies can transform waste fishing gear into valuable secondary raw materials.

Moreover, innovative circular business models such as Product-as-a-Service where services and outcomes are sold instead of the physical product can extend the use phase of fishing gear. Under such models, manufacturers retain ownership of the gear, which incentivizes durability, repairability, and recyclability (Compobasso, 2022).

d) Awareness and Education

Raising awareness among fishers, port authorities, and the general public about the impacts of ALDFG and the benefits of circular economy solutions is crucial. Education and outreach programs can encourage active participation, helping local communities and industry stakeholders become part of the solution.

CONCLUSION AND RECOMMENDATIONS

ALDFG represents a major component of global marine pollution, posing severe threats to marine ecosystems and human health. Issues such as ghost fishing, biodiversity loss, and microplastic contamination highlight the scale of environmental damage caused by derelict fishing gear. The unsustainability of the traditional linear economy model makes it imperative for the fisheries sector to adopt circular economy principles.

The circular economy offers a holistic approach that emphasizes resource efficiency and waste reduction throughout the entire life cycle of fishing gear from design and production to use and end-of-life management. Principles such as eco-design, waste valorization, reuse, and recycling form the foundation of these strategies. Innovative systems like AQUAFIL's ECONYL®

Regeneration System demonstrate the potential to derive high-value products from discarded fishing nets.

However, the absence of clear and consistent legislation and supportive incentives has slowed the transition of the fisheries sector toward circularity. Policies such as green public procurement and minimum recycled content requirements could play a key role in accelerating this transition (Compobasso, 2022). At the global level, organizations such as the United Nations Environment Programme (UNEP) and the International Maritime Organization (IMO) have developed initiatives and guidelines addressing ALDFG. For example, the IMO is working to establish international standards for fishing gear marking and tracking.

At the national level, many countries have implemented action plans and legal frameworks to combat ALDFG. In Türkiye, various regulations and projects are being carried out to prevent marine pollution and improve fisheries waste management (Compobasso, 2022; Hill, 2023). Such measures enhance producer responsibility and strengthen recycling infrastructure, thus promoting the adoption of circular economy principles.

Despite progress, several challenges remain including data gaps, logistical difficulties, insufficient recycling infrastructure, and regulatory shortcomings. Overcoming these barriers requires localized solutions, crossborder cooperation, inclusive stakeholder engagement, and investment in innovative technologies.

Recommendations

- 1. Development of Data Collection and Monitoring Systems: Establish comprehensive data systems to record the quantity, type, and sources of ALDFG. Reliable data are essential for designing targeted intervention strategies and tracking progress.
- 2. Eco-Design and Sustainable Material Use: Encourage the use of durable, repairable, recyclable, and environmentally friendly materials in the design phase of fishing gear. Standards such as the EN17988:2024 series developed in the European Union can serve as useful guidelines.
- 3. Strengthening Recycling and Recovery Infrastructure: Increase investments in facilities for the collection, sorting, and recycling of

- fishing gear. In remote areas, mobile or small-scale recycling units should be supported to reduce transport costs and emissions.
- 4. Regulatory Frameworks and Incentive Mechanisms: Effectively implement EPR schemes to ensure that producers are held accountable for the end-of-life management of their products. Additionally, provide financial incentives for businesses that adopt circular economy practices.
- 5. Education and Awareness Campaigns: Conduct awareness and education programs among fishers, port operators, NGOs, and the public on the environmental impacts of ALDFG and the benefits of circular economy solutions. Such initiatives encourage public participation, promote responsible fishing practices, and enhance reporting and retrieval of lost gear. Global initiatives such as the Global Ghost Gear Initiative (GGGI) play a key role in facilitating knowledge exchange and developing collaborative solutions (GGGI Newsletter, 2024).
- 6. Stakeholder Collaboration: Strengthen cooperation among policymakers, regulators, gear producers, distributors, fishers, port authorities, waste managers, and recycling companies. Robust collaboration enables stakeholders to mutually reinforce each other's efforts (Sala & Richardson, 2023). Successful local examples include coastal cooperatives and NGO partnerships that collect and recycle lost nets, generating both environmental and economic benefits.
- 7. International Cooperation: Since ALDFG and marine plastic pollution are global issues, international collaboration and harmonization of standards are essential. Joint projects between countries can lead to more coordinated and effective solutions.

Implementing these recommendations will help reduce pressures on marine ecosystems, enhance the sustainability of the fisheries sector, and support the goal of achieving cleaner and healthier oceans for future generations.

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CHAPTER 6

INVASIVE NON-NATIVE SPECIES AND BIOSECURITY IN MARINE DIVING

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INTRODUCTION

Invasive non-native species (INNS) are plants, animals, pathogens and other organisms that are alien to an ecosystem, and which may cause economic or environmental harm or adversely affect human health. Invasive alien species introduced and/or spread outside their natural habitats, have affected native biodiversity in almost every ecosystem type on earth and are one of the greatest threats to biodiversity (CBD, 2009). Alien marine species may be introduced into new geographic areas in a number of ways. For example, they may be introduced deliberately for fisheries and other purposes. However, many are introduced unintentionally, for example via ballast water or biofouling, equipment associated with fishing, mariculture or marine diving (GISP, 2008).

Marine wildlife tourism that involves recreational activities and interaction with diverse organisms in underwater ecosystems has grown globally over the last decades. Recreational diving is one of the fastest-growing types of marine wildlife tourism activities, with thousands of new practitioners trained in SCUBA diving each year (Giglio et al., 2020). Marine diving equipment can act as a vector for the introduction and spread of invasive nonnative species (INNS) in marine environments. Diving equipment has many places where pieces of seaweed can easily become entangled and lodged. Many algal pests have the ability to survive without emersion for several days if conditions remain damp, and can regenerate from small fragments. Diving tourism often involves visits to several divergent locations within 2-4 days, and thus diving gear can act as vectors in secondary pathways that enhance the spread of algae (Kinloch et al., 2003).

Biosecurity in marine diving is the general term for measures taken to decontaminate equipment and is an important element of management strategies aimed at preventing the spread of INNS (Bradbeer et al., 2020). Scuba divers and snorkelers can inadvertently transfer species between waters when traveling with dive equipment. All species of algae, aquatic plants, and animals, including microscopic larvae, can be transferred in this way. Therefore, precautions should be taken to reduce the risk of INNS transmission, especially when diving in different waters on the same or repeated days.

2. WORLD MARINE DIVING SECTOR

Marine diving industry is a field in which underwater activities and activities are conducted, either with or without equipment, for recreational or professional purposes. Recreational diving, also known as diving tourism, is a sector that stands out as an alternative to traditional tourism and is growing in popularity (Güney Marmara Kalkınma Ajansı, 2020). Scuba diving tourism is an increasingly popular global industry that supports local economies and promotes marine conservation. The scuba diving industry is estimated to generate annual revenues of between US\$8.5 billion and US\$20.4 billion, while supporting up to 124,000 jobs worldwide (Figure 1) (Schuhbauer et al., 2025).

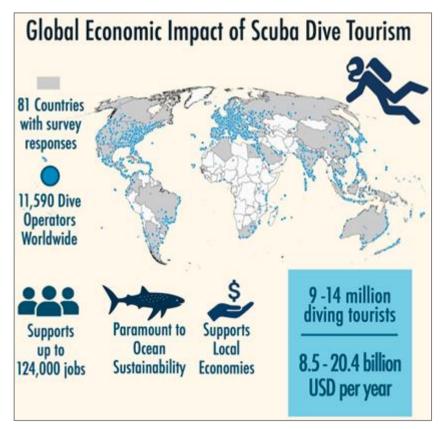


Figure 1. Global Economic Impact of Scuba Dive Tourism.

There are 11,590 dive operators worldwide. An estimated 8.9–13.6 million divers and snorkelers worldwide participate in offshore diving tourism

activities (Schuhbauer et al., 2025). One of the largest global certification agencies for scuba divers, the Professional Association of Diving Instructors (PADI), issued more than 28 million dive certifications between 1968 and 2020, and certifies an additional 1 million new divers each year. Important diving centers in the world are presented in Figure 2 (Cabral, et al., 2025).

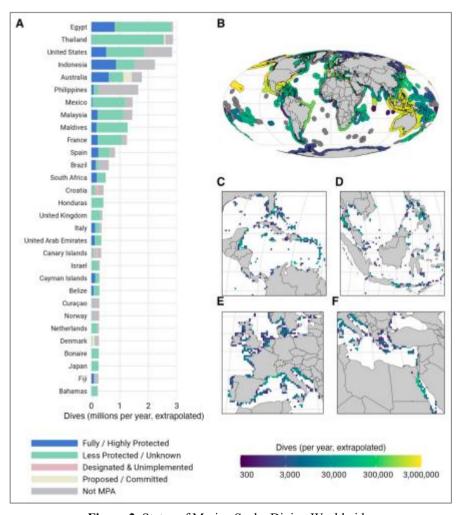


Figure 2. Status of Marine Scuba Diving Worldwide.

3. INVASIVE NON-NATIVE SPECIES IN MARINE DIVING

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). Therefore, species that are transported and established in new habitats via diving equipment during marine dives are considered invasive alien/non-native species when they have biodiversity, ecosystem services, human health or economic impacts.

Wetsuits and equipment provide numerous locations where seaweed fragments, including microscopic algae, can easily become entangled and lodge. Many harmful micro- and macroalgae can survive for several days under moist and favourable conditions. They can also regenerate from small fragments under similar conditions. Dive tourism and activities typically involve visits and/or dives to several different locations over a period of 2–4 days. Therefore, wetsuits and equipment can act as secondary vectors, increasing the introduction/translocation or spread of algae and/or other invasive marine organisms (GISP, 2008).

PADI (The Professional Association of Diving Instructors) has informed its members about five significant invasive non-native species (Figure 3). These species are: The Lionfish (*Pterois volitans*), which is native to the Pacific Ocean and causes serious problems in the Caribbean and contributes to the destruction of coral reefs; The Shore Crab (*Carcinus maenas*), which causes economic damage to aquaculture and fisheries; The Northern Pacific Seastar (*Asterias amurensis*), which is carried in ship ballast water and causes ecological damage; The Rainbow Trout (*Oncorhynchus mykiss*), which can spread disease and feed on native fish; and The Asian Clam (*Corbicula fluminea*), which can cause the collapse of commercial fishing operations (PADI, 2025).

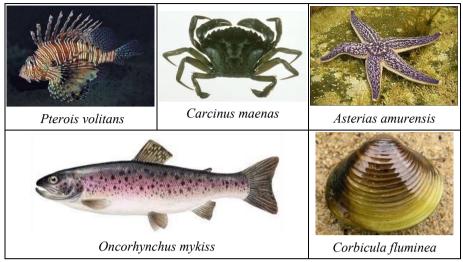


Figure 3. Five of the Most INNS Marine Species

The alien freshwater amphipod of Ponto-Caspian origin, *Dikerogammarus villosus*, also known as the killer shrimp (Figure 4), is recognized as being one of the worst invasive alien species in Europe, representing a major conservation problem. Recently, the species has been reported to invade lakes in the Alps in putative association with overland transport linked with recreational activities. Moreover, the species is able to survive up to three and a half days out of water, between the layers of diving wetsuits (Bacela-Spychalska et al., 2013).



Figure 4. *Dikerogammarus villosus* (Killer Shrimp)

4. BIOSECURITY IN MARINE DIVING

Biosecurity in Marine Diving refers to all precautions to be taken with diving suits and equipment to minimize the risk of transmitting or spreading INNS during sea diving (Bradbeer et al., 2020). Accordingly, to put it more clearly, biosecurity in marine diving or water sports activities, whether with or without equipment, regardless of the purpose, includes all measures that may cause the introduction/transport or spread of non-native or invasive non-native species and the elimination of such creatures from diving equipment.

The overriding principle is that prevention is better than cure, and the key to success in this approach is awareness, education, and training, which play a crucial role in combating invasive alien species. Divers, whether scuba diving, non-scuba diving, training, scientific diving, professional diving (industrial diving), or recreational diving, can unintentionally become vectors for invasive alien species due to contact with water and prolonged exposure to water through the diving equipment or suits they use. Equipment includes, in addition to the diving suit, the boats and engines used during the dive.

For biosafety purposes in marine diving and boat and trailer use:

- Divers should be aware of IYT and participate in training programs designed for this purpose.
- Whenever possible, divers' entry and exit points in dive sites should be limited, preferably to a single point or location.
- If possible, boats and equipment should be provided at the dive site and used in place of personal equipment brought from off-site.
- Biofouling must be completely removed from all hulls before transfer to another site.
- Water accumulated in bilges or hulls must be completely drained before leaving the site.
- Water-cooled engines must be flushed with tap water.

4.1. The "Check, Clean and Dry" Protocol

To date, it has not developed a specific guide or code of conduct for marine diving. Instead, it has published the "Code of Conduct for Recreational Fishing and Invasive Alien Species" (Counsil of Europe, 2014) in a general framework. It has adopted "Check, Clean, Dry" procedures based on public participation, education, awareness-raising, and voluntary work.

Check:

All clothing and equipment (Figure 5) should be thoroughly inspected, and any visible debris (mud, plant matter, or animal matter) should be removed and returned to the body of water where it was found. Pay particular attention to the seams of clothing, boots, and waders. Where this is not possible, drain the accumulated water from a water body (stream, lake, river, canal, etc.) to a point containing an absorbent surface (grass, gravel, etc.) at least 30 m away from the structures.

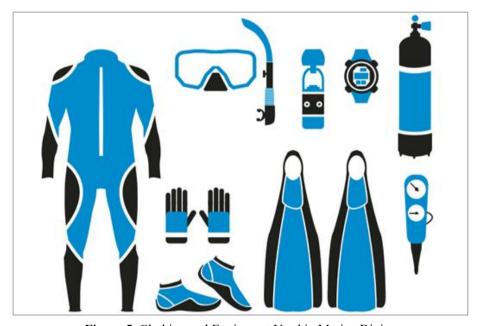


Figure 5. Clothing and Equipment Used in Marine Diving

Clean:

Clothing and equipment should be washed in freshwater on site. Washing waste should not be transferred to another river or drainage system. Used clothing should be washed inside and out in 45°C hot water and soaked in the same hot water for 15 minutes. For marine diving, wash the diving suit in a 5% dishwashing detergent solution and leave it for 30 minutes (Figure 6) (Outsiderview, 2024). Use local water source initially to help remove heavy biofouling (U.S. Fish and Wildlife Service, 2018). Do not wash your diving suit with pressurized water or hot water of 60 °C, as this may damage the seams and joints (U.S. Environmental Protection Agency, 2016). Soak your diving suit

and equipment in a solution prepared with 2-3% household bleach for disinfection purposes for 15 minutes (USGS, 2016). Pay particular attention to cleaning the seams and folds of your diving suit. Use wetsuit shampoos for cleaning. Keep at least 15 L of fresh water in your vehicle.



Figure 6. Cleaning Diving Equipment with 5% Detergent Solution

Dry:

To kill small species that are not easily seen, dry all equipment for at least 5 days or more. If drying is not possible, wipe dry thoroughly and carefully with a towel before reuse. Be aware that some invasive alien species may have microscopic life stages and are not visible to the naked eye (Trujillo, 2021; Sailor for the Sea, 2025).

4.2. Marine Diving and Citizen Science

Citizen science is scientific endeavours conducted in whole or in part by amateur or non-professional scientists whose information is sourced or funded by the public. Citizen science is increasingly used to understand the distribution of INNS and is emerging as a useful tool for early detection. It can also expand the spatial and temporal scale of alien species records (Costello and Trottet,

2023). Monitoring studies provide data on ecosystem changes caused by biological invasions (Figure 7) (Mangelli et al., 2021).



Figure 7. Marine Diving a nd Citizen Science

Divers' willingness to participate in citizen science activities to conserve biodiversity and monitor invasive fishes is a motivating activity for environmentally conscious divers (Carballo-Cardenas and Tobi, 2016). Properly guided exploration transforms divers' dives into useful data collection activities. This could create an additional market for the diving industry and means that scientists can obtain important information needed to monitor and track marine ecosystems at little or no cost. Over the past decade, the use of citizen science has increased, exceeding the number of new species recorded through INNS monitoring efforts that would otherwise be economically or logistically feasible by professional researchers alone. Such studies allow for earlier detection of species and range expansion observations, essential for rapid and effective management (Anderson et al., 2017).

Data recorded by recreational divers have helped inform and inform policy changes regarding the location of marine protected areas and have made significant contributions to the monitoring of unevenly distributed INNS species (Hyder et al., 2015). Citizen science initiatives are promising for recording and monitoring marine INNS. In Florida, fishermen and recreational divers documented lionfish infestations 1–2 years ago during traditional coral reef monitoring programs (Scyphers et al., 2015). In the Mediterranean, recreational divers participating in the "Seawatchers" citizen science project were the first to detect the sergeant major *Abudefduf saxatilis* (Linnaeus, 1758), a small fish native to the tropical Atlantic (Azzurro et al., 2013). In Greece, recreational divers, underwater photographers, and fishermen together recorded 28 alien marine species (subsequently confirmed by taxonomists) in 2012 (Zenetos et al., 2013).

Marine Protected Areas (MPAs) help restore biodiversity and improve fisheries. It is estimated that 33.1 million scuba dives are made in marine environments worldwide each year, 70% of which occur in MPAs (Cabral, et al., 2025). Studies have been conducted in recent years on the introduction or transportation of INNS into marine protected areas and the risks of its establishment. Therefore, collaboration with relevant diving clubs, boat owners, and yachtsmen is crucial for new INNS detections (IUCN, 2012).

5. CONCLUSION

- Marine diving is considered one of the vectors that plays a role in the transmission or spread of invasive non-native species (INNS).
- Marine diving is also a source of crucial field data for the early detection and distribution mapping of INNS through citizen science.
- To encourage responsible marine diving, awareness of regulations should be fostered among divers, managers, policymakers, and other stakeholders through targeted information, education, and training.
- Special attention should be paid to biosecurity and INNS identification / detection and reporting procedures in sea dives.
- All divers have a favorite location and are generally familiar with the local/endemic species in the area. Potential INNS guides can easily detect if they are readily available and provided.
- Adequate signage or guidance should be provided to dive schools, centers, or clubs to inform them of the risks of INNS and advise on how to prevent its spread.

- If divers can identify INNS, it is an important factor in combating INNS.
- Awareness and education programs aimed at informing about INNS in sea diving should be conducted in collaboration with experts.
- Government institutions and officials should collaborate closely with divers and their affiliated federations, institutions, and organizations on INNS-related programs.
- Citizen Science and diver collaboration should be established and ensured in studies related to INNS.
- In cooperation with relevant government agencies and national/international diving centres, the Code of Conduct (Guidelines) for INNS should be established and reviewed periodically and as appropriate, taking into account new developments in INNS.

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CHAPTER 7

BIOGEOGRAPHY AND CONSERVATION STATUS OF KILLIFISH (CYPRINODONTIFORMES) IN TURKIYE

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INTRODUCTION

The name "killfish" is generally used as a general description for species of the order Cyprinodontiformes, which are small in size and oviparous (Nelson, 2006). These species are widely distributed in both freshwater and brackish water ecosystems worldwide and are notable for their high adaptability and distinct physiological tolerance to environmental change (Parenti, 1981; Hrbek and Meyer, 2003). The term "killfish" derives from the Dutch word "kil" or "kill," meaning "small stream." This term emerged as a local expression used by Dutch immigrants to North America to describe fish living in small channels. Over time, it became widespread in the literature and gained its current usage (Huber, 2011).

It is reported that many species belonging to genera such as Nothobranchius, Aphyosemion, Fundulopanchax, Epiplatys, Cyprinodon and Aphanius, which are included in Cyprinodontidae and related killifish groups, are widely preferred in ornamental fish due to their bright colors, distinct sexual dimorphism and rare color variants (Dodzian et al., 2018; Tozzini and Reichard, 2020). Because most of these species are small, relatively peaceful, and prefer still or gently flowing waters, they can be kept with small characins, rasboras or shrimp species with similar ecological requirements. However, due to the territorial defense and splashing behavior commonly observed in many killifish species, single-species aquaria are often considered a safer option (Dodzian et al., 2018). While maintenance difficulties vary significantly among species, annual killifish species such as Nothobranchius require advanced care and experience due to the obligatory diapause phase development. In and during egg contrast, Cyprinodon Rivulus/Kryptolebias species are considered more suitable for beginner aquarists due to their higher environmental tolerance and relatively low maintenance requirements (Tozzini and Reichard, 2020). Many killifish species are highly adapted to planted aquariums, as they naturally inhabit shallow, low-flow habitats densely populated with aquatic plants. Aquatic plants in these environments contribute to the reduction of behavioral stress by providing hiding places and serve as suitable spawning substrates (Tozzini & Reichard, 2020). The diversity of color patterns, the collection value of endemic species, the challenges of breeding protocols, and the fact that some species are currently captured only from limited wild populations contribute

to the high economic value of killifish in the aquarium industry. Furthermore, their potential as model organisms in biological and evolutionary developmental studies further enhances their value (Teletchea et al., 2016; Anjur et al., 2021).

Killifish species are taxonomically classified within the order Cyprinodontiformes, which includes the families Aplocheilidae, Fundulidae, Cyprinodontidae, and Aphaniidae (Wildekamp, 1999; Esmaeili et al., 2014). Turkey exhibits a remarkable level of endemism, particularly due to the abundance of species belonging to the genus Aphanius, the main members of the Aphaniidae family, and therefore occupies an important position for regional biodiversity (Wildekamp et al., 1999). Since Anatolia is located at a transition position between the European and Asian continents, hydrological isolations, geological differentiations and climatic changes have played an important role in increasing the genetic diversity of these species (Hrbek and Meyer, 2003; Pfleiderer et al., 2014).

Cyprinodontiformes is one of the orders within the Osteichthyes that exhibits remarkable biodiversity, both ecologically and systematically. According to current taxonomic assessments, approximately 1,250 fish species worldwide are included in this order (Parenti, 1981; Ghedotti, 2000; Nelson, 2006). Members of this order are known for their generally small size, their inhabitance of coastal areas, lake habitats characterized by marshes and reeds, and brackish and semi-saline biotopes. This group encompasses a wide range of taxonomic diversity, including killifish and livebearers. The order Cyprinodontiformes encompasses a wide range of taxonomic diversity, including many families such as Aphaniidae, Cyprinodontidae, Fundulidae, Profundulidae, Goodeidae, Poeciliidae, Nothobranchiidae, and Rivulidae. Members of this order are particularly notable for forming centers of endemism concentrated in the Afro-Asiatic regions and the Americas. These geographies are critical to the group's evolutionary and biogeographic diversity (Costa, 2013; Hrbek and Meyer, 2003).

Aphanius species in Turkey generally inhabit endorheic lake systems and saltwater or brackish water springs (Küçük and Gülle, 2008). The Lakes Region, Central Anatolia and Eastern Anatolia regions, in particular, stand out as the primary population areas where Aphanius species are distributed. The isolated lake and spring systems in these regions have led to genetic

diversification among different populations, ultimately enabling the scientific description of many new species (Pfleiderer et al., 2014; Yoğurtçuoğlu, 2017). Molecular-based phylogenetic studies conducted in recent years reveal that the number of Aphanius species in Turkey is higher than classical morphological descriptions.

Aphanius species hold a special place in Turkey's freshwater fish fauna, both in terms of their taxonomic diversity and their ecological adaptability. Therefore, determining the current distribution of these species, developing effective conservation strategies, and deciphering their phylogenetic relationships are of great importance for both scientific research and ecological management.

2. CHANGES IN NOMINAL NAMING OF KILLIFISH SPECIES DISTRIBUTED IN TURKEY

2.1. Taxonomic Classification

The taxonomic position of killifish has been subject to numerous revisions throughout history. However, increasing molecular phylogenetic studies have demonstrated that the genus Aphanius is genetically distinct from other Cyprinodontidae and should be classified within the separate family Aphaniidae (Ghedotti, 2000; Hrbek and Meyer, 2003). Currently, most taxonomic studies classify killifish in Turkey as part of the family Aphaniidae (Nelson, 2006; Esmaeili et al., 2014).

Killifish distributed in Turkey have differentiated at the species level based on morphological characteristics, pigmentation patterns, habitat preferences, and genetic data. The geological history of Anatolia, isolation between lakes, and climatic changes are prominent factors in the evolutionary diversity of these species (Hrbek and Meyer, 2003; Pfleiderer et al., 2014). As a result of these isolation processes, numerous endemic Aphanius species have emerged in Turkey.

2.2. Taxonomic History and Nomenclatural Revisions

Scientific descriptions of killifish in Turkey date back to the late 19th century. One of the first species described during this period was *Aphanius mento* (Heckel, 1843), reported from the area around Lake Damascus. In the following years, new species names were proposed and classifications

developed for populations in different geographical regions. For example, Aphanius anatoliae (Leidenfrost, 1912), representing the Central Anatolian populations, was described, and species such as Aphanius chantrei, Aphanius villwocki, Aphanius marassantensis, and Aphanius danfordii were also recognized as separate taxa following subsequent morphological and molecular analyses (Wildekamp et al., 1999; Pfleiderer et al., 2014; Yoğurtçuoğlu, 2017). Taxonomic revisions have accelerated significantly, particularly in the 2000s, with the increase in molecular phylogenetic studies. Genetic analyses of Aphanius species have revealed that traditional morphological classifications remain limited and that many populations exhibit significant genetic differences at the species level (Hrbek and Meyer, 2003). Consequently, some populations in Turkey previously considered subspecies, such as Aphanius anatoliae sureyanus, are now considered independent species (Yoğurtçuoğlu, 2017). In addition, it has been determined that some populations within the Aphanius dispar species complex diverged phylogenetically from the Middle East to Anatolia, and in this context, the Anatolian populations constitute an independent evolutionary line (Esmaeili et al., 2014). According to the International Commission on Zoological Nomenclature (ICZN) rules, the validity of scientific nomenclature for killifish species is based on the earliest description and verification of type specimens. Within this framework, the scientific names of killifish in Turkey have been revised several times over time. For example, the renamings *Lebias* mento (Heckel, 1843) - Aphanius mento (Heckel, 1843) and Lebias anatoliae - Aphanius anatoliae are among the best-known examples of this process (Wildekamp, 1999).

2.3. Taxonomic Diversity and Current Status

According to current literature data, as of 2023, Turkey's freshwater ichthyofauna comprised a total of 427 species belonging to 20 orders, 37 families and 97 genera. While 21 of these species (4.9%) were of foreign origin, 215 (50.4%) were considered endemic to Turkey. The highest species richness among Turkish freshwater fish is observed in the Cypriniformes order with 297 species (69.6%), followed by Cyprinodontiformes (26 species, 6.1%), Salmoniformes (25 species, 5.9%), Gobiiformes (20 species, 4.7%), Siluriformes (13 species, 3.0%), and Clupeiformes (9 species, 2.1%) (Çiçek et

al., 2023). There are 13 different species in the inland waters of Türkiye, especially members of the killifish (Aphaniidae) family (FishBase, 2025). A large proportion of these species inhabit ecologically isolated habitats in localities such as the Lakes Region, Central Anatolia, the Lake Van Basin, and the Aegean Region. This speciation process, supported by genetic analyses, has made Turkey an important center for the evolutionary diversity of Aphanius species (Hrbek and Meyer, 2003; Yoğurtçuoğlu, 2017).

Killifish in Turkey represent a taxonomically rich, highly endemic group of fish whose systematic history is constantly being revised. These characteristics highlight the country's biogeographic diversity and the evolutionary importance of its freshwater ecosystems. Future phylogenetic and population genetic studies will more accurately and clearly establish the taxonomic status of some currently controversial Aphanius species.

The family Cyprinodontidae, within the order Cyprinodontiformes, comprises small-sized, benthopelagic, or littoral species commonly referred to as "toothed cyprinids." Members of the family have a wide geographic distribution in temperate and subtropical climate zones, primarily in North America, Central America, the Caribbean, and Africa (Parenti, 1981; Costa, 2011). Cyprinodontidae constitutes a systematically remarkable group due to their morphological diversity, their adaptability to extreme environmental conditions (high temperature, high salinity, hypoxia), and the endemic distribution of many species (Costa, 2013).

Systematically, the family is divided into several subfamilies such as Cyprinodontinae, Cubanichthyinae, Orestiinae and Garmanellinae, and these subgroups are supported by both morphological and molecular markers in phylogenetic analyses (Parenti, 1981; Hrbek & Meyer, 2003). The increased use of molecular data in recent years has enabled the reassessment of species boundaries and the description of many new species, particularly in closed-basin endemics in the Americas (Costa, 2011). In this context, the systematics of the family Cyprinodontidae is dynamic, and phylogenetic relationships are constantly revised in light of new findings.

3. GEOGRAPHICAL DISTRIBUTION OF KILLIFISH SPECIES IN TURKEY

Turkey, thanks to its biogeographic location at the intersection of three continents, represents an important center of diversity for freshwater fishes (Kosswig, 1955; Geldiay and Balık, 2009). This is also supported by the high species richness and endemism rates of killifishes of the genus Aphanius (Cyprinodontiformes: Aphaniidae) (Wildekamp, 1999; Hrbek and Meyer, 2003). The geological history of Anatolia, orogenic movements, the isolation of lake basins, and different climatic zones stand out as the main factors shaping the genetic and morphological diversity of these species (Pfleiderer et al., 2014; Yoğurtçuoğlu, 2017).

3.1. Distribution Areas and Regional Distribution

Aphanius species in Turkey are concentrated in three main biogeographic regions across the country, from the west to the east. The regional distributions of the species are detailed below.

3.1.1. Western and Southwestern Anatolian Populations (Aegean Region and The Lakes Region)

The species *Aphanius anatoliae, Aphanius villwocki*, and *Aphanius sureyanus* are distributed in this region (Küçük and Gülle, 2008). The closed basin systems that make up the Lakes Region (Burdur, Acıgöl, Salda, Eğirdir) have allowed these species to evolve into isolated populations (Wildekamp et al., 1999). *A. sureyanus*, in particular, is endemic only to the Lake Burdur Basin (Küçük, 2012).

3.1.2. Central Anatolian Populations

In Central Anatolia, the species *Aphanius danfordii, Aphanius marassantensis*, and *Aphanius iconii* have been described. These species inhabit brackish water springs and small lakes around the Kızılırmak and Sakarya Rivers (Pfleiderer et al., 2014). *A. marassantensis*, in particular, is endemic to the Kızılırmak River drainage (Yoğurtçuoğlu, 2017). These species are also notable for their tolerance to high salinity fluctuations (Hrbek and Meyer, 2003).

3.1.3. Eastern and Southeastern Anatolia Populations

Aphanius mento, A. arakensis, and A. transgrediens are distributed in the Eastern Anatolia region. These species inhabit the Lake Van Basin, the Aras River basin, and the saltwater resources around Şanlıurfa (Freyhof & Brooks, 2011; Esmaeili et al., 2014). A. transgrediens, in particular, is endemic to the Lake Acı and is considered critically endangered due to its high adaptability to hypersalinity conditions (Hrbek & Wildekamp, 1996).

3.1.4. IUCN Status and Natural Distribution

The current Red List status of killifish species distributed in Turkish inland waters is given in Figure 1.

	Species	IUCN Red List Status
	Aphanius anatoliae	NT
	Aphanius villwocki	LC
	Aphanius sureyanus	EN
	Aphanius danfordii	CR
	Aphanius transgrediens	CR
RED° LIST	Aphanius splendens	EX
	Aphanius marassantensis	NE
	Aphanius iconii	NE
	Aphanius meandricus	NE
	Aphanius meridionalis	NE

^{*}NT: Near Threatened, LC: Least Concern, EN: Endangered, CR: Critically Endangered, NE: Not Evaluated, EX: Extinct (IUCN, 2025)

Figure 1. Current status of the IUCN Red List of Aphanius species.

The main killifish species naturally distributed in Turkish inland waters (mostly Aphanius and related genera) and their reported localities are summarized in Table 1. Species distribution and classification information was prepared based on FishBase, Killi-Data and current literature (Wildekamp et al., 2017; Çiçek et al., 2023).

Krater Lake

(Wildekamp

Adana, Sevhan

and Ceyhan Basins

(Wildekamp

Ponds and

springs around

1999)

Konya (Yoğurtçuoğlu et al., 2017)

1999)

Turkish / Main Locality / Turkish / Main Locality / Species Species Common Name Basin Common Name Basin Aphanius Anatolian toothed Eğirdir, Tuz Lake, Kızılırmak toothed Kızılırmak and anatoliae Konya Basin marassantensis Yeşilırmak basins (Wildekamp (FishBase, 1999) Wildekamp 1999) Burdur toothed Burdur Lake Fresh water A phanius Aphanius Kaynak too thed (Wildekamp 1999; fontinalis *sureyamus* resources in IUCN, 2023) Afyon (Wildekamp 1999 Acıgöl toothed Denizli-Afyon, Büyük Menderes Büyük Menderes Aphanius Aphanius transgrediens Acıgöl Lake meandricus toothed carp Basin (Wildekamp resources 1999) (FishBase, 2025) Hazar Lake (Elazığ) Aphanius Hazar toothed Aphanius Gölcük to othed Isparta – Gölcük

splendens

Aphanius

meri dional is

Aphanius iconii

carp

Southern Anatolian

Ikonium toothed

carp

toothed carp

Table 1. Killifish species naturally distributed in Turkey

asquamatus

A phanius

danfordii

Aphanius

villwocki

Sultan Marshes

Sakarya toothed

carp

toothed carp

3.2. Ecological Determinants of Distribution

(Wildekamp 1999;

FishBase, 2025)

Kavseri - Sultan

(Yoğurtçuoğlu et

al., 2017)

Marshes, Kızılırmak

Sakarya River Basin

(FishBase, 2025)

The most important ecological factors affecting the distribution of Aphanius species are water salinity, temperature, oxygen level, and habitat isolation (Hrbek and Meyer, 2003; Küçük, 2012). Most species live in shallow lakes and underground spring systems in temperate climate zones and exhibit high adaptability to changes in salinity and temperature.

3.3. Biogeographic Zoning of Species

Studies have demonstrated the distribution of Aphanius species in Turkey more clearly through geographic information systems (GIS) mapping (Yoğurtçuoğlu, 2017). These maps include the coordinates of the lakes, springs, and streams where the species live and clearly distinguish biogeographic clusters (Lakes Region, Central Anatolia, Van Basin).

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CHAPTER 8

INVASIVE NON-NATIVE SPECIES AND BIOSECURITY IN MARITIME TRANSPORT

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INTRODUCTION

Maritime transport about 85% in volume of world trade and moves an estimated more than 10 billion tonnes of ballast water globally each year (UNCTAD, 2025). Vessels are recognised as one of the primary vectors involved in the introduction or translocation of invasive non-native species. The ballast water and sediment carried by ships and biofouiling on the hull can have a devastating impact on marine ecosystems, biodiversity, human health and economy and are very difficult and expensive to tackle (Castello et al., 2022).

Global maritime transport trade has been affected by the Covid-19 pandemic in parallel with the global economy. Throughout 2020, global maritime transport trade experienced a 3.4% decline in tonnes. This decline is the first since 2009 due to the major impacts of the Covid-19 pandemic. However, total volume is projected to reach approximately 12 billion tonnes in 2021, a 4.2% increase (Table 1) (Deniz Ticaret Odası, 2021).

Table 1. World Trade and Maritime Transport

World Trade									
and Maritime	2014	2015	2016	2017	2018	2019	2020	2021	2022
Transport									
World Maritime									
Transport	3.4%	2.2%	3.1%	4.1%	2.7%	0.41%	3.8%	4.2%	3.0%
Development							3.8%		
World Trade									
Volume (billion	12.5	12.78	12.95	13.56	13.95	14.09	12.79	13.71	14.57
tons)									
World Maritime									
Transport	10.56	10.79	11.12	11.57	11.89	11.94	11.51	11.99	12.35
(billion tons)									
World Shipping	040/	84%	86%	85%	85%	85%	000/	87%	85%
Rate by Sea	04%	04%	80%	05%	05%	05%	90%	0/70	05%

International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 has been in force since September 2017. As of July 15, 2021, 86 Countries, representing 91% of the world's merchant marine fleet, have ratified it. The Convention aims to prevent the introduction,

proliferation, and spread of invasive non-native species (INNS) through untreated ballast water discharged from ships. This problem is considered one of the four greatest threats to the world's oceans and is considered to pose a significant threat to biodiversity, have environmental and economic impacts, and could cause serious public health problems if left unaddressed (UNCTAD, 2011 and 2015). Since the Convention entered into force, ships have been required to manage their ballast water according to D-1 and D-2 standards; the oldest ships are required to displace 95% of their ballast water at least in an offshore area; the second restriction imposes restrictions on the maximum amount of living organisms in ballast water discharges up to a certain level and on microbes harmful to human health. The current goal is to ensure effective and uniform implementation of the Convention (UNCTAD, 2020).

The Ballast Water and Sediments Management Convention, 2004, aims to prevent the transport and spread of potentially harmful aquatic species in ballast water. Invasive non-native species, such as marine animals, plants, and algae, can attach themselves to the exterior of ships (e.g., ship hulls) and other marine structures. This occurrence is known as biofouling. When ships and structures are moved to new areas, these species can detach, adapt to the new habitat, proliferate, spread, outcompete local fauna, and become invasive, with adverse effects on local and natural ecosystems. Therefore, biofouling is also important and must be addressed. The Control of Harmful Antifouling Systems on Ships Convention, 2001, defines antifouling systems as "a coating, paint, surface treatment, surface, or device used on a ship to control or prevent the attachment of undesirable organisms." It aims to ban the use of harmful organotin compounds in antifouling paints used on ships and to establish a mechanism to prevent the potential future use of other harmful substances in antifouling systems (UNCTAD, 2020).

This study examines the impacts of maritime transport on the introduction and transport of invasive non-native species (INNS). Biosecurity measures to address ballast water, sediments, and hull fouling (biofouling) are discussed and presented when addressing INNS for the sector.

2. INVASIVE NON-NATIVE SPECIES IN MARITIME TRANSPORT

Oceans are huge natural structures consisting of habitats separated from each other by natural barriers. Oceans are home to a wide variety of species (plants, algae, fish, microorganisms, etc.). However, some species have been intentionally or unintentionally transported to other seas/oceans (habitats) because of human activities. Non-native species transported to new habitats with similar characteristics find opportunities to adapt and thrive. If there are no natural predators in the ecosystem, some non-native species may become dominant due to their competitive advantage and disrupt the biological diversity of the new habitat. When these species have a negative impact on biodiversity, ecosystem services, human health or economic impact, they are referred to as invasive non-native species (INNS) (Carlton, 1996; GloFouling, 2025).

The introduction and establishment of INNS is considered one of the greatest threats to freshwater, coastal and marine ecosystems worldwide. The main vectors for the intentional or unintentional transfer of non-native species in the maritime sector are ships' ballast water and the biofouling of mobile offshore structures (Figure 1). In addition, ballast water and biofouling are also considered one of the main vectors for bioinvasions (GloFouling, 2025).

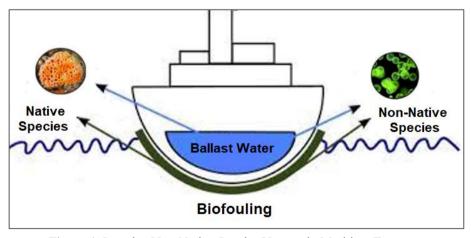


Figure 1. Invasive Non-Native Species Vectors in Maritime Transport

There is a strong correlation between known INNS levels worldwide and global coastal economic zones. Parallel to the increase in global trade in

developed countries, the fourfold increase in maritime transport over the last thirty years has brought with it the highest risk of INNS entry in regional central ports (Figure 2). While natural processes such as currents and winds previously managed alien species, today, opportunities to travel around the world have increased significantly, particularly due to maritime transport. More than 80% of the world's marine ecological regions have been affected by at least one INNS (Challinor et al., 2014; Clear Seas, 2025a)

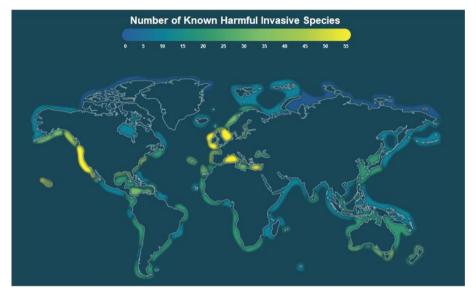


Figure 2. The Relationship between INNS and Global Coastal Economic Zones

INNS may be found freely in ballast water; in the sediment layer accumulated at the bottom of ballast tanks; and in areas of biological contamination on the hull and niche areas of ships. It is thought that more than 50% (some research estimates that up to 69%) of non-native species may have been transported through biofouling. The Figure 3 below provides a list of high-profile invasive aquatic species that are capable of being translocated via biofouling. These species are indicative and there are numerous other species involved in serious invasions, which have been recorded around the world. (IMO, 2025a).

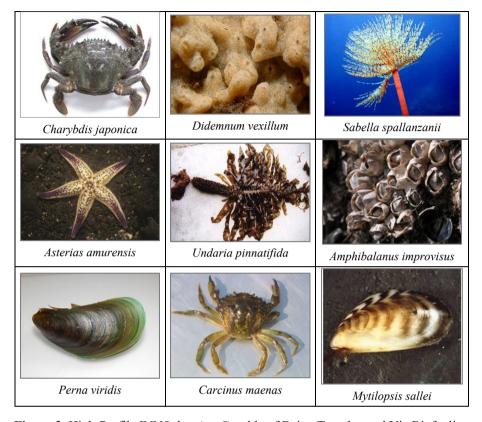


Figure 3. High-Profile INNS that Are Capable of Being Translocated Via Biofouling

2.1. What is Ballast Water and Sediment and Why is it Important?

Ballast water is seawater stored in a separate tank on board a ship to provide stable equilibrium during navigation, submerge the rudder propeller, assist propulsive power and manoeuvrability, and reduce stress on the vessel. It constitutes approximately 30-35% of a ship's carrying capacity (cargo + fuel + etc.) when unloaded. Ballast water is water that is necessary and mandatory for the safe operation of ships. It is used to compensate for the different cargo loads that a ship can carry at different times, including loading and unloading. Ballast tanks are an essential part of ship design, varying in number and size depending on the type and structure of the ship, and are used to add weight to the bottom and sides of the ship when necessary (Figure 4) (Akdoğan, 2018; David, 2015).

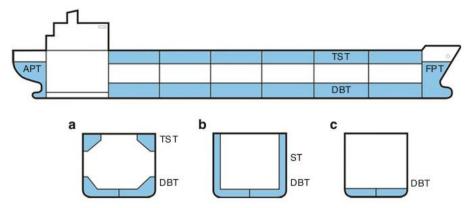


Figure 4. Ballast tanks on: (a) most bulk carriers, (b) tankers, container vessels, and some newest bulk carriers, and (c) Ro-Ro and general cargo vessels. (APT after peak tank, DBT double bottom tanks, FPT forepeak tank, ST side tanks, TST topside tanks or upper wing tanks).

Why is ballast water important?

- Ballast is an important part of ensuring the safety and stability of ships. However, ballast water (seawater) contains numerous microorganisms.
- Every year, 10 billion tonnes of ballast water are transported worldwide.
- The majority of marine species include planktonic stages in their life cycles, meaning that thousands of different marine species can be transported.
- Ballast water contains zooplankton/phytoplankton, bacteria, invertebrates and fish eggs.
- Approximately 7,000 marine species can be transported at a time.
- One cubic metre of ballast water can contain up to 50,000 zooplankton specimens.
- Marine species transported in ballast water tanks can thrive and become invasive in their new habitats.
- It is reported that a new species invasion occurs in the seas every 9 weeks.
- Invasive alien species are one of the greatest threats to global biodiversity.

- They can also cause serious problems for the economy, the environment and human health.
- Sediments can also be transferred with ballast water. These may also contain organisms (Clear Seas, 2025b; GloBallast, 2025a).

Sediment is ballast sludge, which is closely related to ballast water. When a ship takes on ballast water, it also takes on material present in the water. In turbid or shallow waters, this often contains solid material. When this material enters the ballast tank, it settles to the bottom as 'sediment' and provides a substrate for various marine species, particularly dinoflagellates (Figure 5). According to the Ballast Water Management (BWM) Convention, sediment is defined as 'material discharged from ballast water within a ship'. Sediment significantly affects the performance of ballast water treatment systems in terms of particle size and organism content (GloBallast, 2025a).

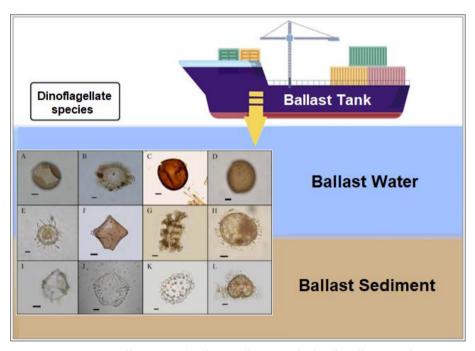


Figure 5. Ballast Water/Ballast Sediment and Dinoflagellate Species

Ship ballast water and sediment contain numerous microorganisms and various organisms, including phytoplankton, zooplankton, and other elements. The 10 most undesirable species in ballast water and sediment are presented in Figure 6. Therefore, the discharge of organisms in this category into the marine

environment will negatively impact new habitats. These animals can become invasive species under favorable conditions (Roy et al., 2022).

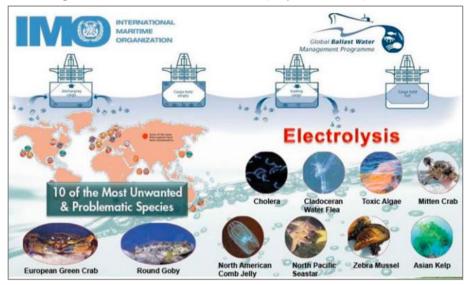


Figure 6. The 10 Most Unwanted Species in Ballast Water and Sediments

2.1.1. How Are Invasive Non-Native Species via Ships' Ballast Water and Sediments?

- When ships discharge cargo in port, they take in seawater and surrounding organisms into their ballast tanks.
- Ballast water becomes "biotic," a living entity where many marine organisms coexist.
- Many of these organisms return to the sea when the ballast is discharged during loading.
- Sediments in ballast tanks cleaned and disposed of in shipyards can also carry organisms.
- These organisms may survive depending on environmental conditions and even become invasive species.
- The extent of IYT transfer becomes more readily apparent when considering global maritime transportation.
- The number of species may vary depending on ship routes and ecosystem conditions (GloBallast, 2017).

2.2. What is Biofouling and Why is it Important?

Biofouling is the accumulation of aquatic organisms, such as microorganisms, plants, and animals, on surfaces and structures immersed in or exposed to the aquatic environment. Biofouling is also known as hull (ship) fouling. Aquatic organisms can be transferred to different locations as biofouling. They can be harmful and invasive in places where they are not naturally found. The transfer of invasive alien species can threaten the seas, human, animal, plant life, and economic and cultural activities. Once invasive aquatic species have established themselves in a new habitat, eradication is generally impossible. The International Maritime Organization (IMO) as "the unintended accumulation of pollution" defines Biofouling (GloFouling, 2025).

2.2.1. How Alien Species Are Transferred via Biofouling on Ships

- Stage 1 Recruitment to Vector: Alien species attach to the ship's hull/niche areas based on the ship's waiting time in port, the presence/condition of the AFS (Antifouling System), the AFS type, and their reproduction.
- Stage 2 Translocation: Species attached to the ship may or may not be transported on the ship during the voyage, depending on their location, the ship's speed, and duration, route, and AFS type.
- Stage 3 Transfer from Vector: At the end of the voyage, transported and surviving organisms move from the ship to their new habitat, depending on environmental conditions (water temperature, salinity, etc.) and the ship's waiting time.
- Stage 4 Colonise and Establish: Alien species establish themselves in their new habitats based on environmental conditions, the availability of sufficient resources for growth and development, and their survival (biotic) resistance.
- Stage 5 Spread and Impact: Established species may begin to spread depending on their population increase with biotic resistance, availability of resources, environmental conditions and additional transfers (Figure 7) (Khodjet et al., 2020).

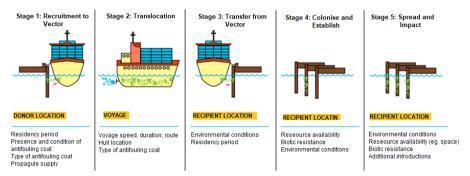


Figure 7. Stages of Introduction of INNS by Vessel Biofouling

Biofouling is classified as micro-pollution (refers to a layer of microscopic organisms, including bacteria and diatoms and the slimy substances they produce) and macro-pollution (refers to multicellular organisms that can be seen with the eye, such as balanus, tubeworms, mussels, algae, etc.) (Figure 8) (2024). In the past, the common view was that ship ballast water was primarily responsible for the introduction of invasive nonnative species. Recent research suggests that biofouling has been underestimated as a potential vector and may, in fact, represent the most common mechanism for the introduction of invasive non-native species.

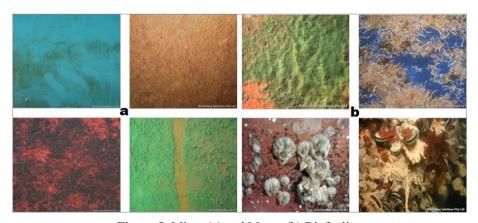


Figure 8. Micro (a) and Macro (b) Biofouling

3. BIOSECURITY IN MARITIME TRANSPORT

Biosecurity risk factors in the maritime transport sector depend on the factors of biofouling within the ship's hull, ballast water and sediment. Biofouling varies depending on the ship type, including the wetted surface area

under water, the time between arrival and departure, the ship's speed depending on the ship type, the ratio and distribution of niche areas to wetted surface areas within the ship's hull, and the accumulation of contamination within the hull. Another risk factor, ballast water, varies depending on the ship's ballast water capacity, ballast discharge volume, frequency, and location of discharge. Ships have ballast water capacities based on their type of operation, structure, and other factors. Studies have determined that the ships with the highest biosecurity risk scores, based on their type, are bulk carriers, heavy lift ships, and general tankers, respectively (Tzeng et al., 2021). Biosecurity in maritime transportation refers to all measures taken to minimize the accumulation of ballast water, sediments, and biofouling on ships, as well as the associated risk of the introduction and spread of invasive non-native species. Two different management methods are implemented for this purpose: ballast water and sediment management and biofouling management.

3.1. Ballast Water and Sediment Management

Ballast water and sediment management is the mechanical, physical, chemical and biological operations carried out on a ship, either individually or in combination, to remove, reduce or preserve harmful aquatic organisms and pathogens containing ballast water and sediment, or to render them harmless. Ballast water carries thousands of aquatic microbes, bacteria, plants, and animals. Discharging untreated ballast water at destination ports poses the risk of introducing invasive species, which can disrupt local ecosystems. To prevent the introduction of invasive species into marine ecosystems, ballast water must be managed, and regulations have been introduced to address this. These regulations include where ballast can be discharged and treatment methods (IMO, 2012).

3.1.1. Ballast Water Management

The International Monetary Fund (IMO) implemented the Ballast Water Management (BWM) Convention (International Convention for the Control and Management of Ship Ballast Water and Sediments, 2004) on 8 September 2017 to regulate ballast water discharge and reduce the risk of introducing invasive aquatic species into the sea. All ships must comply with the D-2 standard by 8 September 2024. The Convention applies to ships taking on and using ballast water on international voyages registered under Contracting

Parties to the BWM Convention. From the date of entry into force, ships in international traffic are obliged to manage their ballast water and sediments according to ship-specific ballast water management plans, meeting specific standards. Ships are required to have:

- Ballast Water Management Plan an operational tool that ship operators must implement and maintain on board to meet the requirements of the Ballast Water Management Convention.
- Ballast Water Record Book a book that records the time ballast water is received on board, the management processes for circulated or treated ballast water, and its discharge into the sea. Records must also be kept of when ballast water is discharged to a reception facility, and any exceptional, accidental, or other discharges.
- International Ballast Water Management Certificate (ships 400 GT and above) a certificate issued by or on behalf of the Administration (Flag State) demonstrating the ship's compliance with the standards for ballast water management in accordance with the BWM Convention, and with an expiration date.

The Ballast Water Management Convention is expected to affect approximately 55,000 ships worldwide and 900 in Turkey. The total global cost of the new regulations is estimated at \$50-55 billion. From the date of entry into force, ships in international traffic are obliged to manage their ballast water and sediments in accordance with ship-specific ballast water management plans and to certain standards. Ballast Water Management Standards: (IMO, 2025b).

- D-1 Ballast Water Exchange Standard: Requires ships to exchange their ballast water at high seas, away from coastal areas. Ideally, this means at least 200 nautical miles from land and at least 200 meters deep. This is believed to reduce the survival of organisms in ships' ballast water and reduce the risk of carrying potentially harmful species. Requires ships to effectively exchange 95% of their ballast water.
- D-2 Ballast Water Performance Standard: Specifies the maximum permissible release of living organisms, including microbes designated as harmful to human health (Table 2).

	· ·	C		
Organism		Permissible Amount of Viable Organisms per unit volume		
Organism size ≥50 μm		<10 adet / m3		
50 μm> Organism size ≥10 μm		<10 adet / ml		
Human health related	Toxigenic Vibrio Chlorae (Serotip O1 ve O 139)	<1 *cfu/100ml (less than 1 cfu/g wet weight in zooplankton samples)		
standards Escherichia coli		<250 *cfu/100 ml		
standarus	Intestinal Enterococci	<250 *cfu/100 ml		

Table 2. Ballast Water Management Convention D-2 Discharge Standards

• D-3 International Ballast Water Management Certificate: This certificate is issued by or on behalf of the Administration (Flag State) to ships of 400 GRT and above, and indicates which standards of the Ballast Water Management (BWM) Convention the ship complies with and has an expiration date.

All ships must manage their sediments in accordance with the Ballast Water Management Plan and remove and dispose of their sediments in approved facilities. All ships must comply with at least the D-1 standard, and all new ships must comply with the D-2 standard. Ultimately, all ships will be required to install a ballast water treatment system in accordance with the D-2 standard. Ballast water treatment systems are commonly categorized as mechanical, chemical, and physical. Most systems use a combination of mechanical, chemical, and physical processes. There are two general approaches to ballast water treatment: physical solid/liquid separation and disinfection.

Solid-liquid separation is the removal of suspended solids from ballast water. It can be used to remove large microorganisms. Separation can be achieved by sedimentation (allowing solids to settle by their own weight) or surface filtration (using a filter to remove solids too large to pass through a material). Disinfection (usually added to the first mechanical stage) removes and/or inactivates microorganisms using a chemical or physical process. Filtration is the most preferred pretreatment method in systems that have received IMO Type Approval. The most significant problems encountered with filtration are compatibility with ballast pump capacity, the necessity of

backwashing due to blockages, pressure drops, and space limitations on the ship. However, it is almost a mandatory option, especially for systems that use UV. For secondary treatment, UV technology is the most preferred method among all systems. However, the efficiency of UV systems faces significant limitations due to seawater turbidity and the structure of the organisms (GloBallast, 2025a).

Factors affecting the efficiency of ballast water treatment systems:

- Composition of the technologies comprising the ballast water treatment system
- Seawater and organisms present at the location of ballast operation
- Meteorological conditions encountered during the voyage and along the ship's route
- Sediment load and particle size of seawater negatively affect filter performance, secondary treatment technologies, and the efficiency of UV systems.
- Organisms at different life stages in the sediment affect chemical treatment efficiency.
- Changing seawater salinities affect energy consumption in electrochemical processes.
- Ballast water and ambient temperature affect metabolic activities and the rate of chemical reactions (Gray et al., 2006; Raikow et al., 2006; Hess-Erga et al., 2008; Yonsel et al., 2014; Chen et al., 2016).

3.1.2. Sediment Management

Seawater damage to ballast tanks can lead to the erosion of silt and invasive species. If left untreated, the formation of a heavy sediment layer in ballast tanks can significantly increase the disinfection process and make it difficult to treat ballast water. The composition of ballast water varies greatly among ships, depending on the ship's ballast water composition and the tank's operation. Generally, ballast sediments include eight main types: clay ($\leq 2 \mu m$), silt (particles 2-63 μm), sand (particles 63-2 μm), larger particles (>2 μm), products of processes in tanks and associated piping, fragments of preservatives, non-living organic and living organisms (bacteria, algae,

crustaceans, snails, worms, fish), and many other organisms (organized organisms) (GEF-UNDP-IMO, 2017).

- Ballast tank sediment management plans must be included in a ship's overall Ballast Water Management Plan in accordance with Regulation B-5 of the Convention.
- Sediment management plans are only valid when ships are in the shipyard. Treatment or removal of ballast tank sediments is not required during normal operation.
- The Convention requires Member States to designate ballast tank reception facilities for sediment removal/treatment operations. These operations are typically carried out in shipyards providing ballast tank cleaning services. The Convention does not require Member States to establish port reception facilities for ballast water.
- Sediments may only be removed at a "sediment reception facility" that is a Party to the Convention.
- Sediments should be removed as "sludge" by shovels, if possible.
- Sediment disposal areas should be planned in areas away from the coast, rivers, and surface waters.
- The ship is responsible for the sediments. The shipyard is merely a service provider.
- Sediments must be cleaned, stored in a secure area, and then transported to a permanent disposal site. Sediments cannot be discharged into local waters.

3.2. Biofouling Management

The amount and growth of biofouling in the hull and niche areas of ships are generally affected by environmental factors (salinity, water temperature, water depth and distance from the shore), hull condition, niche areas and antifouling systems (Table 3) and the operating characteristics and design/structure of the ship (Table 4) (New Zealand Ministry for Primary Industries, 2015).

Ship Hull Condition Niche Areas Anti-Fouling System	Specific risks
Hull roughness	The dense 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 3. Hull Condition, Niche Areas and Anti-Fouling System Affecting Biofouling

Table 4. Operational Characteristics and Design/Structure of the Vessel Affecting Biofouling

Ship's Operating			
Characteristics and	Specific risks		
Design/Structure			
Usage rate,	Depending on local environmental conditions and		
anchorage/cruise time,	antifouling coating, long anchoring/marina stays may		
location where	increase biofouling (calcareous organisms are		
the ship is kept	retained within 5-8 days).		
Smood	Navigat,,on at lower speeds than normal may cause		
Speed	increased biofouling.		
Damaga to the entifolding	Ships that frequently visit ports and/or areas with high		
Damage to the antifouling coating	tides are more likely to have their anti-fouling coating		
	damaged by friction.		
Areas sensitive to fouling	Rudders, propellers and propeller shafts are more		
Areas sensitive to fourning	sensitive areas		

Biofouling management is to minimise the risk of biological contamination through good maintenance practices appropriate to the ship's operational profile (IMO, 2025c).

Biofouling management:

- Antifouling system effectiveness. Application of AFC (Antifouling Coatings) to the ship's hull and niche areas. The ship's operational profile (speed, time out of operation, operational environmental conditions) and selection of the appropriate AFC.
- Monitoring the ship's performance, conducting inspections and cleaning.

- Effective surface before AFC application and operating.
- Monitoring ship performance. Proactive maintenance and cleaning of the microfouling layer on the hull prevents the settlement of larger organisms.
- Contingency plans, such as in-water inspections and in-water cleaning, should be in place for interruptions in the ship's operating profile and damage to AFC. Repairing even minor damage to AFC.
- Replacing AFC within the specified service life.
- Maintaining/cleaning pipelines / sea chests or using MGPS for biofouling.

Every ship must have a biofouling management plan. The biofouling management plan must be specific to each ship and must include the ship's operational documentation. The plan should include the following information (MEPC, 2011).

- Details of AFC and processes implemented in niche areas, including biofouling-sensitive areas, planned inspections, repairs, maintenance, and renovations.
- Recommended antifouling systems and operational practices appropriate to operating conditions,
- Details of crew safety, including the antifouling system used,
- A Biofouling Record Book with the necessary documentation to verify any recorded processes.

A Biofouling Logbook must be maintained for each ship. This document assists the ship owner/operator with operational practices and allows government authorities to quickly and efficiently assess potential biofouling risks. The information that should be included is:

- Details of the antifouling systems and operational practices used,
- Details of the beaching and relaunching dates, if any, and precautions, repairs, and maintenance,
- Dates and locations of in-water inspections, inspection results, and any interventions and repairs,
- Dates and notes of inspections, maintenance, and observations of internal seawater and cooling systems,
- Detailed processes outside the ship's normal operating profile and profile.

The selection of antifouling coatings (AFC) is one of the most important criteria in biofouling management. Biofouling reduces ship speed, increases resistance, and increases fuel consumption and greenhouse gas emissions (Figure 9). Biofouling accumulation varies in different parts of the ship. AFC degrades quickly on the ship's boot topping, facilitating macroalgae growth. Niche areas are more prone to biofouling due to different hydrodynamic forces.



Figure 9. Heavily Biofouled Ship Hull

3.2.1. Biosecurity Risk Factors of Ships in Maritime Transport

Biosafety risk factors in maritime depend on biofouling, ballast water, and sediment. Biofouling varies by ship type (Table 5). The ships with the highest biosafety risk scores were identified as bulk carriers, heavy-lift ships, and general tankers, respectively (Tzeng et al., 2021; GloBallast, 2025b).

Table 5. Biosafety Risk Factors in the Transfer of INNS by Ships

Invasive Non-Native Species Biosecurity Risk Factors in Maritime				
Biofouling		Ballast Water and Sediment		
Importance		Importance		
1	- physical and a second a second and a second and a second and a second and a second a second and a second and a second and a second and a second and a second and a second and a second and a second and a second a	1		
	Ship hull wetted surface area		Ballast water capacity	
2	Waiting/staying time in port	3	Ballast discharge volume	
2		3		
	Vessel's type-specific speed		Ballast discharge frequency	
3	Ship hull complexity			
3	Amount of biofouling			

4. CONCLUSION

• Biofouling on ships, ballast water, and sediments are a significant pathway for the introduction of invasive non-native species, which

- can have significant detrimental effects on biodiversity, ecosystem services, human health, and economic impacts.
- Biosecurity in maritime refers to all measures taken to minimize the risk of the introduction and spread of invasive non-native species.
- Ballast water and sediment management = Biosecurity in maritime.
- Every ship must have a Ballast Water and Sediment Management Plan.
- Every ship must maintain a Ballast Water Record Book.
- Every ship must hold an International Ballast Water and Sediment Management Certificate.
- Ballast Water Management Technology must be ship-specific.
- Sediments should only be cleaned at authorized sediment reception facilities.
- Biofouling management = Biosecurity in maritime.
- Every ship must have a Biofouling Management Plan.
- Every ship must maintain a Biofouling Record Book.
- AFS (Anti Fouling Systems) should be according to the ship's operational profile and type.
- Biocide-free AFS (Anti Fouling Systems) must be used.
- An AFS Certificate or AFS Declaration must be kept on board.

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CHAPTER 9

FISH CYTOKINES: MASTER REGULATORS OF IMMUNITY AND AQUACULTURE HEALTH

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INTRODUCTION

Aquaculture plays a vital role in global food security, currently providing more than half of the seafood consumed worldwide. However, the intensive and high-density nature of modern aquaculture systems creates favorable conditions for the rapid spread of infectious diseases caused by viruses, bacteria, and parasites. These diseases remain one of the most significant constraints to the sustainability and economic viability of the aquaculture industry (Austin 2023; Mair et al., 2023; FAO, 2024). In response to growing concerns over antibiotic resistance and environmental contamination, aquaculture is increasingly embracing preventative strategies such as immunostimulation, genetic selection, and vaccination, as sustainable alternatives to routine antibiotic use. Immunostimulants like β-glucans have been widely studied for their capacity to enhance fish innate immunity: for instance, β-glucan supplementation boosts macrophage respiratory burst and degranulation, even under stress, improving resistance to pathogens in various species (Hadiuzzaman et al., 2022). Recent in vitro work with tilapia macrophages has shown that β-glucan-enriched diets help maintain their functionality even after exposure to chemical stressors, a clear demonstration of immune priming (Fierro Castro et al., 2024). Selective breeding for disease resistance further supports this shift; genetic selection programs have achieved significant heritable gains in resistance against major pathogens, reducing dependence on treatments (Nguyen, 2024; Jin & Liu, 2024). The development of new adjuvant technologies, particularly novel nanoparticle-based adjuvants, is critical for advancing vaccination. These specific adjuvants offer a safe method to boost immunogenicity in teleost fish, resulting in immune responses that are both stronger and more targeted (Tammas et al. 2024a, Tammas et al. 2024b). These strategies often work by modulating cytokine networks such as IL-1 β , TNF- α , IL-6, and IFNs, thereby priming immune cells for faster and stronger responses upon pathogen exposure (Wang & Secombes, 2013). Altogether, integrating cytokine biology into immunostimulation, genetic improvement, and vaccination presents a powerful, sustainable pathway to reduce antibiotic reliance, improve fish health, and increase productivity in aquaculture.

Consequently, the focus of health management has shifted from reactive therapeutic interventions, which are costly, inefficient, and environmentally unsustainable, to proactive and holistic strategies. Central to these approaches is the enhancement of the fish's natural defense mechanisms. Thus, understanding and manipulating immune responses have become essential for promoting animal welfare, reducing disease-related losses, and achieving sustainable aquaculture production (Harikrishnan et al., 2012a; Harikrishnan et al., 2012b)

Teleost fish display a sophisticated immune system that enables them to cope with the diverse pathogens present in aquatic environments. The immune system of teleosts, which constitute the majority of aquatic vertebrates, is a complex and efficient defense network critical for survival in pathogen-rich habitats. While teleosts share many fundamental immune components with mammals, they also exhibit unique anatomical and evolutionary adaptations (Uribe et al., 2011). Fish immunity is broadly categorized into two interdependent arms: innate (non-specific) and adaptive (specific) immunity. The innate immune system provides the first line of defense through physical barriers (skin, gills, and mucus), humoral factors (lysozyme, complement, and acute-phase proteins), and cellular mechanisms such as phagocytosis, oxidative burst, and natural killer-like cell activity (Uribe et al., 2011; Mokhtar et al., 2023). In contrast, the adaptive immune system, although less developed than that of mammals, relies on T and B lymphocytes, major histocompatibility complex (MHC) molecules, and immunoglobulins (IgM, IgD, and IgT/Z), which contribute to long-term, pathogen-specific protection (Reyes-Cerpa et al., 2012; Zhang et al., 2010; Kordon et al., 2022). A deeper understanding of these immune systems is vital for improving disease resistance and advancing effective vaccination strategies in aquaculture (Secombes & Wang, 2012; Zou & Secombes, 2016). Regulation of immune responses in fish involves a complex interplay of signaling molecules, transcription factors, and cellular mediators that maintain immune homeostasis, coordinate defense mechanisms, and prevent excessive inflammation (Secombes & Wang, 2012; Uribe et al., 2011). Among these regulatory molecules, cytokines play pivotal roles in modulating immune responses, facilitating communication among immune cells, and balancing inflammation with tissue repair (Zou & Secombes, 2016). Cytokines are small, secreted signaling proteins with growth, differentiation, and activation functions that regulate the nature of immune responses (Reyes-Cerpa et al. 2012). Cytokines act as master regulators of the immune system,

mediating the initiation, amplification, and resolution of immune reactions (Zou & Secombes, 2016; Li et al., 2023).

In teleost fish, cytokine-mediated communication determines when, where, and how immune responses are activated or suppressed. For example, pro-inflammatory cytokines such as interleukin- 1β (IL- 1β) and tumor necrosis factor-alpha (TNF- α) initiate rapid immune cell recruitment to sites of infection, while anti-inflammatory cytokines such as IL-10 are essential for resolving inflammation and preventing tissue damage. Chemokines, a specialized subgroup of cytokines, function as chemoattractants that guide immune cells (including macrophages and neutrophils) from lymphoid organs (e.g., head kidney and spleen) to infected or inflamed tissues such as the gills and gut. Particularly important are interferons (IFNs), which form the cornerstone of the antiviral defense system. Following viral recognition, infected cells release IFNs that activate antiviral mechanisms in neighboring cells, thereby limiting viral replication and spread (Secombes et al. 2011b; Zou & Secombes, 2016; Li et al., 2023; Dai et al., 2025).

In the aquaculture setting (high stocking densities, fluctuating water quality, and handling stress elevate disease susceptibility) understanding cytokine-mediated immune regulation is critical for designing sustainable disease management strategies. Cytokines are now recognized not only as immunological mediators but also as practical biomarkers and therapeutic targets for maintaining health under intensive farming conditions. Modulation of cytokine expression through nutritional immunostimulants, probiotics, or vaccination provides a sustainable approach to enhancing disease resistance without reliance on antibiotics or chemicals (Zou & Secombes 2016; Sakai et al., 2021; Baloch et al., 2022; Tian et al., 2023).

Recent research demonstrates that targeted modulation of cytokine expression particularly IL-1β, IL-6, TNF-α, and IL-10 can enhance resistance to infection and improve resilience to environmental stress in teleosts, which confirm that immunostimulants, probiotics, feed additives, and vaccine adjuvants exert their effects largely through the manipulation of cytokine networks, reinforcing the concept that cytokines are practical targets rather than merely mechanistic markers in aquaculture health management (Awad et al., 2011; Wang & Secombes, 2013; Mansoori et al., 2024; Awad, 2025; Abdul Kari, 2025; Karataş, 2025)

To date, more than 100 cytokines have been identified in humans, and genomic studies reveal that teleost fish contain nearly all of the major cytokine families found in mammals (Savan & Sakai, 2006; Zou & Secombes, 2016). Accordingly, this chapter focuses on cytokines in teleost fish, emphasizing their functional diversity and central roles in immune regulation. A thorough understanding of cytokine biology provides the foundation for developing innovative immunomodulatory and vaccination strategies, ultimately contributing to improved fish health and sustainable aquaculture production.

2. FISH IMMUNE SYSTEM

Teleosts demonstrate a highly organized and evolutionarily advanced immune system that integrates innate and adaptive components, with cytokines serving as central regulators of communication between these two arms. Their immune architecture is built upon a network of primary and secondary lymphoid organs that coordinate hematopoiesis, immune cell maturation, antigen recognition, and effector responses.

The head kidney (pronephros) is the major hematopoietic organ in teleosts and functionally parallels the mammalian bone marrow. It supports the development of myeloid and lymphoid lineages, including macrophages, neutrophils, B and T lymphocytes, and natural killer-like cells, making it a pivotal site for both innate and adaptive immunity (Uribe et al., 2011; Zou & Secombes, 2016). Complementing this, the thymus serves as the primary site for T-cell maturation, generating functional CD4⁺ and CD8⁺ T lymphocytes that orchestrate cell-mediated immunity and regulate innate responses through cytokine-driven activation of phagocytes (Zou & Secombes, 2016).

Among secondary lymphoid organs, the spleen acts as a major immunological filter for blood-borne antigens and supports antigen presentation, phagocytosis, and B-cell activation leading to antibody production (Secombes et al., 2011b; Han et al. 2024). Teleosts also have diverse mucosa-associated lymphoid tissues (MALTs), including gut-associated (GALT), gill-associated (GIALT), skin-associated (SALT), and nasopharynx-associated (NALT) lymphoid tissues. These tissues house macrophages, neutrophils, mast cells, granulocytes, and both B and T lymphocytes, enabling rapid localized responses to mucosal pathogens and contributing to long-term

immunological memory (Das & Salinas, 2020; Salinas et al., 2021; Dezfuli et al., 2023).

The teleost innate immune system constitutes the first line of defense and includes cellular components such as macrophages, neutrophils, mast cells, granulocytes, and natural killer-like (NK) cells. These cells mediate pathogen clearance through phagocytosis, production of lytic enzymes, and generation of reactive oxygen species (ROS) (Mokhtar et al., 2023; Hopo et al., 2024). Neutrophils rapidly migrate from hematopoietic tissues to infection sites to engulf pathogens and initiate inflammatory responses.

Pathogen detection is mediated largely by pattern-recognition receptors (PRRs), including NOD-like receptors (NLRs), which activate cytokine-driven inflammatory pathways (Swain & Miryala, 2025). Proinflammatory cytokines such as IL-1 β , TNF- α , and IL-6 promote immune activation, whereas immunoregulatory cytokines—including IL-10 and TGF- β —modulate inflammation to prevent tissue damage (Zou & Secombes, 2016; Li et al., 2023).

The teleost adaptive immunity is mediated primarily by B and T lymphocytes. B cells differentiate into subsets expressing immunoglobulin isotypes such as IgM, IgD, and IgT/IgZ, with IgM dominating systemic responses and IgT playing a specialized role in mucosal immunity (Zhang et al., 2010; Yu et al., 2020; Salinas et al., 2021; Chen et al., 2020). Unique among vertebrates, teleost fish B cells bridge innate and adaptive immunity by combining potent phagocytic activity with classical antibody-mediated responses (Han et al., 2024).

T lymphocytes, including cytotoxic T lymphocytes (CTLs) and helper T cells, develop in the thymus and express T-cell receptors (TCRs) essential for antigen-specific recognition (Yamaguchi et al. 2019; Kordon et al., 2022; Cao et al., 2023).

3. CYTOKINES IN TELEOST

Recent research demonstrates that cytokine networks in teleosts are highly conserved with mammalian immune systems. Teleost fish express major cytokines (Table 1), including IFN- γ , IL-4/13, IL-17, IL-10, and TGF- β , which supports the existence of Th1-like, Th2-like, Th17-like, and regulatory-like CD4⁺ T-cell subsets (Wang & Secombes 2013; Tian et al., 2023; Cao et al.,

2023; Cao et al., 2025). Functional CD4⁺ T cells have been documented in species such as zebrafish and salmonids, with differentiation governed by conserved transcription factors and their associated cytokine pathways (Tian et al., 2023; Cao et al., 2025).

Cytokines serve as central mediators linking innate pathogen detection to adaptive immune activation. They orchestrate inflammation, regulate leukocyte trafficking, shape T- and B-cell responses, and maintain homeostasis in mucosal tissues (Sakai et al., 2021; Mokhtar et al., 2023; Kong et al., 2024). In mucosa-associated lymphoid tissues (MALTs), such as gut-, gill-, skin-, and ocular-associated lymphoid structures, rapid and localized cytokine responses are essential for protection against mucosal pathogens (Dezfuli et al., 2023).

Table 1. The major cytokines & functions in teleost

Cytokines	Type / Function	Key Role in Fish
IL-1β	Pro-inflammatory	Activates macrophages, induces other cytokines, promotes inflammation
IL-2	T cell growth factor	Stimulates T cell proliferation and adaptive immunity
IL-6	Pro- & anti- inflammatory	Acute phase response, B cell differentiation, macrophage activation
IL-10	Anti-inflammatory	Suppresses excessive immune responses, regulates macrophages
IL-12	Pro-inflammatory	Promotes Th1-like responses, induces IFN-γ production
IL-17	Pro-inflammatory	Recruits neutrophils, protects mucosal surfaces
IL-18	Pro-inflammatory	Stimulates IFN-γ production, enhances NK/T cell function, macrophage activation, and antiviral immunity
IFN-α / IFN-β	Type I IFN	Antiviral, activates antiviral genes, inhibits viral replication
IFN-γ	Type II IFN	Activates macrophages, promotes Th1 responses
TNF-α	Pro-inflammatory	Activates macrophages, induces apoptosis, recruits immune cells
CXCL8 (IL-8)	Chemokine	Recruits neutrophils to infection sites
CCL19 / CCL21	Chemokine	Guides lymphocytes to lymphoid tissues

Interleukin-1Beta (IL-1 β) is produced mainly by activated macrophages after pathogen recognition via Toll-like receptors (TLRs). IL-1 β binds to the IL-1 receptor type I (IL-1RI), activating key intracellular signaling pathways. Induces inflammatory responses (e.g., leukocyte recruitment, fever-like responses) in Teleosts. Also, upregulates antimicrobial peptides and acutephase proteins. In species like zebrafish and rainbow trout, IL-1 β expression is found to be increased after bacterial or viral infection. (Zou & Secombes, 2016; Cao et al., 2025)

Interleukin-2 (**IL-2**) is a crucial immunoregulatory cytokine in fish, fundamentally governing the adaptive immune response by acting as the primary T cell growth and survival factor, a role that is highly conserved across vertebrates. IL-2 drives the essential proliferation and differentiation of T lymphocytes, thereby expanding the pool of antigen-specific cells needed to combat infection. Also, it helps balance the immune response by regulating the expression of both Th1 (cell-mediated) and Th2 (humoral) cytokines, and it also contributes to B lymphocyte activation and antibody secretion (Wang & Secombes, 2013; Zou & Secombes, 2016; Cao et al., 2025)

Interleukin-6 (IL-6) binds to IL-6 receptor (IL-6R) and glycoprotein 130, triggering the most important communication systems in the immune system of fish. This cytokine stimulates acute-phase protein production in the liver and promotes B-cell differentiation and macrophage activation. Also plays a dual role as pro-inflammatory during infection, but also supports recovery and tissue repair. (Zou & Secombes, 2016;Cao et al. 2025)

Interleukin-10 (IL-10) engages IL-10 receptor (IL-10R1/IL-10R2), activating signal transducer and activator of transcription 3 (STAT3) signaling to suppress pro-inflammatory cytokines (like IL-1 β , TNF- α , and IFN- γ). Acts as a key anti-inflammatory cytokine, preventing excessive immune damage and regulating macrophage cell activity. The expression increases after viral infection to balance inflammation. (Wei et al., 2014; Zou & Secombes, 2016; Cao et al., 2023)

Interleukin-12 (IL-12) is a heterodimeric cytokine that binds to IL-12R, activating signal transducer and activator of transcription 4 (STAT4) signaling and promoting Th1-like responses. Functions as an inducer of IFN-γ production from Natural Killer-like cells and T cells, also bridges innate and adaptive

immunity, and enhances cytotoxic responses against viruses and intracellular bacteria (Zou & Secombes, 2023)

Interleukin-17 (IL-17) acts through IL-17 receptor complex (IL-17RA/IL-17RC), activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways. Function as promotes neutrophil recruitment and mucosal defense and upregulates pro-inflammatory mediators (IL-1 β , TNF- α , chemokines) in fish. Also plays a key role in gut and gill immunity, maintaining barrier function. (Corripio-Miyar et al., 2012; Wang et al., 2020; Baloch et al. 2022; Han et al. 2025).

Interleukin-18 (IL-18) is produced as an inactive precursor activated by caspase-1 (inflammasome pathway) and binds to IL-18R, stimulating IFN-γ production via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Synergizes with IL-12 to induce strong cell-mediated immune responses in fish. Also enhances macrophage activation and antiviral immunity (Yamaguchi et al. 2019; Baloch et al. 2022).

Interferons (IFNs) Types: especially type I and II interferons, are critical for antiviral defence in fish. They trigger antiviral states in neighbouring cells and regulate downstream gene expression of interferon-stimulated genes (ISGs).

Type I IFNs induce antiviral state through janus kinase / signal transducer and activator of transcription (JAK/STAT) signaling, leading to interferon-stimulated gene (ISG) expression. It is critical for early antiviral defense for example, viral hemorrhagic septicemia virus, VHSV. Beside, promotes macrophage activation and antigen presentation (Robertsen, 2006; Zou &Secombes 2011).

Type II IFN (IFN-γ) is produced by T and NK-like cells; it binds to interferon gamma receptor (IFNGR), activating signal transducer and activator of transcription 1 (STAT1) signaling. Functions: activates macrophages and induces nitric oxide synthase expression and upregulates MHC class II and antigen presentation. (Grayfer et al. 2018; Zou & Secombes, 2016)

Tumor Necrosis Factor (TNF-\alpha) pro-inflammatory; stimulates apoptosis, activates macrophages, and recruits immune cells to infection sites. It binds tumor necrosis factor receptors (TNFR1/2), triggering nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and caspase pathways.

It promotes inflammatory cytokine production and leukocyte recruitment and may induce apoptosis of infected cells in fish. (Hong et al., 2013; Secombes et al., 2011b)

Chemokines (CC and CXC Chemokines); Chemokines (a subclass of cytokines) regulate the migration of immune cells to sites of infection or damage, thus coordinating the spatial aspects of the innate immune response. it binds to G-protein-coupled receptors (GPCRs), activating mitogen-activated protein kinase (MAPK) pathway that guides leukocyte migration. Its functions coordinate cell trafficking to infection sites, CCL19/21-like chemokines regulate lymphoid tissue organization, and CXCL8 (IL-8) recruits neutrophils during bacterial infection (Bird & Tafalla, 2015).

Transforming Growth Factor-Alpha (TGF-\alpha); TGF- α is primarily a growth and differentiation factor, but it also influences immune cell functions indirectly. Functions in immune modulation, tissue repair & regeneration, cell growth, and enhances responsiveness to IL-6, TNF- α , and other growth/immune mediators. (Secombes & Wang 2012; Zou & Secombes 2011).

4. CYTOKINES IN INNATE AND ADAPTIVE IMMUNITY

The innate immune system constitutes the first line of defense in teleosts and relies heavily on cytokine-mediated signaling. Innate immune cells in mucosal tissues rapidly upregulate pro-inflammatory cytokines (including IL-1β, TNF-α, and IL-6) upon pathogen detection, initiating inflammation and recruiting phagocytic cells (Mensah et al., 2024). Chemokines, particularly CXCL8/IL-8, coordinate neutrophil and leukocyte migration to infection sites, while type I interferons activate interferon-stimulated genes (ISGs) and type II interferon (IFN-γ) enhances macrophage activation and antigen presentation. Transcriptomic studies of the ocular mucosa in rainbow trout challenged with *Ichthyophthirius multifiliis* reveal strong local upregulation of cytokines, chemokines, and T- and B-cell markers, reflecting sophisticated immune orchestration (Kong et al., 2023). Comparable findings across other mucosaassociated lymphoid tissues indicate that these ancient vertebrate immune strategies are highly conserved in teleosts (Kong et al., 2024; Mensah et al., 2024).

Cytokines also regulate adaptive immunity in teleosts. B cells, primarily expressing IgM along with IgD and IgT/IgZ in some species, are regulated by IL-4/13, which promotes proliferation and differentiation. TNF superfamily members, such as B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), support B-cell survival, long-term plasma cell maintenance, and antibody production (Kordon et al., 2022). In T cells, cytokines including IL-12, IFN-γ, IL-17, IL-10, and TGF-β guide differentiation into functional subsets analogous to mammalian Th1, Th17, and regulatory lineages. IL-17 and IL-23-like pathways are particularly important at mucosal surfaces, where extracellular pathogens are prevalent (Jenberie et al., 2024).

The teleost cytokines constitute a molecular network connecting innate and adaptive immunity, with distinct roles such as:

- Pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) initiate inflammation and enhance antigen presentation.
- Anti-inflammatory cytokines (IL-10, TGF-β) resolve inflammation and prevent tissue damage.
- Interferons activate antiviral programs and enhance phagocyte function.
- Differentiation cytokines (IL-12, IL-4/13, IL-17, IL-23) direct T-cell lineage commitment.
- Growth and survival cytokines (BAFF, IL-2-like, GM-CSF) supporting lymphocyte maturation (Zou & Secombes, 2016).

Cytokines are very important molecules that play a role in pathogen detection and alarm reactions, rapidly converting early recognition signals into coordinated immune activation. Innate immune cells detect microbial patterns through pattern-recognition receptors, inducing pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6) that initiate inflammation, increase vascular permeability, and recruit phagocytic cells (Mensah et al., 2024; Swain & Miryala, 2025). Chemokines such as CXCL8/IL-8 further amplify these alarm responses by directing leukocyte migration. Type I interferons trigger antiviral defense, while IFN- γ enhances macrophage activation and antigen presentation, establishing a highly responsive alarm system for rapid pathogen containment (Mensah et al., 2024).

Chemokines

Chemotaxis is the directed migration of immune cells toward chemical signals, which is a fundamental component of the teleost immune response, facilitating rapid recruitment of leukocytes to sites of infection or tissue damage. In teleosts, chemokines, a specialized class of cytokines, act as primary mediators of chemotaxis, with conserved CXC and CC families coordinating distinct immune cell movements (Zou & Secombes, 2016; Mensah et al., 2024). CXC chemokines, particularly CXCL8/IL-8, strongly promote neutrophil migration during bacterial infections and are rapidly upregulated in mucosal tissues, including gills, gut, and skin (Zou & Secombes, 2016). CC chemokines, such as CCL19-like, CCL20-like, and CCL25-like molecules, attract monocytes, macrophages, and lymphocytes, playing a central role in the organization of mucosa-associated lymphoid tissues (MALT) and the strategic positioning of adaptive immune cells (Mensah et al., 2024). Pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, amplify chemotaxis by inducing chemokine production, whereas IFN-y and type I interferons enhance leukocyte recruitment and activation during antiviral responses (Swain & Miryala, 2025). Chemokine-driven migration is particularly critical at mucosal surfaces, supporting neutrophil phagocytosis and ROS production, macrophagemediated pathogen clearance, antigen presentation, and the coordinated positioning of T and B cells for adaptive immune responses (Zou & Secombes, 2016; Mensah et al., 2024).

Understanding cytokine networks has practical implications for aquaculture. Dietary interventions, immunostimulants, and vaccines that modulate cytokine signaling can enhance disease resistance and fish health. Cytokine profiles are sensitive to environmental stress, diet, pathogen exposure, and stocking density. For instance, Atlantic salmon fed β -glucan immunostimulants show strong upregulation of IL-1 β , TNF- α , and IL-6 in the head kidney and spleen, reflecting activation of innate immune pathways (Mensah et al., 2024). Functional feeds, probiotics, plant extracts, and nanoparticles modulate cytokine networks, enhancing disease resistance and reducing antibiotic reliance (Harikrishnan et al., 2012a; 2012b). Vaccines and adjuvants also act through cytokine pathways, promoting antigen presentation, lymphocyte activation, and long-lasting immunity (Zou & Secombes, 2016). Recombinant cytokines, including IL-1 β , interferons, and other interleukins,

can be administered directly or via DNA vaccines to stimulate innate immunity, enhance phagocyte activity, and improve adaptive responses, reducing mortality from bacterial and viral pathogens (Secombes et al., 1999; Wangkahart et al., 2019; Deng et al., 2024; Torrealba et al., 2024). Anti-inflammatory cytokines such as IL-10 and TGF-β are explored to regulate excessive inflammation under stressful culture conditions (Dambatta et al., 2024). Innovative delivery methods, such as nanoparticles and inclusion bodies, provide controlled cytokine release, sustaining immune activation in species like salmonids and grass carp (Wei et al., 2014; Mensah et al., 2024). Collectively, these approaches highlight cytokine-based strategies as central tools for enhancing fish health and promoting sustainable aquaculture practices (Secombes et al., 1999; Wang et al., 2022).

5. CONCLUSION

Cytokines are fundamental regulators of immune function in teleost fish, orchestrating both innate and adaptive responses to maintain homeostasis and defend against pathogens. Their evolutionary conservation across vertebrates underscores their essential roles in pathogen recognition, inflammatory signaling, immune-cell recruitment, and coordination between innate and adaptive immunity. Pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and chemokines like CXCL8 initiate rapid responses to infection, while interferons establish antiviral states. Adaptive immune responses are further shaped by cytokines such as interleukins and TGF-β, which influence B-cell survival, antibody production, and T-cell differentiation. Together, these signaling molecules that are mediators a highly integrated signaling network that defines the timing, magnitude, and quality of immune responses in teleost fish. In aquaculture, cytokines serve not only as mechanistic regulators but also as practical tools for fish health management. Their sensitivity to environmental stressors, diet, pathogen exposure, and husbandry practices makes them powerful biomarkers for disease monitoring and immune status assessment. Moreover, modulation of cytokine networks through immunostimulants, probiotics, functional feeds, plant extracts, nanoparticles, vaccines, or selective breeding provides a sustainable strategy to enhance disease resistance and reduce reliance on antibiotics. This is particularly relevant for intensive farming systems, where rapid pathogen spread and stress can compromise fish health.

As aquaculture continues to expand globally, integrating cytokine-based knowledge into breeding programs, health management strategies, and dietary interventions will be essential for building more robust and welfare-oriented, environmentally sustainable systems. Further research is needed to fully characterize the functional diversity of teleost cytokines, elucidate their roles in mucosal and systemic immunity, and identify innovative approaches for immunomodulation. Ultimately, understanding cytokine networks in teleost fish will continue to provide both mechanistic insights and practical solutions, supporting improved fish health, productivity, and sustainable aquaculture production worldwide.

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CHAPTER 10

RESTORATIVE AQUACULTURE: A SYSTEM APPROACH FOR SUSTAINABLE GLOBAL FOOD PRODUCTION

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INTRODUCTION

Inadequate food production has become one of the most critical problems worldwide. It is estimated that the world population will rise to 10 billion by 2050 and food demand will increase by 50% as a result of this growth (Roth and Galyon, 2024); (Falcon et al., 2022). Seas and oceans, covering more than 70% of the world's surface, are a very important resource for sustainable food production through aquaculture. Aquaculture is one of the fastest-growing food production sectors in the world, providing more than 50% of all seafood consumed. According to FAO reports, global aquaculture production reached 223 million tons in 2022, more than the production obtained through fishing (FAO, 2024).

Despite its rapid growth, traditional aquaculture faces significant challenges regarding its sustainability. The global distribution of aquaculture is geographically uneven. Asian countries account for 91.6% of world production, followed by the Americas (3.5%), Europe (2.7%), Africa (2%), and Oceania (0.2%). This geographical concentration in Asia creates inefficiencies in global seafood supply chains. This situation limits economic development in regions with adequate water resources but underdeveloped aquaculture sectors. In addition, rising sea temperatures, ocean acidification (decreased pH), and changing ocean currents due to climate change are giving additional problems to aquatic life by affecting the health, growth rates and reproductive cycles. These challenges require innovative developments for aquaculture that will address environmental issues while producing and improving them in a positive direction (Ruben et al., 2025); (Verdegem et al., 2023). One such innovative approach is restorative aquaculture.

THE CONCEPT OF RESTORATIVE AQUACULTURE

Restorative aquaculture is a system that integrates ecological, social, and economic components, setting it apart from traditional production systems which primarily focus on capacity. While sustainable aquaculture aims to minimize harm, restorative aquaculture seeks to provide net ecological gains. Restorative aquaculture represents a sustainable aquatic food production, going beyond resource conservation to include active environmental restoration. These practices address methods that can actively contribute to ecosystem health and minimize the negative impacts of traditional aquaculture production

systems. The Nature Conservancy states that "when the right practices are used in the right places, aquaculture can help improve ocean health, support economic development, and increase food production worldwide" (The Nature Conservancy, 2023); (The Nature Conservancy, 2021).

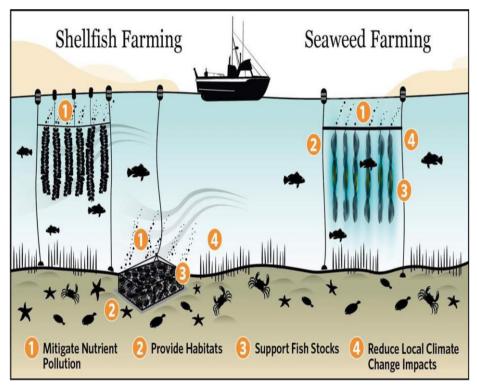


Figure 1. Restorative aquaculture methods and their effects on the ecosystem. **Source:** (Costa-Pierce, 2024-https://v4.infofish.org/index.php/article-ii-3-2024-restorative-aquaculture-for-people-profit-and-planet)

Certain principles have been established to guide industry stakeholders and regulators on how to implement restorative aquaculture activities for the development of restorative aquaculture. These principles demonstrate how equipment and management strategies can be used to increase environmental benefits for both newly established or expanding and existing aquaculture activities (The Nature Conservancy, 2021); (Alleway et al., 2023). These principles are classified as follows.

Strategic Site Selection: Aquaculture farms should be established in areas with the highest potential environmental benefits. The health and characteristics of the local ecosystem are of vital importance in this regard. For example, a farm established in an area with degraded and limited natural habitats is more likely to be a critical resource for new habitats than a farm established in an area with healthy existing natural habitats.

Appropriate Species Selection: The selection of species to be cultivated is the most fundamental factor determining the type and magnitude of benefits to be achieved. Different species have functions that directly affect the environmental benefits they can provide. For example, the filtration capacity of bivalve mollusks or the uptake of nutrients from the water by seaweed are directly important for the desired target.

Beneficial Equipment Design: Cultivation equipment, methods, and infrastructure that increase positive environmental impacts during cultivation should be used. Some methods can provide breeding, feeding, and shelter areas for wild species. It is very important to select equipment that maximizes positive impacts on local fauna while minimizing negative risks such as pollution.

Adaptive Management Practices: A production management approach that benefits the environment surrounding the farming area should be implemented. Since the construction, production, harvesting, maintenance planning, and methods of the farming facility, as well as the shape of the farm, can affect ecological outcomes, all these stages must be carefully planned.

Optimal Scale and Density: Production activities should be carried out at a scale that is compatible with the ecosystem capacity and needs of the production area. Production capacity and methods should be implemented with capacity selection appropriate to environmental impact assessments.

Knowledge Sharing and Community Participation: Sufficient data and technical expertise should be provided to identify, recognize, and disseminate the environmental, social, and economic benefits of production. For restorative aquaculture to be successful, it must be recognized and supported by communities and managers. In this way, the shared responsibility that will be assumed is important not only for ecological benefits but also for maximizing social and economic opportunities, such as livelihoods, education, and equality.

Within the framework of these principles, a comparison of restorative aquaculture and traditional aquaculture is provided in Table 1.

	Traditional Production	Restorative Production
Primary objective	To maximize production	To improve or balance the
	capacity and output	health of the ecosystem while
		producing
Environmental	Minimized or mitigated	Protecting the environment in a
impact		positive way
System design	Production with linear	Cyclical integrated systems
	inputs and outputs (such	(such as the consumption of
	as feed-fish)	fish waste by mussels)
Biodiversity	Generally, decreases	Increases are observed
Resource use	Focused on efficiency	Focused on efficiency

Table 1. Comparison of Traditional and Restorative Aquaculture Approaches

The applications required for the development of restorative aquaculture systems are fundamentally parallel to those required for traditional aquaculture.

Applications for community participation and benefit

Generally limited

Social participation

For example, AI-supported systems are increasingly being used to optimize feeding practices, reduce feed and other resource waste, and increase productivity. Computerized monitoring methods and algorithms have reached a level where they can assess fish size and numbers to optimize feed quantities. These automated systems are also important for restorative aquaculture because they reduce feed loss and its environmental impact while improving feed conversion rates. Similarly, underwater imaging drones and sensors provide real-time data that enables rapid detection and action by monitoring fish health and water quality parameters. Such devices measure oxygen, salinity, temperature, and light, while showing personnel the fully controlled structure of each farm system. All this data ensures that operations are kept under constant control and that the impact on the environment is minimized (Gokulnath et al., 2024).

Recirculating aquaculture systems (RAS) are perhaps the most significant technological advancement in terrestrial aquaculture production in recent years. These systems recycle water through coarse and biofiltration systems and use up to 95% less water than traditional fish farming systems. In addition, the water used is cleaned of waste by filtration systems before being discharged, minimizing environmental impact. Modern RAS incorporates

many technological components, such as biofilters, drum and cartridge filters, UV and ozone sterilization systems, to maintain optimal water quality and prevent the spread of disease. With these structures, closed-loop farming systems are also an important component of restorative aquaculture (Bregnballe, 2022).

Integrated Multi-Trophic Aquaculture (IMTA) is another innovative method closely aligned with the principles of restorative aquaculture. IMTA brings together species at different trophic levels (fish, shellfish, seaweed, etc.), creating synergistic relationships where the waste of one species becomes food for another. This production system mimics natural ecosystem processes, reducing waste and increasing the overall system efficiency of production. Research indicates that IMTA systems can significantly reduce environmental impacts and potentially increase overall productivity and profitability (Giangrande et al., 2021); (Azhar and Memis, 2023).

Alternative feed technologies represent a critical innovative application for reducing the environmental footprint of aquaculture. Feed typically accounts for 50-60% of operational costs and traditionally relies on components such as fish meal and fish oil, which have significant ecological and economic impacts. Therefore, alternative products to fish meal and oil are crucial for sustainable growth and the environment. Alternative sources that can be used in fish feed include insect-derived raw materials, microalgae, and single-cell proteins. Studies on many species show that proteins obtained from insects can be used in place of fish meal by many fish species without any adverse effects. Similarly, it has been reported that the use of fish processing waste and byproducts (heads, intestines, bones, skin) in fish meal and oil production is not only a high-nutrient source but also a practice that reduces waste and increases economic efficiency (Serra et al., 2024); (Maulu et al., 2022).

Energy systems and carbon footprint reduction are important issues for restorative aquaculture activities. Aquaculture activities have a significant advantage over other animal production in terms of carbon footprint. Aquaculture is among the food production systems with the lowest carbon footprint due to high feed conversion rates, the use of wetlands instead of terrestrial areas, and low methane gas emissions from digestive systems. A large portion of carbon emissions from aquaculture originates from other production stages such as feed production, processing, and transportation. Using energy sources such as solar energy to reduce these emissions can lead to a reduction of 4-61% in already low values (Li et al., 2025); (Cortes et al., 2025).

Genetic and breeding applications are other important methods contributing to the advancement of restorative aquaculture. Selective breeding and breeding programs provide benefits, particularly in feed efficiency and adaptation to climate change, by developing desired traits such as growth rate, disease resistance, and environmental tolerance. Recently, gene editing technologies have enabled the rapid development of economically important traits by modifying key genes in fish. This provides a robust framework for meeting food demand. However, researchers also warn that challenges remain in understanding, regulating, and improving the efficiency of complex traits influenced by multiple genes and environmental factors, as well as in addressing ethical and safety issues (Malik et al., 2025); (Friedman et al., 2022). All of the approaches mentioned above are summarized in Table 2.

Table 2. Technological innovations and system design approaches in restorative aquaculture

Technology Class	Key Innovations	Restorative Benefits
Monitoring and Control	AI-supported feeders,	Reduction of waste and
	underwater drones,	mortality rates,
	cameras, IoT sensors	improvement of product
		health
System Design	RAS, IMTA, open sea	Reduced water usage,
	systems	nutrient recycling, habitat
		creation
Feed Technology	Alternative feeds,	Reducing pressure on
	microalgae, use of fish	wild fisheries, circular
	processing by-products	economy
Genetic Improvement	Fish farming methods,	Improved disease
	gene editing	resistance, stress
		tolerance, increased feed
		efficiency, reduced
		environmental impact
Energy Systems	Solar-powered	Low carbon footprint,
	operations, efficient	reduced costs
	pumps	

THE ENVIRONMENTAL AND ECOSYSTEM BENEFITS OF RESTORATIVE AQUACULTURE

Restorative aquaculture methods aim to provide a wide range of benefits that contribute to the ecosystem and environment in addition to production

targets. These benefits represent a fundamental shift towards systems that produce positive environmental outcomes by focusing on minimizing the negative impacts of traditional aquaculture systems (Sara et al., 2025).

The most substantial ecological benefit that restorative aquaculture provides is the enhancement of water quality. Bivalves such as oysters and mussels, as well as seaweed, function as natural biofilters, thereby eliminating suspended solids, excess nutrients (e.g. nitrogen and phosphorus), and pollutants from the water. Research indicates that a single adult oyster is capable of filtering 50 gallons (189 litres) of water on a daily basis, thereby removing nitrogen, phosphorus, and suspended particulate matter. This filtration capacity has been demonstrated to enhance water clarity and quality, particularly in eutrophic waters. The cultivation of bivalves and seaweed has been demonstrated to be capable of reducing nitrogen at a rate of \$84–505 per tonne. This has significant implications for both economic and ecological considerations (Oesterling & Petrone, 2012; Barrett et al., 2022).

Another important ecosystem service provided by restorative aquaculture is habitat creation or restoration. Shellfish and seaweed farms created through aquaculture activities provide habitats that support the life of various wild fish and invertebrate species. These habitats increase local biodiversity and support wild fish populations by providing shelter, feeding, and breeding area. Research indicates that habitats created by aquaculture activities can provide approximately 300-1,100 kilograms of additional fish per hectare per year compared to reference habitats, which could translate to approximately \$1,000-3,000 USD in additional income per hectare per year for commercial or recreational fishermen (The Nature Conservancy, 2021); (Barrett et al., 2022).

Increasing biodiversity is another important environmental benefit of well-designed restorative aquaculture systems. Unlike monoculture systems, which can reduce local biodiversity, restorative systems typically aim to bring together multiple species, creating heterogeneous habitats. Integrated Multi-Trophic Aquaculture (IMTA) systems, which bring together species from different trophic levels, can increase biodiversity in and around the production area by creating multifaceted ecological relationships and habitat structures. (Hughes and King, 2023); (Theuerkauf et al. 2021).

Carbon sequestration is an increasingly important ecosystem service provided by restorative aquaculture activities such as seaweed cultivation. Seaweed is among the world's fastest-growing plants, capable of using large amounts of carbon dioxide through photosynthesis. These characteristics

demonstrate that seaweed cultivation has significant potential not only for carbon reduction but also for the production of biomass that can be used for commercial purposes (food, cosmetics, medicine, etc.). In recent years, it has been reported that the expansion of seaweed cultivation could make a meaningful contribution to efforts to combat climate change. The increase in macroalgae in aquatic environments is thought to contribute to increased carbon dioxide absorption through photosynthesis, which in turn could help solve the problem of ocean acidification, or in other words, the decrease in pH (Troell et al., 2022).

Nutrient cycling and waste reduction are fundamental ecosystem services provided by restorative aquaculture systems that use circular aquaculture systems such as IMTA. In these aquaculture systems, waste products resulting from the metabolic activities of one species become valuable inputs for another species. For example, waste feed or metabolic waste (such as nitrogen and phosphorus) from fish farming can be consumed by seaweed or bivalve mollusks, thereby reducing potential eutrophication and pollution effects while obtaining additional valuable products from production. This approach transforms linear production systems into systems that maximize efficiency while minimizing environmental impacts (Ogella et al., 2024).

The restoration of endangered species and ecosystems is the most direct environmental benefit of restorative aquaculture methods. Aquaculture is a good solution for increasing populations by producing offspring of species whose populations have seriously declined due to overfishing, habitat loss, or disease, and by creating new reef habitats. Similarly, seaweed aquaculture can help restore lost kelp forest habitats that support various marine communities. These restoration activities contribute to the recovery of threatened species and ecosystems while often providing additional benefits by improving water quality and habitat for other species (Overton et al., 2024).

The cumulative impact of the ecosystem services provided by restorative aquaculture will become even more significant as activities expand. If current bivalve and seaweed aquaculture activities triple their current capacity by 2050, the economic value of removing waste nutrients from the water column and the positive impact on habitats will reach US\$17-56 billion annually. Alongside its environmental benefits, this significant economic value highlights the potential of restorative aquaculture to make a meaningful contribution to both environmental protection and economic development goals (Barrett et al., 2022).

CASE STUDIES AND GLOBAL APPLICATIONS

Restorative aquaculture is practiced in various forms in different parts of the world, depending on environmental conditions and needs, cultural preferences, and market structures.

In China, traditional polyculture systems integrated with agriculture have been practiced for centuries. Fish farming in rice paddies, in particular, is one of the most classic methods. In recent years, modern innovations have increased the restorative potential of such polyculture farming systems. Eutrophication problems in Qiandao Lake, which plays an important role in supplying drinking water in China, have been solved by farming silver carp and bighead carp in the lake. In this method, the fish consume algae and become valuable protein sources, while the reduction in the amount of phytoplankton in the water improves water quality. As a result of these efforts, 5,000 tons of carp are farmed in the lake annually, while 200,000 tons of algae, 500 tons of nitrogen, and 175 tons of phosphorus are removed from the environment (The Nature Conservancy, 2021).

In another example, people living in coastal areas of Belize have established seaweed farms as an alternative livelihood to fishing, and this activity has resulted in many beneficial improvements to the aquatic ecosystem. Farmers in Placencia have provided habitat for commercially important shellfish and lobster species, whose numbers had declined due to previous fishing activities, through their seaweed (particularly Eucheuma isiforme) operations. The three-dimensional structure of seaweed farms has increased species diversity by attracting various fish and invertebrate species to these areas, while also creating a refuge for these species. These businesses have provided direct economic benefits through seaweed sales and indirect benefits through the development of fishing. The Belize example demonstrates how restorative aquaculture can support struggling fishing communities (The Nature Conservancy, 2021).

Another example of restorative aquaculture was implemented in the United States. In the Chesapeake Bay region, where wild oyster populations had declined to 1-2% of their original levels, restorative aquaculture was implemented through oyster farming initiatives. In this application, aquaculture activities not only produced valuable food but also improved water quality at the river mouth thanks to the filtration provided by the oysters. A single adult oyster filters up to 50 gallons of water per day, removing particulate matter and excess nutrients that contribute to the bay's water quality degradation. As a result of the benefits provided, the program has been formally incorporated into

the Chesapeake Bay Total Maximum Daily Load (TMDL) management strategy, with state and federal regulations recognizing and encouraging its benefits to water quality. This application is also a fine example of law enforcement agencies encouraging the development of restorative aquaculture (The Nature Conservancy, 2021).

SOCIO-ECONOMIC DIMENSIONS AND COMMUNITY INTERACTION

Restorative aquaculture practices involve significant socio-economic issues in addition to their environmental importance. Understanding and addressing these issues is critical to the successful development and operation of the system.

The creation of livelihoods and economic development are vital for the advancement of restorative aquaculture activities. The aquaculture sector is already a significant economic resource, generating approximately \$264 billion in revenue and providing employment to 20 million people worldwide. Restorative approaches can increase these economic benefits by creating new value streams linked to ecosystem services, expanding market opportunities for products, and improving production stability through improved environmental conditions. Restorative aquaculture can create important livelihoods, particularly in developing regions and coastal communities with limited economic development opportunities, while also addressing environmental degradation issues (The Nature Conservancy, 2021).

Community participation is a critical success factor for restorative aquaculture activities. Unlike traditional aquaculture activities, which sometimes encounter resistance and opposition from local communities, restorative aquaculture approaches have greater potential for community participation and support. Involving local communities in planning, implementation, and benefit sharing ensures that activities are aligned with local values and priorities while also helping to distribute benefits more equitably. The Nature Conservancy emphasizes that "the participation of all stakeholders will be critical to the meaningful scale-up of restorative aquaculture" (The Nature Conservancy, 2021).

Market dynamics and consumer preferences are becoming increasingly important for the development of restorative aquaculture. Consumers' growing awareness and concerns about the environmental and social impacts of food production are creating expanding market opportunities for sustainably produced food products. Certification programs, sustainability standards, and

traceability systems, which have become widespread in the aquaculture sector in recent years, provide mechanisms to communicate the restorative benefits of products to consumers and potentially create market differentiation and premium pricing opportunities. Recent research shows that consumers want more information about the origin and production methods of seafood. This indicates strong market potential for restorative aquaculture products (Schrobback et al. 2025).

Policies and regulators significantly influence the development of restorative aquaculture activities. Supportive policies, financial incentives (such as subsidies, low-interest loans, or grants), technical assistance, and simplified permitting processes can encourage the adoption of these approaches.

Gender equality and increased participation of women in economic life are recognized as important factors in the development of aquaculture. In many regions, women play important roles in the aquaculture value chain but face constraints in accessing resources, credit, education, and decision-making authority. Removing these constraints and ensuring equal participation and benefit sharing can increase the social sustainability of restorative aquaculture initiatives and contribute to broader social development goals (Adam and Njogu, 2023).

Education and capacity building are fundamental to the widespread adoption of restorative aquaculture approaches. Technical training programs can increase management efficiency and improve farmers' ability to implement these systems. Similarly, educating consumers about the environmental benefits of restorative aquaculture can help build market support for these approaches. Academic institutions and research organizations play important roles in developing the scientific basis for restorative approaches, while also playing important roles in training the next generation of practitioners and researchers.

CONCLUSION

Restorative aquaculture overcomes many of the challenges faced by traditional aquaculture while also providing significant environmental benefits. Although it shares many similarities with polyculture aquaculture, restorative aquaculture goes beyond sustainability by contributing to the improvement of ecosystems through activities such as water filtration, nutrient capture, habitat provision, and enhancing ecosystem functions through carbon sequestration.

With this structure, it is seen as a solution-oriented resource in addressing issues such as food security, climate change, and ocean health.

Restorative aquaculture practices require targeted technological systems, supportive legal regulations, economic incentives, and continuous research and development. These enabling factors will maximize the potential of restorative approaches, increasing the social acceptance of aquaculture alongside food production.

Restorative aquaculture will play an increasingly important role in global food systems, especially considering the effects of climate change on terrestrial agriculture and fisheries. It is clear that aquaculture has great potential when you consider that the 50 million square kilometers of ocean covering most of the Earth's surface is a suitable resource for restorative aquaculture (The Nature Conservancy, 2023). Realizing this potential will require coordinated efforts among researchers, industry participants, regulators, and communities. Restorative aquaculture offers a way to improve rather than degrade ocean ecosystems by creating positive feedback between food production and environmental health. By adopting this approach, it should be remembered that aquaculture activities are not merely a food production method, but also an agricultural practice that contributes to cleaner waters, healthier ecosystems, and more resilient coastal communities.

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CHAPTER 11

EFFECTS OF MARINE BROWN MACROALGAE AS FUNCTIONAL FEED ADDITIVES ON HEMATOLOGICAL PARAMETERS OF FISH

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INTRODUCTION

Aquaculture is one of the rapidly growing sectors worldwide. This growth is related to increasing seafood demand and reduced availability of wild marine populations. The interest has shifted toward feed additives that can support growth, health, and disease resistance in fish as the industry expands. For this reason, sustainable aquaculture is becoming important. The development of farming methods and fish health is essential for sustainable systems. (Stentiford et al., 2020). Hematological parameters, including erythrocyte count, hemoglobin level, and hematocrit, are widely used indicators for monitoring the physiological condition of fish (Mohammadian et al., 2025; Yeganeh & Adel, 2019). Immune parameters show immune activity and the ability to respond to stressors or pathogens (Ashour et al., 2020; Cortes et al., 2024; Gupta et al., 2021; Rani et al., 2020). Changes in these values indicate alterations related to stress, disease, or nutritional factors. These parameters are therefore used to assess the effects of feed additives on fish health.

Marine macroalgae are used as functional feed supplements in aquaculture (Gazali et al., 2023; Holdt and Kraan, 2011; Remya et al., 2022; Zbakh et al., 2020). These multicellular phototrophic organisms distribute mainly in littoral zones. Their biomass contains polysaccharides, proteins, polyunsaturated fatty acids, vitamins, minerals, and secondary metabolites (Figure 1). Macroalgae also have been reported to possess several beneficial activities such as immunostimulatory, antiinflammatory, antioxidant, antibacterial and growth enhancement properties (Biris-Dorhoi et al., 2020; Holdt & Kraan, 2011). When added to fish diets, they supply nutrients such as vitamins and minerals. They also contain compounds that can support immune activity (Abdelrhman et al., 2022; Maghawri et al., 2023; Zulfiqar et al., 2025). This review presents studies investigating the effects of marine brown macroalgae and brown algal polysaccharides on hematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, MCHC) in several fish species. The available experiments include freshwater and marine species and report outcomes based on algal species, extract type, dose, and feeding duration. These compiled data may serve as a reference for researchers studying the hematological effects of macroalgae in fish.

2. BROWN MACROALGAE

Marine macroalgae are multicellular organisms that live in coastal ecosystems. They are classified into three major taxonomic groups according to their dominant pigment composition (Dos Santos et al., 2021). Within these groups, brown algae contain not only chlorophyll a and c. They also contain brown colored pigment fucoxanthin (Sun et al., 2024). About 1,800 species of brown macroalgae have been identified worldwide. These species include small foliose forms as well as large kelps that may reach several tens of metres in length (Pereira, 2021). Their structural diversity and having a wide range of bioactive compounds make them important for ecological and biotechnological studies (Ak, 2023; Pereira, 2021). They contain a range of nutrients. The amounts of these nutrients vary depending on taxonomy, species, and local conditions. In brown macroalgae, carbohydrates constitute a large portion of the dry weight, followed by moderate protein levels, low lipid content, and essential amounts of minerals and dietary fibre (Biris-Dorhoi et al., 2020; Holdt & Kraan, 2011). Carbohydrate levels are usually 30–50%, protein levels range between 5-35%, and lipid levels are generally 1-5% (Healy et al., 2023; Öztaşkent & Ak, 2021; Türker et al., 2022). Nutritional differences exist among the brown algae, such as Cystoseira or Sargassum, which typically contain 5-15% protein (Ak et al., 2022; Healy et al., 2023; Öztaşkent & Ak, 2021). Lipid content is generally low (1–10%), but the lipid fraction contains polyunsaturated fatty acids (PUFAs). Some brown algae contain arachidonic acid (20:4 n-6) and EPA (20:5 n-3) (Öztaşkent & Ak, 2021).

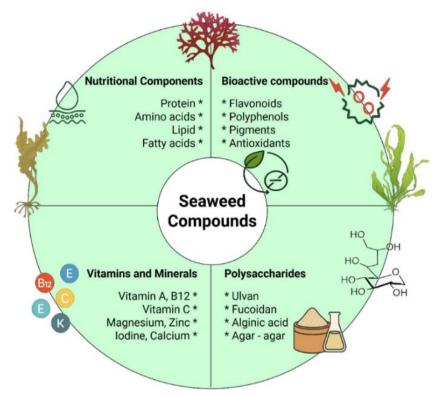


Figure 1. Main compound groups found in marine macroalgae.

One of the most notable biochemical features of brown macroalgae is the complex polysaccharides they produce in a species-specific manner. These compounds can have both structural and storage functions (Jönsson et al., 2020; Pereira, 2021). They mainly contain alginate, laminarin, and fucoidan in their cell walls. Alginate provides structural support; laminarin is a storage polysaccharide made of glucose whereas fucoidan is a sulfated, fucose-rich polysaccharide (Healy et al., 2023; Jönsson et al., 2020). The structural properties of these polysaccharides depend on several features, such as monosaccharide composition, linkage type, degree of sulfation, branching, and solubility. For example, alginates in brown algae are made of blocks of α -L-guluronic acid and β -D-mannuronic acid in different ratios, and this ratio directly affects their gel-forming capacity (Ak, Koru, et al., 2022; Jönsson et al., 2020). Laminarin is a β -(1 \rightarrow 3)-glucan made of glucose units. It has a branched structure with short side chains, works as a storage polysaccharide, and is easily soluble in water. Fucoidan is rich in fucose and shows different

sulfation patterns and substantial structural variability. The position of sulfate groups is a key factor influencing the biological activity of fucoidan (Ak et al., 2022; Jönsson et al., 2020; Peñalver et al., 2020).

On the other hand, brown macroalgae have a relatively high ash content, indicating a rich mineral composition. The ash content is usually about 15–40% of dry weight and is much higher than in land plants (Ak et al., 2022; Healy et al., 2023; Türker et al., 2024). Because they take up minerals directly from seawater, they can accumulate high levels of essential elements, including iodine, calcium, potassium, magnesium, sodium, iron, and zinc. Brown algae contain much higher iodine levels than the other macroalgal groups (Healy et al., 2023). They are also rich in vitamins A, B-group, C, and E (Caf et al., 2019; Peñalver et al., 2020). The antioxidant vitamins in brown algae, together with polyunsaturated fatty acids in the lipid fraction, may help to reduce oxidative stress (Ak & Turker, 2018a, 2018b). They are not only rich in basic nutrients but also contain many different bioactive compounds. These include polyphenols, carotenoids, phlorotannins, flavonoids, terpenes, sterols, alkaloids and phenolic acids (Ak & Turker, 2018b; Ak et al., 2022; Monroy-García et al., 2025). Phlorotannins are found only in brown algae and are polymerized phenolic compounds; together with fucoxanthin, they are characteristic of this group (Monroy-García et al., 2025). Carotenoids occur in all macroalgal groups; however, fucoxanthin is especially abundant in brown algae (Peñalver et al., 2020). These compounds are protective metabolites produced by them to cope with environmental stress. They are also of interest in human nutrition and pharmaceutical applications due to their potential health effects. They have also anti-inflammatory, anticancer, antibacterial and antiviral effects (Cotas et al., 2020; Subbiah et al., 2023).

3. BASIC HEMATOLOGICAL PARAMETERS

Hematological parameters used to evaluate fish health provide information not only on the general physiological condition of the fish but also on their responses to environmental conditions, feeding strategies, and potential pathogenic stressors (Ahmed & Thompson, 2019). Fish hematology commonly includes measurements of several blood parameters. These include red and wite blood cell counts (RBC and WBC), hemoglobin (Hb), hematocrit (Hct), and the erythrocyte indices MCV, MCH, and MCHC.

3.1. Red Blood Cell Count (RBC)

Red blood cells, or erythrocytes, are responsible for oxygen carriage and metabolic functions (Esmaeili, 2021). These cells are nucleated and have a distinct red color due to hemoglobin which is complex compound composed of globin, a colorless protein part, and colored heme pigment with iron (Esmaeili, 2021; Nazarudin et al., 2020). The magnitude of RBCs is a critical biomarker in fish, representing their oxygen carrying ability and physiological reactions to multiple environmental stressors (Esmaeili, 2021; Madibana et al., 2017). Hence, RBC as a parameter associated with respiratory physiology is commonly used in the determination of fish health (Cortes et al., 2024; Esmaeili, 2021; Sattanathan et al., 2024). In the literature, normal RBC levels in fish have been reported as 0.4-5.2×106/mm³ (Esmaeili, 2021).

Brown algae can produce both increasing and decreasing responses in RBC values, depending on species and dose (Table 1). Gazali et al. (2023) reported that in O. niloticus, Sargassum sp. at 2, 2.5, and 3 g/kg increased RBC levels significantly after 30 days (p<0.05). The highest RBC value was 1.76±0.03×106 cell/mm³ at 3 g/kg. Yeganeh and Adel (2019) tested 5, 7.5, and 10% Sargassum ilicifolium in Huso huso. They found that only the 10% dose increased RBC (1.22±0.12×10⁶/mm³) significantly compared to the control $(1.05\pm0.09\times10^6/\text{mm}^3)$ (p<0.05). And lower doses did not show significant differences (p>0.05). Zulfigar et al. (2025) reported that in *Labeo rohita*, all doses (3, 5, and 7%) of S. ilicifolium significantly increased RBC compared to the control $(3.750\pm0.250\times10^{-6}/\mu l)$ (p=0.0088**), and RBC showed a dosedependent increase, reaching the highest value at 7% (5.250±0.250×10⁻⁶/ μ l). In contrast, Yangthong et al. (2022) found that in Scatophagus argus, Sargassum sp. at 0.25-1.0 g/kg produced numerical increases in RBC (5.07±1.05-6.17±0.9×10¹⁰ cell/ml at 0–1 g/kg), but these differences were not statistically significant (p>0.05). Mohammadian et al. (2025) found that when fed to *Lates* calcarifer the diets containing 5 and 10% Spirulina + Sargassum resulted in a significant decrease of RBC from the control group (2.1±0.4×10⁶/μl) to $1.9\pm0.1\times10^{6}$ /µl at the end of experiment for fish fed with 10% treatment level. The decrease was, however, not statistically significant (p>0.05). In contrast, Maghawri et al.

Table 1. Changes in RBC, WBC and Hb of freshwater and marine fish fed diets supplemented with brown macroalgae WDC

Algae Species	Fish	Dose	RBC WBC		Hb	References
	Species					
Sargassum sp.	О.	0 g/kg	1.40±0.04 ^a ×10 ⁶ cell/mm ³	$1.89\pm0.01^{ab}\times10^{4}cell/mm^{3}$	7.20±0.20a g/dl	Gazali et al.
	niloticus	2 g/kg	$1.57\pm0.04^{b}\times10^{6} \text{ cell/mm}^{3}$	$1.91\pm0.01^{b}\times10^{4}cell/mm^{3}$	$7.67\pm0.12^{b}g/dl$	(2023)
		2.5g/kg	$1.65\pm0.03^{c}\times10^{6} \text{ cell/mm}^{3}$	$1.94\pm0.02^{c}\times10^{4}cell/mm^{3}$	$7.73\pm0.12^{b}g/dl$	
		3 g/kg	$1.76\pm0.03^{d}\times10^{6}~cell/mm^{3}$	$1.99\pm0.02^{d}\times10^{4}cell/mm^{3}$	$7.93\pm0.12^{b}g/dl$	
S. ilicifolium	H. huso	0 %	$1.05\pm0.09^{a}\times10^{6}/mm^{3}$	$11.84\pm0.42^{a} \times 10^{3}$ /mm ³	8.62±0.26ª g/dl	Yeganeh and
		5 %	$1.14 \pm 0.08^{ab} \times 10^{6/}mm^3$	$11.96{\pm}0.53^a{\times}10^3/mm^3$	$8.78{\pm}0.20^{a}g/dl$	Adel (2019)
		7.5 %	$1.18{\pm}0.10^{ab}{\times}10^{6}/mm^{3}$	$12.22{\pm}0.64^{ab}{\times}10^{3}/mm^{3}$	$9.02\pm0.16^{b}g/dl$	
		10 %	$1.22{\pm}0.12^b{\times}10^6/mm^3$	$12.56 \pm 0.82^{b} \times 10^{3} / mm^{3}$	$9.36 \pm 0.22^{b} \text{ g/dl}$	
S. ilicifolium	L. rohita	0 %	$3.75\pm0.25^{d}\times10^{-6}/\mu1$	$4.85\pm0.15^{d} \times 10^{3}/\mu l$	12.30±0.20 ^d g/dl	Zulfiqar et al.
		3 %	$4.00\pm0.30^{c}\times10^{-6}/\mu1$	$8.45{\pm}0.45^{c}{\times}10^{3}/\mu l$	$12.90\pm0.10^{bc}g/dl$	(2025)
		5 %	$4.20{\pm}0.40^{b}{ imes}10^{-6}/\mu 1$	$9.85{\pm}0.45^{b}{ imes}10^{3}/{\mu}l$	$13.00\pm0.30^{b}g/dl$	
		7 %	$5.25{\pm}0.25^a{ imes}10^{-6}/\mu 1$	$11.45{\pm}0.45^a{\times}10^3/\mu l$	$16.50\pm0.50^{a}g/dl$	
Sargassum sp.	S. argus	0 g/kg	$5.07\pm1.05^{a}\times10^{10}cell/ml$	$6.84 \pm 1.58^{a} \times 10^{8} \text{ cell/ml}$	NA	Yangthong et
		0.25 g/kg	$5.39 \pm 1.42^{a} \times 10^{10} cell/ml$	$6.02\pm0.81^{a}\times10^{8} \text{ cell/ml}$	NA	al. (2022)
		0.5 g/kg	$5.68 \pm 1.38^a \times 10^{10} cell/ml$	$6.02\pm0.95^{a}\times10^{8} \text{ cell/ml}$	NA	
		0.75 g/kg	$5.35\pm1.55^{a}\times10^{10}cell/ml$	$6.19\pm0.40^{a}\times10^{8} \text{ cell/ml}$	NA	
		1 g/kg	$6.17\pm0.9^a \times 10^{10} \text{ cell/ml}$	$6.17\pm0.91^{a}\times10^{8} \text{ cell/ml}$	NA	
Sargassum +	L.	0 %	$2.1\pm0.4 \times 10^{6}/\mu l$	$3.3\pm0.6^{b} \times 10^{3}/\mu l$	6.4 ±0.5 g/dl	Mohammadian
Spirulina	calcarifer	5 %	$2.0{\pm}0.3{ imes}10^6/\mu l$	$6.6{\pm}0.5^{\rm a}{\times}10^{3}/\mu l$	6.0±0.3 g/dl	et al. (2025)
		10 %	$1.9 \pm 0.1 \times 10^6 / \mu l$	$3.5{\pm}0.3^b \times 10^3/\mu l$	6.4±0.4 g/dl	

NA: Not available. Superscript letters indicate statistically significant differences reported in the original studies.

Table 1 continued

Algae Species	Fish Species	Dose	RBC	WBC	Hb	References
P. pavonica O. niloticus		0 g/kg	$1.84\pm0.06^{b}\text{mm}^{3}$	58.1±0.31° μl	$9.55\pm0.78^{d}g/dl$	Maghawri et al.
		2 g/kg	$2.55{\pm}0.05^{a}mm^{3}$	92.3 \pm 0.34 $^{\mathrm{a}}\mu l$	$12.87{\pm}0.05^{a}g/dl$	(2023)
		4 g/kg	$1.60\pm0.03^{\circ}\text{mm}^{3}$	$34.6{\pm}0.5^{d}\mu l$	$7.68\pm0.04^{e}g/dl$	
		6 g/kg	$1.77{\pm}0.04^bmm^3$	$21.98 \pm 0.09^{e} \mu l$	$10.6 \pm 0.04^{b} g/dl$	
		8 g/kg	$1.81{\pm}0.04^{b}mm^{3}$	$71.7{\pm}0.45^{\rm b}\mu l$	$9.94{\pm}0.08^{c}g/dl$	
D. ciliolata	C. gariepinus	0 g/kg	$2.53\pm0.25^{a}\times10^{6}/\mu l$	137.07±15.96 ^a 10 ³ /μl	9.63±0.65ª g/dl	Aderolu et al.
		0.25 g/kg	$3.23{\pm}0.21^b{\times}10^6{/\mu}l$	$164.53{\pm}12.27^b10^3/\mu l$	$11.80 \pm 1.15^{b} g/dl$	(2025)
		0.5 g/kg	$2.90{\pm}0.1^{ab}10^6/\mu l$	$145.27{\pm}4.37^{ab}\ 10^{3}/\mu l$	$11.93{\pm}1.50^{b}g/dl$	
		1 g/kg	$2.60{\pm}0.30^{\rm a}10^{\rm 6}/\mu l$	$142.63{\pm}12.69^a10^3/\mu l$	$10.57{\pm}0.76^{ab}$	
					g/dl	
S. muticum	O. niloticus	0 ml/kg	$0.203\pm0.173 \times 10^{12}/1$	$4.43\pm2.57 \times 10^9/1$	31.00±13.89 g/l	Cortes et al.
		15 ml/kg	$0.147{\pm}0.10{\times}10^{12}/l$	$2.80\pm2.10 \times 10^9/1$	17.00±9.82 g/l	(2024)

NA: Not available. Superscript letters indicate statistically significant differences reported in the original studies.

(2023) found an increase in RBC count from 1.84±0.06 mm³ in the control group of O. niloticus to 2.55±0.05 when the diet was supplemented with 2 g/kg Padina pavonica (p<0.05). However, higher concentration of P. pavonica at 4, 6, and 8 g/kg resulted in reduction of RBC count when compared to control groups. The decrease at 4 g/kg was significant (p<0.05), suggesting a possible adverse effect at higher doses. For *Dictyota ciliolata*, Aderolu et al. (2025) reported that in Clarias gariepinus, RBC reached the maximum value at 0.25 g/kg (3.23±0.21×10⁶/μl), which was significantly higher than the control $(2.53\pm0.25\times10^6/\mu l)$ (p<0.05). No significant changes were found at 0.5 and 1 g/kg (p>0.05). In a comparative study including several algae (Cortes et al., 2024), it was reported that in O. niloticus, Sargassum muticum resulted in the lowest RBC value (0.147±0.10×10¹²/l), while Kappaphycus alvarezii (15 ml/kg) increased RBC from 0.203±0.173×10¹²/l in the control to 0.313±0.188×10¹²/l, giving the highest RBC value in the trial. The same study showed that *Ulva reticulata* produced an RBC value (0.31±0.19×10¹²/l) similar to the control. The study reported an optimal RBC range of 3.8–5.8 for O. niloticus and found that the measured values did not fall within this range, suggesting that the experimental conditions may have influenced erythrocyte counts.

Studies on brown algal polysaccharides, especially purified or fractionated forms, have increased in recent years, and their effects on RBC levels have been widely investigated (Table 2). These studies show that these compounds can modulate RBC responses in fish, both alone and when combined with environmental stressors. Abdel-Warith et al. (2021) found that in O. niloticus, the control RBC value was 3.72±0.11×106/mm³. Fucoidan increased RBC to 4.17±0.04×10⁶/mm³ (p<0.05), which was the highest value. Fish fed the basal diet and exposed to atrazine showed the lowest RBC level at 3.14±0.06×10⁶/mm³ (p<0.05). In the fucoidan + atrazine group, RBC was 3.64±0.04×10⁶/mm³, and this value was not different from the control but was significantly higher than the atrazine group (p<0.05). Rani et al. (2020) examined fucoidan fractions (0.1, 0.2, and 0.3%) obtained from Padina tetrastromatica and Sargassum oligocystum in O. mossambicus for 15, 30, 45, and 60 days. Fucoidan from P. tetrastromatica increased RBC from $2.76\pm0.12\times10^{6}/\mu l$ (control) to $2.86\pm0.02\times10^{6}/\mu l$ at day 60. Fucoidan from S. oligocystum produced RBC values between 2.54±0.13 and 2.71±0.08×10⁶/μl.

However, none of these changes were statistically significant (p>0.05). Abdelrhman et al. (2022) tested *Sargassum dentifolium* polysaccharides (SP; 0, 10, 20, and 30 g/kg) in Red tilapia and reported significant differences among doses. The lowest RBC value was 10 g/kg, while 30 g/kg produced the highest RBC level and was statistically distinct from the other groups. In general, increasing the SP dose increased RBC, except at the lowest dose (p<0.05).

3.2. White Blood Cell Count (WBC)

White blood cells (leukocytes) are essential indicators of immune function and general health in fish. Their numbers are lower than erythrocytes. Reported values range from 10–116.5×10³/μl, depending on species and physiological condition (Esmaeili, 2021; Gazali et al., 2023). Leukocytes occur as agranulocytes (lymphocytes, monocytes, thrombocytes) and granulocytes (neutrophils, eosinophils, basophils), each contributing to immune defense through antibody production, phagocytosis, or clotting functions (Adel et al., 2021; Yeganeh & Adel, 2019; Zulfiqar et al., 2025). Neutrophils and lymphocytes are the predominant cell types involved in innate immune responses, and their proportions can shift during infection or stress. Leukocyte levels often increase under acute stress or pathogen exposure and decrease when immune function is suppressed (Adel et al., 2021; Yeganeh & Adel, 2019). Consequently, the white blood cell (WBC) counts are widely used as biomarkers for assessing both physiological stress and disease status in fish (Adel et al., 2021; Cortes et al., 2024; Maghawri et al., 2023).

In recent years, studies on the effect of different algae and their preparations have demonstrated that changes in WBC levels were species dependent as well to dose dependent. Therefore, marine brown macroalgae as feed additives have been increasingly considered in several aquaculture studies evaluating their effects on immune parameters. The main findings of these studies, specifically in relation to WBC level in fish stressed being exposed to brown algae, are presented in Table 1. In *Oreochromis niloticus*, *Sargassum* sp. at 2, 2.5, and 3 g/kg increased WBC from $1.89\pm0.01\times10^4$ cell/mm³ in the control to $1.99\pm0.01\times10^4$ cell/mm³ at 3 g/kg, and these increases were significant (p<0.05) (Gazali et al., 2023). In *Huso huso*, only the 10% dose of *Sargassum ilicifolium* increased WBC to $12.56\pm0.82\times10^3/\text{mm}^3$ compared to the control value of

Table 2. Changes in RBC, WBC and Hb of freshwater and marine fish fed diets supplemented with brown algal polysaccharides

Algae Species	Fish Species	Dose	RBC	WBC	Hb	References
Fucoidan	O. niloticus	Control	$3.72\pm0.11^{b}\times10^{6}\text{mm}^{3}$	39.19±0.50b	11.90±0.15 ^b	Abdel-
				$\times 10^3 / \text{mm}^3$	g/100 ml	Warith et al.
		Fucoidan	$4.17\pm0.04~^{a}\times10^{6}mm^{3}$	41.39 ± 0.52^a	12.49 ± 0.19^a	(2021)
				$\times 10^3 / \text{mm}^3$	g/100 ml	
		Basal diet	$3.14\pm0.06~^{c}\times10^{6}mm^{3}$	35.67 ± 0.57^{c}	10.01 ± 0.18^d	
		and Atrazine		$\times 10^3 / \text{mm}^3$	g/100 ml	
		Fucoidan	$3.64\pm0.04^{b}\times10^{6}mm^{3}$	$38.48\pm0.69^{b}\times10^{3}/mm^{3}$	11.05±0.11°	
		and Atrazin			g/100 ml	
Fucoidan	О.	0 %	2.76±0.12 10 ⁶ /μl	3308±22/μl	5.59±0.13 g/dl	Rani et al.
(P.	mossambicus	0.1 %	$2.75\pm0.08\ 10^6/\mu l$	$3573\pm23/\mu l$	5.61±0.03 g/dl	(2020)
tetrastromatica)		0.2 %	$2.78\pm0.06\ 10^6/\mu l$	$3580{\pm}15/\mu l$	5.69±0.13 g/dl	
		0.3 %	$2.86\pm0.02\ 10^6/\mu l$	$3817{\pm}33/\mu l$	$5.67 \pm 0.02 \text{ g/dl}$	
Fucoidan (S.	О.	0 %	2.70±0.15×10 ⁶ /μl	$3313\pm90\times10^{3}/\text{mm}^{3}$	5.54±0.06 g/dl	Rani et al.
oligocystum)	mossambicus	0.1 %	$2.54{\pm}0.13 \times 10^6/\mu l$	$3443\pm18 \times 10^{3}/mm^{3}$	5.62±0.11 g/dl	(2020)
		0.2 %	$2.59{\pm}0.08 \times 10^6/\mu l$	$3383\pm90 \times 10^{3} / \text{mm}^{3}$	5.64±0.01 g/dl	
		0.3 %	$2.71{\pm}0.08 \times 10^6/\mu l$	$3453\pm36 \times 10^{3}/mm^{3}$	5.69±0.12 g/dl	
Polisaccharides	О.	0 g/kg	$206.50\pm17.50^{ab}\times10^{6}/\mu l$	NA	40.93±9.90° g/dl	Abdelrhman
(Sargassum	mossambicus	10 g/kg	$155.67 \pm 44.79^{c} \times 10^{6}/\mu l$	NA	$31.77\pm3.66^{\circ}g/dl$	et al. (2022)
dentifolium)	\times O .	20 g/kg	$199.33 \pm 58.69^{b} \times 10^{6}/\mu l$	NA	$39.10\pm7.76^{b}g/dl$	
	niloticus	30 g/kg	$259.00\pm42.00^{a}\times10^{6}/\mu l$	NA	$38.20 \pm 1.95^{b} \text{ g/dl}$	

nuoucus 50 g/kg 259.00±42.00"×10"/μι NA 38.20±1.95" g/dl

NA: Not available. Superscript letters indicate statistically significant differences reported in the original studies.

11.84±0.42×10³/mm³ (p<0.05) (Yeganeh & Adel, 2019). In *Labeo rohita*, Sargassum ilicifolium at 3, 5, and 7% significantly increased WBC compared to the control $(4.85\pm0.15\times10^3/\mu l)$ (p=0.0012**), and the highest WBC value was recorded at 7% with $11.45\pm0.45\times10^3/\mu l$ (Zulfigar et al., 2025). In Scatophagus argus, the control group showed the highest WBC value (6.84±1.58×10⁸ cell/ml), and all doses of the extract produced lower levels. Still, these changes were not statistically significant (p>0.05) (Yangthong et al., 2022). In mixed applications, Spirulina + Sargassum (5 and 10%) in Lates calcarifer increased WBC from $3.3\pm0.6\times10^3/\mu l$ in the control, with the highest value at 5% $(6.6\pm0.5\times10^{3}/\mu l)$. The 5% dose $(6.6\pm0.5\times10^{3}/\mu l)$ showed a significant increase compared with the control (p<0.05), whereas the 10% dose $(3.5\pm0.3\times103/\mu l)$ did not differ from the control (p>0.05) (Mohammadian et al., 2025). In O. niloticus, Padina pavonica at 2 and 8 g/kg significantly increased WBC (p<0.05), with the highest value at 2 g/kg (92.3±0.34 μl). In contrast, 4 and 6 g/kg significantly decreased WBC (p<0.05), with the lowest value at 6 g/kg (21.98±0.09 µl) (Maghawri et al., 2023). In Clarias gariepinus, Dictyota ciliolata at 0.25 g/kg produced the maximum WBC value of $164.53\pm12.27\times10^{3}/\mu l$ compared to the control at $137.07\pm15.96\times10^{3}/\mu l$ (p<0.05). Only the 0.25 g/kg dose showed a significant increase; the 0.5 and 1 g/kg doses did not differ from the control (p>0.05) (Aderolu et al., 2025). Cortes et al. (2024) evaluated different algal groups in O. niloticus. With the brown alga Sargassum muticum, WBC decreased to 2.80±2.10×10⁹/l compared to the control (Table 1). With the red alga Kappaphycus alvarezii, WBC decreased more strongly and remained far below control values. In contrast, in the green alga *Ulva reticulata*, WBC increased to 9.27±9.27×10⁹/l, a significant increase compared to the control. The study stated that the optimum WBC range for O. niloticus is $4-10\times10^9/1$.

The effects of brown algal polysaccharides on WBC levels in fish have been demonstrated mainly through studies using fucoidan fractions (Table 2). Fish exposed to atrazine showed a significant decrease, while the fucoidan + atrazine group did not differ from the control but remained higher than the atrazine-only group (Abdel-Warith et al., 2021). Similar trends were reported in studies using sulfated polysaccharide fractions from different algal sources. In *O. mossambicus*, fucoidan fractions (0.1, 0.2, and 0.3%) from *Padina tetrastromatica* and *Sargassum oligocystum* were tested (Rani et al., 2020).

WBC values were higher than in the control group, but none of the increases were statistically significant (p>0.05). At day 60, WBC increased from $3308\pm22\times10^3/\mu l$ in the control to $3817\pm33\times10^3/\mu l$ at 0.3% in *P. tetrastromatica*. In *S. oligocystum*, values ranged from $3313\pm90\times10^3/\mu l$ in the control to $3453\pm36\times10^3/\mu l$ at 0.3%. None of the increases were significant (p>0.05).

2.3. Hemoglobin (Hb)

Hemoglobin (Hb) is the primary molecule responsible for transporting oxygen in fish blood, and its concentration largely determines the oxygen-carrying capacity of the circulatory system (Esmaeili, 2021). Most of the oxygen in circulation is carried by Hb rather than dissolved in plasma (Cortes et al., 2024; Esmaeili, 2021). Beyond oxygen transport, Hb also contributes to carbon dioxide exchange and helps maintain acid–base balance. Reductions in Hb are commonly associated with anemia, nutritional deficiencies, or impaired physiological status (Cortes et al., 2024; Esmaeili, 2021; Nazarudin et al., 2020). Because of its close association with respiratory efficiency and overall metabolic condition, Hb level is widely used as a biomarker for evaluating fish health and physiological stress responses (Sattanathan et al.,2024). Recent findings indicate that macroalgal supplements can alter Hb levels in a species-and dose-dependent manner.

Findings on the effects of brown algae on fish hemoglobin (Hb) show both increasing and decreasing responses depending on species and dose (Table 1). In *Oreochromis niloticus* fed *Sargassum* sp., Hb in the control group was 7.20±0.20 g/dl, while at 2, 2.5, and 3 g/kg it increased to 7.67±0.12, 7.73±0.12, and 7.93±0.12 g/dl, and all increases were significant (p<0.05) (Gazali et al., 2023). In *Huso huso* fed *Sargassum ilicifolium*, Hb increased from 8.62±0.26 g/dl in the control to 9.02±0.16 and 9.36±0.22 g/dl at 7.5% and 10% doses, and these increases were significant (p<0.05) (Yeganeh & Adel, 2019). In *Labeo rohita*, *S. ilicifolium* at 3, 5, and 7% increased Hb significantly from the control value of 12.30±0.20 g/dl (p=0.022*), and the highest value was recorded at 7% with 16.50±0.50 g/dl (Zulfiqar et al., 2025). In *Lates calcarifer* fed *Spirulina* + *Sargassum* mixtures, Hb values ranged from 6.0±0.3–6.4±0.4 g/dl, and neither 5% nor 10% doses produced significant changes compared to the control (p>0.05) (Mohammadian et al., 2025). *Padina pavonica*, of the same species, showed a more precise dose-response. Hb increased significantly at 2, 6, and 8

g/kg (p<0.05), with the highest value at 2 g/kg (12.87±0.05 g/dl). At 4 g/kg, Hb decreased below the control and showed a significant reduction (p<0.05) (Maghawri et al., 2023). In *Clarias gariepinus* fed *Dictyota ciliolata*, Hb increased from 9.63±0.65 g/dl in the control to 11.80±1.15 and 11.93±1.50 g/dl at 0.25 and 0.5 g/kg, and these increases were significant (p<0.05) (Aderolu et al., 2025). Cortes et al. (2024) reported that in *O. niloticus*, Hb was 31.00±13.89 g/l in the control and 17.00±9.82 g/l with *Sargassum muticum* (Table 1). In contrast, the red alga *Kappaphycus alvarezii* produced the highest Hb value (48.0±14.53 g/l). The green alga *Ulva reticulata* produced a similar increase (31.33±12.24 g/l). The authors noted that the Hb values did not reach the normal range for hemoglobin concentration (Cortes et al., 2024).

Studies evaluating the effects of sulfated polysaccharide fractions on hemoglobin (Hb) show dose- and time-dependent responses (Table 2). Abdel-Warith et al. (2021) reported that Hb was highest in the fucoidan group, while atrazine produced the lowest value; the fucoidan + atrazine group showed an intermediate level, lower than the control but higher than the atrazine-only group (p<0.05). In *O. mossambicus*, fucoidan fractions from *Padina tetrastromatica* and *Sargassum oligocystum* (0.1, 0.2, and 0.3%; 15, 30, 45, 60 days) produced limited changes. In *P. tetrastromatica* fractions, Hb increased from 5.59±0.13 (control) to 5.67±0.02 g/dl (0.3 %). In *S. oligocystum* fractions, Hb ranged between 5.54±0.06 (control) and 5.69±0.12 g/dl (0.3 %). None of these changes were statistically significant (p>0.05) (Rani et al., 2020). Hb in Red tilapia fed *Sargassum dentifolium* polysaccharides was 40.93±9.90 g/dl in the control, and it showed the lowest value at 31.77±3.66 g/dl at 10 g/kg (p<0.05); the 20 and 30 g/kg groups' values were lower than the control but not as low as the 10 g/kg group (Abdelrhman et al., 2022).

3.4. Hematocrit (Hct)

Hematocrit (Hct) is the percentage of red blood cells (erythrocytes) in the total blood volume. It is often used to diagnose anemia and is preferred because it is easy to measure (Esmaeili, 2021; Magnoni et al., 2017). Hct decreases in cases such as poor nutrition and anemia, and increases in dehydration (Baleta & Bolaños, 2019). Stress factors can also increase hematocrit levels. For this reason, Hct can be used as an indicator of the

organism's secondary physiological responses to environmental pollutants and irritant substances (Baleta & Bolaños, 2019; Magnoni et al., 2017).

Studies on the effects of brown algae on Hct show clear species- and dose-dependent patterns (Table 3). Diets containing Sargassum sp. increased Het in *Oreochromis niloticus*: the control value was 28.33±0.58%, and feeding 2.5 and 3 g/kg raised Hct to 31.00±1.00% and 31.67±0.67%, respectively (p<0.05) (Gazali et al., 2023). When Huso huso received Sargassum ilicifolium at 5–10%, Hct values stayed close to the control (33.25±1.10%), with the highest value only slightly increasing to 33.72% at 10%, showing no significant difference (p>0.05) (Yeganeh & Adel, 2019). A stronger response was reported for Labeo rohita. Feeding this species with Sargassum ilicifolium at 3, 5, and 7% increased Hct from the control value of 35.50±0.50% to significantly higher levels at all doses (p=0.0001**), reaching $54.50\pm0.50\%$ at 7% (Zulfigar et al., 2025). Scatophagus argus showed a more stable pattern; supplementation with Sargassum sp. at 0.25–1.0 g/kg produced values between 35.53±2.94% (1 g/kg) and 36.80±1.44% (0.5 g/kg), close to the control and without significant differences (p>0.05) (Yangthong et al., 2022). A significant decrease in Hct value was found in *L. calcarifer* given Spirulina+Sargassum supplementation. The Hct fell from 44.0±3.0% in the control animals to become 34.2±2.2% at a concentration of 5% (p<0.05) (Mohammadian et al., 2025). Contrariwise, the addition of *Padina pavonica* to the same species produced diversified effects on Hct (both increment and significant decrease with dose). Significant increments were seen at 2,6 and 8 g/kg (p<0.05) with the highest Hct level of 42.18±0.24% from the 2 g/kg dosage. But a 4 g/kg dose decreased Hct to 23.34±0.07%, a value that was substantially different compared to the control group (Maghawri et al., in press). Cortes et al. (2024) reported that Sargassum muticum increased Hct in O. niloticus from 0.83±0.83% to 1.43±1.47% (Table 1). In another study, the macroalga species Kappaphycus alvarezii had an Hct value of $2.96\pm2.12\%$ and *Ulva reticulata* had an Hct value of $1.73\pm0.69\%$. The authors noted that none of these values were within the reported normal range of 30-50% for O. niloticus, again suggesting that experimental or environmental conditions could have influenced outcomes.

Studies on polysaccharide fractions from algae show dose-dependent patterns in Hct responses (Table 4). Fucoidan exposure in *O. niloticus* produced more precise shifts. The Hct value of the control group was 36.33±0.33%, and

fucoidan alone increased it to 39.00±0.58% (p<0.05). Atrazine reduced Hct to 32.67±0.88%, a significant decrease (p<0.05), while the fucoidan + atrazine group showed a value close to the control with no significant difference (p>0.05) (Abdel-Warith et al., 2021) In Red tilapia fed *Sargassum dentifolium* polysaccharides at 10, 20, and 30 g/kg, significant increases were recorded at 10 and 30 g/kg, with the highest value at 10 g/kg (42.77±8.16%; p<0.05). The remaining dose was similar to the control (Abdelrhman et al., 2022).

3.5. Mean Corpuscular Volume (MCV)

MCV (Mean Corpuscular Volume) is a parameter that expresses the average volume of erythrocytes. It is calculated with the formula Hct (%) × 10/RBC (106/mm³) (Aderolu et al., 2025). MCV is used to evaluate circulatory features such as heart rate and blood flow in fish. It is also a physiological parameter that reflects their osmoregulatory status (Manikandan et al., 2022). MCV may increase or decrease depending on the type of anemia. For this reason, it is regarded as an important part of hematological evaluation (Maghawri et al., 2023; Sotoudeh & Jafari, 2017).

Studies on the effects of brown algae on MCV show species- and dosedependent responses in Table 1. In Labeo rohita receiving Sargassum ilicifolium at 3, 5, and 7%, the control value was 77.50±0.20 fl, and all doses caused significant increases (p=0.0004**), reaching 98.50±0.50 fl at 7% (Zulfigar et al., 2025). Feeding Spirulina + Sargassum mixtures at 5% and 10% to Lates calcarifer produced a different pattern. The control MCV was 247.9±56.7 fl, and both doses resulted in values comparable to the control without significant differences (p>0.05) (Mohammadian et al., 2025). When Padina pavonica was added to the diet of Oreochromis niloticus at 2, 4, 6, and 8 g/kg, the control value was 162.86±0.25 μm³. Significant increases occurred at 2, 6, and 8 g/kg, with the highest value recorded at 2 g/kg ($203.54\pm0.88 \mu m^3$). At 4 g/kg, MCV decreased significantly to 147.82±0.24 μm³ (p<0.05) (Maghawri et al., 2023). In Clarias gariepinus fed Dictyota ciliolata at 0.25, 0.5, and 1 g/kg, the control MCV was 135.37±6.62 fl, and all doses produced values similar to the control without significant differences (p>0.05) (Aderolu et al., 2025).

Table 3. Changes in Hct, MCV, MCH and MCHC of freshwater and marine fish fed diets supplemented with brown macroalgae

Algae	Fish	Dose	Hct	MCV	MCH	MCHC	Refrences
Species	Species						
S.	L. rohita	0 %	35.50±0.50 ^d %	77.50±0.20 ^d fl	25.50±0.50 ^d pg	30.80±0.20 ^d %	Zulfiqar et al.
ilicifolium		3 %	45.75±0.75° %	$79.90\pm0.10^{c}\mathrm{fl}$	29.35±0.05° pg	$33.10\pm0.40^{\circ}\%$	(2025)
		5 %	$48.65 \pm 0.35^{b}\%$	$88.70{\pm}0.30^b{\rm fl}$	$31.10\pm0.40^{b}pg$	$35.05 \pm 0.75^{b} \%$	
		7 %	54.50±0.50 ^a %	$98.50{\pm}0.50^a{\rm fl}$	$34.50\pm0.50^{a}pg$	$37.50 \pm 0.50^{a} \%$	
Sargassum	L.	0 %	44.0±0.3ª%	247.9±56.7 fl	35.9±8.2 pg/cell	14.6±0.4 g/dl	Mohammadian
+ Spirulina	calcarifer	5 %	$34.2 \pm 2.2^{b} \%$	$182.1 \pm 16.4 \text{ fl}$	33.6±5.3 pg/cell	$18.1 \pm 1.6 \text{ g/dl}$	et al. (2025)
		10 %	$35.7 \pm 1.9^{b} \%$	190.5±15.7 fl	34.3±2.8 pg/cell	$18.3{\pm}1.6~g/dl$	
Р.	O. niloticus	0 g/kg	29.8±0.59 ^d %	$162.86\pm0.25^{d}\mu\text{m}^{3}$	62.38±0.31ª g/dl	30.71±0.38° g/dl	Maghawri et al.
pavonica		2 g/kg	42.18 ± 0.24^{a0} %	$203.54{\pm}0.88^a\mu m^3$	$62.57{\pm}0.08^ag/dl$	$33.5 \pm 0.08^{a} g/dl$	(2023)
		4 g/kg	$23.34 \pm 0.07^{e} \%$	$147.82 \pm 0.24^{e} \mu m^{3}$	$48.2{\pm}0.31^{d}g/dl$	$32.24\pm0.31^{b}g/dl$	
		6 g/kg	$31.87 \pm 0.09^{\circ} \%$	$187.38{\pm}0.27^b\mu m^3$	$49.9 \pm 0.35^{c} g/dl$	$30.56 \pm 0.08^{\circ} g/dl$	
		8 g/kg	$33.68 \pm 0.09^{b} \%$	$172,86\pm0,31^{c}\mu\text{m}^{3}$	$51.1 \pm 0.46^{b} g/dl$	$30.2 \pm 0.42^{c} g/dl$	
D. ciliolata	C.	0 g/kg	NA	135.37±6.62ª fl	29.13±1.15ª pg	NA	Aderolu et al.
	gariepinus	0.25 g/kg	NA	$127.30\pm5.99^{a}\mathrm{fl}$	$31.07\pm2.07^{a}pg$	NA	(2025)
		0.5 g/kg	NA	$123.07{\pm}12.9^afl$	$30.37{\pm}1.23^apg$	NA	
		1 g/kg	NA	$126.83{\pm}1.60^afl$	$30.97\pm3.43^{a}pg$	NA	
S. muticum	O. niloticus	0 ml/kg	0.83±0.83 %	NA	NA	NA	Cortes et al.

NA

NA

NA

(2024)

NA: Not available. Superscript letters indicate statistically significant differences reported in the original studies.

1.43±1.47 %

15 ml/kg

Data on the effects of polysaccharide-based applications on MCV in fish are limited, but the available studies indicate species- and dose-dependent responses (Table 4). In *Oreochromis niloticus*, applications of fucoidan and atrazine produced only minor changes. The control value was 97.71±2.28 fl, and the fucoidan-only, ATZ-only, and fucoidan + ATZ groups all showed values comparable to the control without significant differences (p>0.05) (Abdel-Warith et al., 2021). In red tilapia receiving *Sargassum dentifolium* polysaccharides at 10, 20, and 30 g/kg, significant increases were observed at 10 and 30 g/kg (p<0.05), with the highest value at 10 g/kg (2.83±0.57 fl). The remaining dose produced a value similar to the control (Abdelrhman et al., 2022).

3.6. Mean Corpuscular Hemoglobin (MCH)

MCH is an indicator used to detect anemia and evaluate respiratory function, and it indicates the average amount of hemoglobin per erythrocyte (Aderolu et al., 2025). Mean corpuscular hemoglobin (MCH) is used together with mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) as an erythrocyte index. It provides basic information on the physiological status and blood characteristics of fish (Adel et al., 2021; Sotoudeh & Jafari, 2017).

The effects of brown algae on MCH in several fish species are shown in Table 1. MCH was 25.50±0.50 pg in the control group of *Labeo rohita* exposed to dietary inclusion levels of 3, 5, and 7% *Sargassum ilicifolium*. And all doses caused significant increases (p=0.0004**), with the highest value recorded at 7% (34.50±0.50 pg) (Zulfiqar et al., 2025). For *Lates calcarifer*, the control MCH was 35.9±8.2 pg/cell when Spirulina + Sargassum mixtures were included in the diet at 5 and 10%. Both treatment groups showed values similar to the control, without significant differences (p>0.05) (Mohammadian et al., 2025). Feeding *Padina pavonica* to *Oreochromis niloticus* at 4, 6, and 8 g/kg increased the control value of 62.38±0.31 g/dl significantly (p<0.05). The highest value was observed at 8 g/kg (51.1±0.46 g/dl) (Maghawri et al., 2023). The study on *Clarias gariepinus* by Aderolu et al. (2025) evaluated *Dictyota ciliolata* but reported Hct rather than MCH. The control value was 29.13±1.15%, and both 0.25 and 0.5 g/kg produced values similar to the control with no significant differences (p>0.05).

Table 4. Changes in Hct, MCV, MCH and MCHC of freshwater and marine fish fed diets supplemented with brown algal polysaccharides

Algae	Fish Species	Dose	Het	MCV	MCH	MCHC	Refrences
Species							
Fucoidan	O. niloticus	Control	36.33±0.33 ^b	97.71±2.28μm³/cell	32.00±0.50 pg/cell	32.74±0.18	Abdel-
			%			%	Warith et al.
		Fucoidan	$39.00{\pm}0.58^a$	$93.49\pm2.28~\mu m^3/cell$	29.93±0.60 pg/cell	32.02 ± 0.39	(2021)
			%			%	
		Basal diet and	32.67 ± 0.88^{c}	$103.95 \pm 2.56 \mu m^3/cell$	31.87±0.87 pg/cell	30.66 ± 0.45	
		Atrazine	%			%	
		Fucoidan and	$36.33{\pm}0.88^{b}$	99. 96 \pm 3.10 μ m ³ /cell	30.39±0.03 pg/cell	30.46 ± 0.90	
		Atrazin	%			%	
Polysacchar	О.	0 g/kg	27.10±7.10°	$1.57\pm0.50^{c}\mu m^{3}/cell$	2.30±0.10 ^a pg	177.10±28.	Abdelrhman
ides	mossambicus		%			$80^{a}g/dl$	et al. (2022)
(Sargassum	× O. niloticus	10 g/kg	$42.77{\pm}8.16^{a}$	$2.83{\pm}0.57^{a}\mu m^{3}/cell$	$2.23{\pm}1.01^apg$	88.27 ± 14.0	
dentifolium)			%			$5^{d} g/dl$	
		20 g/kg	$26.87{\pm}7.89^{c}$	$1.40 \pm 0.01^{c} \mu m^{3} / cell$	$2.10\pm0.87^{a}pg$	155.40±63.	
			%			$26^{b}g/dl$	
		30 g/kg	37.60 ± 4.30^{b}	$1.97 \pm 0.90^{b} \mu m^{3} / cell$	$1.97 \pm 0.83^{b} pg$	102.77±17.	
			%			$01^{c} g/dl$	

NA: Not available. Superscript letters indicate statistically significant differences reported in the original studies.

Findings on the effects of brown macroalgal polysaccharides on MCH in fish indicate that responses vary by compound type and dose (Table 4). In *Oreochromis niloticus*, applications of fucoidan and atrazine produced only minor shifts. The control value was 32.00±0.86 pg, and the fucoidan-only, basal diet + atrazine, and fucoidan + atrazine groups all showed values comparable to the control, with no significant differences (p>0.05) (Abdel-Warith et al., 2021). A different pattern was observed in red tilapia (*O. mossambicus* × *O. niloticus*) receiving *Sargassum dentifolium* polysaccharides at 10, 20, and 30 g/kg. MCH declined progressively with increasing dose, and only the 30 g/kg group differed significantly from the control value of 2.30±0.10 pg, reaching 1.97±0.83 pg (p<0.05). The remaining doses produced values close to the control (Abdelrhman et al., 2022).

3.7. Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC is a hematological parameter that reflects the ratio of the amount of hemoglobin inside the erythrocyte to the cell volume. This value indicates the intracellular hemoglobin concentration and is used to evaluate conditions such as hypochromic or hyperchromic anemia (Sattanathan et al., 2024a). The changes in MCHC can also indicate nutritional status and environmental stressors in fish. MCHC is calculated using hemoglobin (Hb) and hematocrit (Hct) values with the following formula:

MCHC (g/100 ml) = [Hb (g/dl) × 100] / Hct (%) (Mohammadian et al., 2025). MCHC values differ according to species and dietary dose (Table 3). In *Labeo rohita* receiving *Sargassum ilicifolium* at 3, 5, and 7%, the control value of 30.80±0.20% increased significantly at all doses, with the highest value recorded at 7% (37.50±0.50%; p=0.0029**) (Zulfiqar et al., 2025). For *Lates calcarifer*, the Spirulina + *Sargassum* mixture produced a different outcome. The control group measured 14.6±0.4 g/dl, and diets containing 5% and 10% led to values that remained close to the control, with no significant differences reported (p>0.05) (Mohammadian et al., 2025). A study on *Oreochromis niloticus* fed *Padina pavonica* showed that the control MCHC (30.71±0.38 g/dl) increased significantly at 2 g/kg, reaching 33.5±0.08 g/dl (p<0.05). The 4 and 6 g/kg doses also produced increases, but these were not statistically distinguishable from the control (p>0.05) (Maghawri et al., 2023).

Studies investigating brown macroalgal polysaccharides show that their effects on MCHC vary with both the compound and the applied dose (Table 4). In *Oreochromis niloticus* exposed to fucoidan and atrazine, the control group measured 32.74±0.18%. The fucoidan-only, basal diet + atrazine, and fucoidan + atrazine treatments produced values that remained close to this baseline, and none of the differences were statistically meaningful (p>0.05) (Abdel-Warith et al., 2021). A more apparent decline was noted in red tilapia fed polysaccharides extracted from *Sargassum dentifolium*. The control MCHC was 177.10±28.80 g/dl, and the most substantial reduction occurred at 10 g/kg, where the value fell to 88.27±14.05 g/dl, a significant decrease (p<0.05). The 20 and 30 g/kg treatments produced results similar to the control, with no substantial differences (Abdelrhman et al., 2022).

4. CONCLUSION

This review article provides an overview of hematological responses—red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH), and MCHC—in several fresh and marine water fish fish treated with brown sea macroalgae or its polysaccharide fractions. Results from the current studies display great variations, since multiple factors including fish species, experimental conditions, algal populations and feeding behaviors are involved. Increases in RBC, Hb and Hct were observed in some studies when ground whole brown algae were included at low (or moderate) dietary levels. Additionally, WBC counts within accepted physiological ranges for the species studied have also been reported in some trials. However, other researchers found nonsignificant changes or decreases in these parameters when the dietary level was higher or applied a specific extract.

Polysaccharide fractions such as fucoidans and other sulfated polysaccharides have been reported to induce a more regulated hematoresponse than whole algal biomass. Some experiments showed that exposure to these compounds increased total RBC, Hb and Hct, whereas a few studies found partial recovery of blood indices affected by chemicals, i.e. such as atrazine. In the other studies, these effects were either small or not statistically significant. RBC, WBC, Hb and Hct had a significant range between studies notably

compared to MCV, MCH and MCHC which showed generally smaller ranges of values and a larger proportion of non-significant differences between treated groups.

The heterogeneity between studies in published works is due to fish species of interest, developmental stage, algal species used, biochemical composition, extraction method and experimental dosage. The extent of this variability is also influenced by differences in feeding duration, tank environment, water cleanliness and co-existing exposure to other environmental perturbations. The same algal species gave different results for various fish species in a few specific cases which indicate interspecific differences in digestion, metabolism and blood physiology. Another level of complication stems from different units of measurement, sampling periods or reporting formats that prevent easy comparison across studies.

For future work, we recommend the development of standardized protocols for these factors (sampling times, analytical procedures and reporting formats). For the determination of limits of inclusion that have quantifiable effects in growth performance and physiology, dose—response studies covering a broad range of levels are required. Further prolonged exposure during feeding under commercial farm conditions would also provide stronger extrapolation of results to the field for practical applications. These methods will help to calculate species-specific inclusion levels of the macroalgae-based feed additives as well as ensuring that hematological data can be better compared to aquaculture research studies.

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CHAPTER 12

A CLOSER LOOK AT PLANT-BASED PROTEINS IN FISH NUTRITION

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INTRODUCTION

The significance of fish meal and fish oil in the feed industry cannot be overstated, given their rich history and impressive nutritional profile. With a high protein content ranging from 68 to 72%, these products boast over 90% digestibility, making them a staple in the production of animal feed. Their balanced amino acid and fatty acid profiles, coupled with an array of essential vitamins and minerals, have solidified their position as performance benchmarks in the industry. The International Fishmeal and Fish Oil Organisation (IFFO, 2025) notes that new components are being developed to supplement these products, supporting sustainable growth in aquaculture.

In 2024, global fishmeal production reached an impressive 5.2 million tonnes, while fish oil production ranged from 1 to 1.3 million tonnes annually. The prices of these commodities were substantial, with fishmeal costing around 1600 USD per ton and fish oil commanding a price of 2800 USD per ton. Peru and Chile emerged as key players in the global market, collectively accounting for 28% of the world's fishmeal production and 23% of global fish oil output. This rebound was particularly noteworthy for Peru, which had experienced an unusually low production year due to unfavorable fishing conditions. Given Peru's minimal domestic consumption, the majority of its fishmeal and fish oil are exported to international markets, catering to the demand for high-quality feed ingredients.

In contrast, Chile's production is largely utilized locally, with a significant portion being used to produce feed for salmon and trout farming. This domestic utilization underscores the importance of these products in supporting the growth of aquaculture in the region. The dynamics of fishmeal and fish oil production and trade are complex, with various countries playing different roles in the global supply chain. For instance, Türkiye experienced a sharp decline in its production of fishmeal and fish oil in 2024, with outputs reaching approximately 11,300 tonnes and 3,400 tonnes, respectively. To meet its domestic demand, Türkiye relied heavily on imports, with fish meal imports totaling 160,000 tons and fish oil imports totaling 79,000 tons, according to data from the Turkish Statistical Institute (TÜİK, 2025).

The reliance on imports highlights the global interconnectedness of the fishmeal and fish oil market, where production fluctuations in one region can have ripple effects on the supply chain. As the aquaculture industry continues

to grow, the demand for high-quality feed ingredients like fishmeal and fish oil is expected to increase. This trend underscores the need for sustainable production practices and the development of new components to supplement traditional sources. The work of organizations like IFFO (2025) and EUFOMA (2025) is crucial in this context, as they provide valuable insights and data on the industry, facilitating informed decision-making among stakeholders. The future of the fishmeal and fish oil industry looks promising, with ongoing research and development aimed at enhancing the nutritional profile and sustainability of these products. As the global community seeks to balance the need for food security with environmental stewardship, the role of fishmeal and fish oil in supporting sustainable aquaculture practices will only continue to grow. With their rich history, impressive nutritional profile, and evolving applications, fishmeal and fish oil remain indispensable components of the feed industry, poised to meet the challenges and opportunities of a rapidly changing world. By understanding the complexities of the global fishmeal and fish oil market, we can better navigate the path towards a more sustainable and foodsecure future.

Soybean and its by-products are considered the second most important raw material in aquafeed formulations. In 2024, the world produced 424.2 million tons of soybeans, while Türkiye produced around 180,000 tons. In the same period, Türkiye imported 3.7 million tons of soybean products (excluding seed) at an average price of 470 USD/ton. The world average export price was 445 USD/ton, and the import price was 498 USD/ton for soybeans (Trademap, 2025). Although fishmeal, fish oil, and soybean by-products are widely used in aquafeed rations, there is continuous research on partial and total replacement of fishmeal and soybean meal due to increasing needs for sustainable protein sources. This study focuses on the promising alternative plant-based feed ingredients that have emerged during the last years.

Global pea production in 2023 reached 13.8 million tons, with the top three countries contributing to this total being Russia with 3.7 million tons, followed by Canada at 2.6 million tons, and China with 1.5 million tons. Under the grain legumes category, Türkiye's production of peas came out to be 3,798 tons (OurWorldinData.org, 2025). The average price per ton was at 460 USD for the 2023–2024 period, whereas it reduced to 405 USD for 2024–2025 (STC and AAFC, 2025). Pea (Pisum sativum) is the second most important crop in

the Fabaceae family because of its nutritional profile. The seeds are rich in protein (20–25%), contain a small amount of fat (1.5–2.0%), carbohydrates mainly in the form of starch (24–49%), and a substantial amount of dietary fiber (60–65%), containing 10–15% insoluble fraction and 2–9% soluble fraction. Moreover, under alkali extraction and isoelectric precipitation, its protein yield can be significantly enhanced ranging between 62.6% and 80% (Shanthakumar et al., 2022)

Accordingly, peanuts (*Arachis hypogaea* L.) have become the prime crop ingredient in agricultural economies, food security, and nutritional plans, due to their importance in oil production and the food industry. Peanuts are grown in more than 100 countries around the world. The FAO (2025) and USDA (2025) estimated that the global production of oilseed peanuts would reach approximately 54.27 million tons in 2023 and 52.24 million tons in 2024. With 26% protein, 48% fat, 3% dietary fiber, high calcium, thiamine, and niacin, the peanut will provide a very valuable opportunity as an inexpensive dietary supplement to fight malnutrition. The peanut meals used in fish feed contain 30.6% protein and 10.3% lipid (Yıldırım et al., 2014).

Worldwide cottonseed production in 2025 reached 41.5 million tons. China was the largest producer and contributed 11.1 million tons, while Türkiye accounted for 1.045 million tons, representing approximately 3% of global output (USDA, 2025). The crude protein content of cottonseed meal is reported at 42.1%, while that of cottonseed protein concentrate achieves as high as 69.5% (Prabu et al., 2021). The average market price of cottonseed protein concentrate is about 1,200 USD per metric ton (Pmarketresearch, 2025)

In the 2024-2025 season, sunflower seed production amounted to 52.1 million tons in the world. Russia, with 16.90 million tons, and Ukraine, with 13.0 million tons of production, together comprised about 57% of the production. Meanwhile, the contribution of Türkiye to world production was 1.35 million tons (USDA, 2025). Sunflower protein concentrate contains approximately 54.5 % crude protein, which is much higher than that of sunflower meal, which contains about 38 % protein (Lovatto et al., 2017).

Aquaculture nutrition has, during recent years, placed a growing emphasis on the search for sustainable fishmeal alternatives. This research undertakes an in-depth review of the major feed ingredients being increasingly used as plant-based protein sources within aquafeeds. Such plant-based feed

ingredients are recognized not only to reduce the dependency on marine resources with limited supply but also for enhancing economic efficiency and environmental sustainability. By analyzing the nutritional composition and digestibility of selected plant proteins, the research strengthens the growing evidence base supporting their incorporation into contemporary aquaculture diets, thereby promoting further development of robust and high-performance feeding strategies.

PLANT-BASED CONCENTRATES

The most typical examples of plant-based protein products are soy protein derivatives. These products are used as alternatives to animal protein and serve as effective substitutes, particularly in aquafeeds. All soy protein products are derived from dehulled soybeans, and in fish nutrition, soybean meal, soy protein concentrate, soy protein isolate, and soy protein hydrolysate are commonly utilized as feed ingredients.

Following extraction, plant-based proteins can be processed into concentrates, isolates, or hydrolysates. Protein concentrates, typically containing 65–80% protein, are derived from plant materials such as soybean, pea, or alfalfa. Isolates are produced from sources like soybean, pea, or sunflower seed using techniques such as alkaline extraction, isoelectric precipitation, or membrane filtration to obtain highly purified proteins. Hydrolysates are generated by enzymatic (e.g., alcalase, pepsin) or chemical hydrolysis, breaking proteins into peptides with protein content ranging from 70–90%, and digestibility reaching up to 95%. For instance, soy protein hydrolysate is produced via enzymatic hydrolysis of soy protein concentrate or isolate, converting insoluble proteins into soluble forms (Hertrampf and Piedad-Pascual, 2000; Figure 1).

General procedures for peptide production from animal and plant proteins. Peptides can be produced from proteins found in animal products (e.g., by-products) or plant feeds (e.g., soybeans) by chemical, enzymatic, or microbial hydrolysis (Hou et al., 2017; Figure 2).

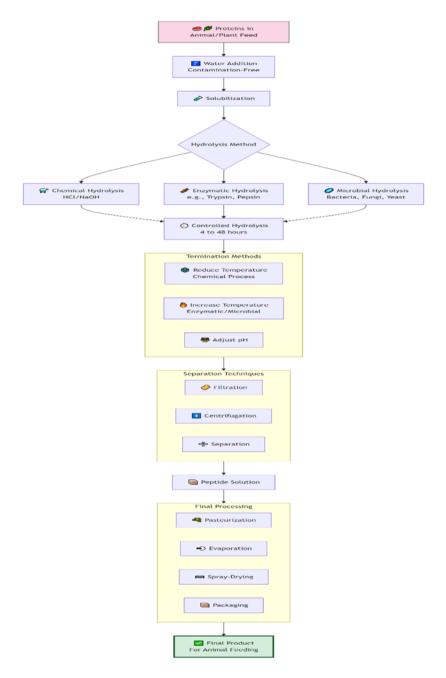


Figure 1. General procedures for the production of peptides from animal and plant proteins.

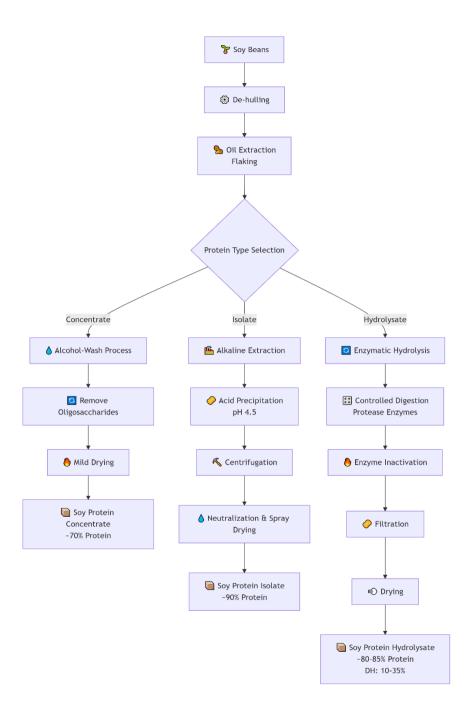


Figure 2. Diagram of soya protein products.

The search for sustainable alternatives to fishmeal in aquafeeds has led to the emergence of soybean protein concentrate (SPC) as a promising candidate. A comprehensive study on rainbow trout, initially weighing 60 ± 2 g, reared under controlled water conditions, has yielded significant insights into the potential of SPC as a fishmeal substitute. The research demonstrated that SPC could effectively replace 50% of fishmeal when incorporated at 20% inclusion level, maintaining growth performance metrics and achieving excellent survival rates of 95-98%. However, high SPC inclusion slightly reduced nutrient digestibility parameters, which could potentially limit its use as a fishmeal substitute. To overcome this limitation, the researchers supplemented the SPC-based diet with 0.25% umami flavor enhancer, which not only compensated for the digestibility challenges but also enhanced protein and energy utilization beyond baseline levels. Mechanistic investigations indicated that this improvement was mediated through the activation of gutbrain axis pathways, leading to improved palatability and nutrient metabolism. The combination of high SPC inclusion with umami supplementation represents an optimized approach for formulating sustainable, fishmealreduced aquafeeds that maintain zootechnical performance while supporting environmental sustainability in aquaculture operations. The use of SPC and umami flavor enhancer can help mitigate the environmental impact of aquaculture by reducing the demand for wild-caught fish and minimizing waste generated by fishmeal production. This study highlights the importance of complementary additives in maximizing the potential of plant-based proteins in aquafeeds. The findings demonstrate the potential of SPC as a fishmeal substitute in rainbow trout diets and underscore the need for further research into the use of complementary additives in aquafeeds to develop sustainable and environmentally friendly aquaculture practices (Calo et al, 2024).

The quest for sustainable and environmentally friendly alternatives to traditional fishmeal in aquaculture has led to the exploration of various plant-based protein sources. Among these, soy protein concentrate (SPC) has emerged as a promising ingredient due to its high protein content and superior nutritional profile. SPC is produced from defatted soybean meal through alcohol-water extraction or mild acid leaching, resulting in a product that contains more than 65% protein on a dry matter basis. Research has demonstrated the potential of SPC as a primary alternative to fishmeal in

aquafeeds. A study on Coho salmon post-smolts found that SPC achieved the highest apparent digestibility coefficients among tested plant proteins, with values of 72.90% for dry matter, 86.76% for crude protein, 87.72% for crude lipid, and 74.12% for gross energy. The essential amino acid digestibility exceeded 85%, indicating that SPC nutrients are highly bioavailable. These findings support the use of SPC as a sustainable and high-performance ingredient in aquafeeds (Lu et. al. 2025). However, excessive replacement of fishmeal with SPC can have negative consequences. A study on juvenile Largemouth Bass found that replacing up to 50% of fishmeal with SPC did not affect growth performance, feed utilization, or intestinal morphology. However, replacement levels of 75% or higher led to significant negative outcomes, including inhibited growth, lower survival rates, and poor feed conversion. This highlights the importance of balancing the use of SPC with other protein sources to ensure optimal nutritional outcomes (Liang et al., 2024).

Other plant-based protein sources, such as alfalfa nutrient concentrate (ANC) and cottonseed protein concentrate (CPC), have also been explored as alternatives to fishmeal. ANC is a processed plant protein derived from alfalfa leaves, with a crude protein content of up to 50%. However, research has shown that inclusion of ANC at levels equal to or exceeding 10% can impair nutrient digestibility, diminish protein utilization, and adversely affect fecal physical characteristics, leading to reduced growth performance in fish (Alfonso et al., 2023). The utilization of Alfalfa Nutrient Concentrate (ANC) as a fishmeal replacement in aquaculture has garnered significant attention in recent years. ANC is a processed plant protein derived from alfalfa leaves, designed to enhance its nutritional profile for use in animal feeds. The production process involves mechanical pressing of fresh alfalfa to separate the nutrient-rich leaf juice from the fibrous solid fraction, followed by concentration and drying steps to isolate the protein and other soluble nutrients. This results in a concentrated powder with a crude protein content of up to 50%, significantly higher than that of raw alfalfa. A study conducted by Chen et al. (2025) aimed to comprehensively evaluate the potential of using ANC as a fishmeal replacement in rainbow trout diets. The research assessed the impacts of ANC on feed management, palatability, nutrient digestibility, growth performance, and fish metabolism. The results showed that ANC has a higher protein content

and lower levels of indigestible nutrients like crude fiber and phytic acid compared to the original whole alfalfa crop. However, fish fed diets containing 10% to 20% ANC exhibited significantly lower specific growth rates and higher feed conversion ratios compared to those fed diets containing 0% or 5% ANC. The apparent digestibility coefficients (ADC) for dry matter and phosphorus were markedly reduced in fish receiving the 20% ANC diet. Notable alterations in hepatic metabolism were observed in fish fed the 20% ANC diet relative to the 0% ANC group, particularly in pathways associated with the tricarboxylic acid (TCA) cycle, amino acid metabolism, and energy regulation. The presence of phytate and indigestible carbohydrates in ANC may have contributed to the observed reduction in nutrient digestibility. Additionally, ANC-based diets contained elevated iron concentrations compared to the control diet, which may exert toxic effects and contribute to growth suppression. The metabolic shifts in the liver, including increased levels of branched-chain amino acids and TCA cycle intermediates, suggest disruptions in nutrient utilization and energy homeostasis. The results show that inclusion of ANC at levels equal to or exceeding 10% impairs nutrient digestibility, diminishes protein utilization, and adversely affects fecal physical characteristics, ultimately leading to reduced growth performance in fish. These findings highlight the importance of careful consideration when using ANC as a fishmeal replacement in aquaculture. Furthermore, the study's results have significant implications for the aquaculture industry, as the use of ANC as a fishmeal replacement could potentially lead to reduced growth rates and increased feed conversion ratios. This could result in decreased profitability for farmers and increased environmental impacts due to the need for more feed. Therefore, it is essential to carefully evaluate the nutritional profile of ANC and its effects on fish metabolism and growth performance before implementing it as a fishmeal replacement. In conclusion, while ANC has a higher protein content and lower levels of indigestible nutrients compared to raw alfalfa, its use as a fishmeal replacement in aquaculture is not without limitations. The reduced nutrient digestibility, impaired protein utilization, and adverse effects on fecal physical characteristics observed in fish fed ANC-based diets highlight the need for careful consideration and further research into the use of ANC in aquaculture. As the demand for sustainable and environmentally friendly aquaculture practices continues to grow, it is crucial to develop and implement

effective and efficient feed management strategies that prioritize the nutritional needs of fish while minimizing environmental impacts.

FCSM is a potential alternative protein source in aquafeeds and is produced by microbial fermentation to diminish antinutritional factors such as free gossypol and to improve nutritional quality. Wang et al. (2023) studied the effects of dietary substitution of FM with FCSM on intestinal health, digestive enzyme activity, immune modulation, and gut microbiota composition in juvenile golden pompano (Trachinotus ovatus). In an 8-week experiment, 375 fish (initial weight: 5.6 ± 0.14 g) were randomly assigned to five iso-proteic and iso-lipidic diets with graded FCSM inclusion levels of 0%, 12.5%, 25%, 50%, and 100% under optimal seawater conditions. The 25% replacement level gave rise to the best results: increased activities of amylase and chymotrypsin, downpro-inflammatory cytokines expression, up-regulated antiregulated inflammatory IL-10, and up-regulated tight junction proteins, indicating improved intestinal integrity and immunity. At this inclusion level, a stable gut microbiota composition was obtained, but increasing this further disrupted the digestive function and impaired the balance between microbial communities. This supports the inclusion of FCSM at 25% as a promising and health-friendly fish meal substitute in golden pompano diets.

Lupin protein concentrate (LPC) and pea protein concentrate (PPC) have emerged as promising candidates. A comprehensive study by Zhang et al. (2012) on rainbow trout demonstrated the potential of LPC and PPC as replacements for fish meal in aquaculture feeds. The researchers evaluated the effects of different ratios of LPC and PPC on the growth performance, feed efficiency, and health of rainbow trout. The study revealed that at a lower inclusion level of 300 g/kg plant protein, all combinations of LPC and PPC performed equivalently to fish meal, with no significant differences observed in feed conversion ratio (FCR), nutrient digestibility, or health parameters. However, at a higher replacement level of 500 g/kg plant protein, distinct patterns emerged, with PPC showing enhanced dry matter, protein, starch, and energy digestibility, increased plasma cholesterol levels, and improved overall performance. In contrast, diets with high LPC ratios demonstrated reduced starch digestibility, decreased mid-intestinal trypsin activity, and impaired feed efficiency. Another promising plant-based protein source is sunflower meal protein concentrate (SMPC). Lovatto et al. (2018) developed a simple and costeffective method for producing SMPC, which was then evaluated in a nutritional study with silver catfish fingerlings. The results showed that SMPC can replace up to 25% of animal protein in fishmeal-free diets without compromising growth, daily weight gain, or survival. Peanut meal is also a promising candidate due to its high protein content and favorable amino acid profile. However, its application is limited by the presence of anti-nutritional factors, unbalanced essential amino acids, and susceptibility to mycotoxin contamination. Microbial fermentation has emerged as an effective bioprocessing technique to enhance protein quality, increase small peptide content, and reduce anti-nutritional factors. Fan et al. (2023) examined a novel fermented peanut meal product created through a co-fermentation method and found that it can serve as a partial substitute for soybean meal in the diets of juvenile carp. Vo et al. (2020) also investigated the effects of replacing fishmeal with various levels of untreated, fermented, and germinated peanut meals on juvenile barramundi. The results demonstrated that fermented peanut meal can successfully replace up to 60% of fishmeal in the diet without compromising growth, feed conversion ratio, survival, or liver and gut health. In contrast, high inclusion levels of germinated and untreated peanut meals led to reduced growth, increased feed conversion ratio, higher mortality, and histopathological alterations. Overall, the studies demonstrate that plant-derived protein concentrates, such as LPC, PPC, SMPC, and fermented peanut meal, have the potential to replace fish meal in aquaculture feeds. These alternatives can provide a sustainable and environmentally friendly solution to the growing demand for protein sources in aquaculture. However, further research is needed to optimize the production and utilization of these protein sources, as well as to evaluate their effects on fish health and performance. The development of these plant-based protein sources can contribute to a more sustainable and environmentally friendly aquaculture industry, reducing the dependence on marine resources and minimizing the environmental impact of fish farming.

Pea protein concentrate is a very refined plant ingredient containing a high protein content and reduced levels of antinutritional factors compared to standard pea meal, while wheat gluten is a protein-rich, viscoelastic ingredient that also plays the role of natural binder in feed pellets. These two ingredients were combined in the ratio 65:35 PPC:WGM, respectively, in order to establish an amino acid profile complementing each other as well as supporting

functional properties. A feeding trial of 11 weeks was carried out with European sea bass (*Dicentrarchus labrax*) with initial body weight of 23.3 ± 1 g, reared in an indoor, partially recirculating marine water system at a water temperature of 24.1 ± 0.9 °C and salinity of $28 \pm 2\%$, to evaluate six isonitrogenous (approx. 50% crude protein) and iso-lipidic (approx.18.5% crude fat) experimental diets. Such a mixture of PPC-WGM can replace up to 75% of the dietary fishmeal protein provided that the diet is adequately supplemented with essential amino acids such as methionine and tryptophan. Justification for this high inclusion level is that it did not compromise growth performance and protein retention while providing considerable benefits in feed efficiency and environmental sustainability regardless of the original quality of the fishmeal (Tulli, 2007).

Air-processed PPC is manufactured through the dry milling and air classification of dehulled peas. This method of physical separation boosts the protein content by eliminating starch, but in contrast to wet-processing techniques, it fails to effectively remove anti-nutritional factors (ANFs), leading to a final product that still maintains fairly high levels of compounds like phytic acid, non-starch polysaccharides, and saponins. A 125-day feeding experiment was carried out using juveniles of sharpsnout sea bream with a starting weight of 14 grams. Pea protein concentrate (PPC) is one such alternative that has been explored as a potential substitute for fishmeal in the diets of various fish species. However, the efficacy of PPC as a fishmeal replacement varies depending on the species, processing method, and inclusion level. This study on sharpsnout sea bream juveniles found that the use of airprocessed PPC as a fishmeal substitute resulted in significant growth impairment, even at the lowest inclusion level of 16% of the diet (Nogales-Mérida et al., 2016). The diets with PPC were supplemented with synthetic methionine, but the growth performance was still negatively affected. Furthermore, higher inclusion levels of PPC caused significant gut histomorphological alterations, making it an inappropriate fishmeal substitute for this species. In contrast, a study on European sea bass found that PPC can replace up to 60% of fishmeal protein without compromising growth performance or feed efficiency (Tibaldi et al., 2005). The discrepancy in the results can be attributed to the differences in species, processing methods, and nutritional requirements. Pea protein concentrate is a refined product obtained from peas through processing that removes a significant portion of the starch

and fiber, resulting in a high-protein ingredient. The concentration process improves the protein content and reduces the levels of certain anti-nutritional factors compared to raw or simply dehulled pea meal. However, the airprocessing method used in the sharpsnout sea bream study may have affected the nutritional quality of the PPC, leading to the observed growth impairment. The European Sea bass study demonstrated that the apparent digestibility of protein and energy was similar across all diets, and the PPC-based diets were supplemented with L-methionine to meet the essential amino acid requirements. The results showed that up to 60% of dietary fishmeal protein can be effectively replaced by PPC without compromising growth performance or feed efficiency. The primary limiting factor for higher inclusion was reduced palatability, not nutrient digestibility or utilization. This suggests that PPC can be a valuable ingredient in aquaculture feeds, provided that it is adequately supplemented with the limiting essential amino acid, methionine. The differences in the results of the two studies highlight the importance of considering the specific nutritional requirements and species-specific characteristics when evaluating the efficacy of PPC as a fishmeal substitute. Further research is needed to optimize the processing methods and inclusion levels of PPC in different fish species to ensure that it can be used as a sustainable and effective alternative to fishmeal. Additionally, the development of more efficient processing methods and the identification of other plant-based protein sources can help to reduce the reliance on fishmeal and promote the sustainability of the aquaculture industry. In conclusion, the use of pea protein concentrate as a fishmeal substitute in aquaculture feeds shows promise, but its efficacy depends on various factors, including the species, processing method, and inclusion level. While PPC can replace up to 60% of fishmeal protein in European sea bass diets without compromising growth performance or feed efficiency, its use in other species, such as sharpsnout sea bream, may require further optimization. As the demand for sustainable and environmentally friendly feed options continues to grow, the development of effective and efficient plant-based protein sources will play a critical role in promoting the sustainability of the aquaculture industry.

CONCLUSION

The growing shortage and escalating prices of fishmeal, along with environmental worries about its production, have heightened the necessity for eco-friendly substitutes in the formulation of aquafeed. Recent studies indicate that a wide variety of plant-based protein concentrates can significantly replace large amounts of fishmeal when properly processed to reduce antinutritional components. These plant-based protein concentrates include fermented peanut meal, cottonseed protein concentrate, soy protein concentrate, sunflower protein concentrate, alfalfa protein concentrate, pea protein concentrate, and lupin concentrate (Table 1). The reported levels of replacement range from roughly 20% to 60%, depending on the specific ingredient and its processing techniques, highlighting their practical viability in aquaculture nutrition. The use of these vegetable proteins can help decrease dependence on limited ocean resources, reduce stress on wild fish populations, and encourage sustainable farming methods for aquatic organisms. Sunflower protein concentrate, for example, boasts excellent digestibility and a well-rounded amino acid composition, making it an attractive alternative to fishmeal. Alfalfa protein concentrate, on the other hand, offers beneficial bioactive compounds that aid in supporting both immune function and gut health. Pea protein concentrate, with its desirable amino acid profile and minimal allergenic properties, broadens the array of effective plant-derived choices. In addition to their dietary benefits, these plant-based protein concentrates can also help to reduce the environmental impact of aquaculture. The production of fishmeal is a significant contributor to the depletion of wild fish populations and the degradation of marine ecosystems. By replacing fishmeal with plant-based protein concentrates, aquaculture operations can reduce their reliance on these limited resources and help to mitigate the environmental impacts of their activities. Furthermore, the use of plant-based protein concentrates can also help to reduce the carbon footprint of aquaculture operations, as the production of these ingredients typically requires less energy and generates fewer greenhouse gas emissions than the production of fishmeal. The combination of various plant protein concentrates, including soy, cottonseed, peanut, sunflower, alfalfa, and pea, offers a hopeful approach to sustainable aquaculture. Embracing these options not only tackles the challenges of fishmeal shortages but also fosters the creation of robust, economical, and environmentally responsible feeding practices that correspond with long-term objectives for global food security and environmental care. By adopting these plant-based protein concentrates, aquaculture operations can help to ensure the long-term sustainability of their activities, while also contributing to the development of more environmentally friendly and socially responsible food systems. Moreover, the use of plant-based protein concentrates in aquafeed formulation can also help to improve the quality of aquatic products. The nutritional content of plant-based protein concentrates can be tailored to meet the specific needs of different aquatic species, allowing for the production of higher-quality products with improved nutritional profiles. This can help to increase consumer confidence in the safety and quality of aquatic products, while also providing a competitive advantage for aquaculture operations that adopt these sustainable and environmentally friendly practices. In conclusion, the growing shortage and escalating prices of fishmeal, along with environmental worries about its production, have highlighted the need for ecofriendly substitutes in the formulation of aquafeed. The use of plant-based protein concentrates, such as fermented peanut meal, cottonseed protein concentrate, soy protein concentrate, sunflower protein concentrate, alfalfa protein concentrate, pea protein concentrate, and lupin concentrate, offers a promising solution to this challenge. By embracing these sustainable and environmentally friendly alternatives, aquaculture operations can help to ensure the long-term sustainability of their activities, while also contributing to the development of more environmentally friendly and socially responsible food systems.

Table 1. Different plant protein sources and their effects on fish.

Fish Species	Initial Weight (g)	Trial Duration	Substitute Ingredient & Inclusion Level	Growth Effect	Threshold for Adverse Effects	Recomme nded Inclusion Level	Reference
Rainbow Trout (Oncorhy nchus mykiss)	60 ± 2	4 weeks	Soy Protein Concentrat e (SPC) 20% of diet, replacing 50% of fishmeal.	No significant difference in growth performanc e, feed intake, or FCR compared to high fishmeal diet.	No adverse effects on growth or feed intake at 20%.	Up to 20% dietary inclusion is well-tolerated. Suppleme ntation with 0.25% umami tastestimulate d.	Calo et al, (2024)
Largemo uth Bass (Micropt erus salmoide s)	17.03 ± 0.01	56	Soy Protein Concentrate (SPC) Replacing 0%, 25%, 50%, 75%, 100% of Fish Meal	No significant difference in growth up to 50% FM replacemen t. Significant inhibition at ≥75% replacemen t.	≥50% replacement: Reduced antioxidant capacity and initiated inflammatio n. ≥75% replacement: Impaired nutrient metabolism, severe inflammatio n, and intestinal damage.	Up to 50% of dietary fish meal can be replaced by SPC.	Liang et al. (2024)
European sea bass (Dicentra rchus labrax)	40.9 ± 0.8	(~14 months)	Soy Protein Concentrate : 18.4% (Fishmeal reduced from 20% to 12%; Replaced with a mix of SPC,	Slight reduction in SGR (0.67%/d vs. 0.69%/d) and final weight (~8%). No significant	No major health, welfare, or mortality issues were observed.	A soy protein concentra te inclusion of 18.4% as part of a blend effectivel y	Alfonso et al. (2023)

			fermented soy, sunflower meal, and yeast protein)	difference in FCR.		replacing 40% of marine protein is recomme nded.	
Coho Salmon (Oncorhy nchus kisutch)	183.17 ± 3.29	8	Protein Concentrat e (SPC) at 30% of diet.	Positive	-	At least 30%	Lu et. Al, (2025)
Oncorhy nchus mykiss (Rainbo w trout)	19.0 ± 0.2 (Trial 2)	9 weeks (Growth Trial)	Alfalfa Nutrient Concentrat e (ANC) replacing dietary fishmeal.	No negative effect. 5%	≥10% no recommenne d.	Up to 5% of the diet.	Chen et al. (2025)
Oncorhy nchus mykiss (Rainbo w trout)	35.00 ± 0.05	8 weeks	CPC & CAP Mixture (1:1) replacing dietary fishmeal	No negative effect on WG, FCR, or SGR when replacing up to 50% FM.	Replacing >50% FM (i.e., FM-5 and FM-0 diets) significantly reduced WG, PER, and antioxidant capacity, and impaired intestinal morphology.	The mixture can effectivel y replace 50% of dietary FM (i.e., reduce FM from 200 g/kg to 100 g/kg in the diet).	Huang et al. (2023)
Rainbow trout (Oncorhy nchus mykiss)	58	62 (growth) + 20 (digestibi lity)	Lupin Protein Concentate (LPC) & Pea Protein Concentrate (PPC) at L/P ratios of 3:0, 2:1, 1:2, 0:3	No significant difference in weight gain or feed intake at 300 or 500 g/kg inclusion.	Reduced starch digestibility and higher FCR with high LPC at 500 g/kg.	Up to 300 g plant protein/k g dietary protein for any LPC/PPC combinati on.	Zhang et al. (2012)
Silver catfish (Rhamdi a quelen)	14 ± 0.26	49	Sunflower Meal Protein Concentrate (SMPC) replacing	No negative impact on final weight or daily gain at 25% or 50%	>25% replacement (for optimal FCR).	Replace 25% of animal protein with SMPC	Lovatto et al. (2018)

			25-50% of	replacemen			
			animal protein.	t. Feed conversion was impaired at			
				50% replacemen			
				t.			
Golden Pompano (Trachin otus ovatus)	5.6 ± 0.14	8 weeks	Fermented Cottonseed Meal (FCSM): 25%(Repla cing fish meal in the diet)	Improved digestive enzyme activity and nutrient absorption at 25% substitution	Significant reduction in digestive enzymes and increased inflammatio n at ≥50% substitution.	substituti on of fish meal with FCSM is recomme nded for optimal intestinal health and function.	Wang et al (2023)
Common carp (Cyprinu s carpio)	4.83 ± 0.02	56	Fermented Peanut Meal (FPM)repla cing 25- 100% of Soybean Meal	Positive; significantl y higher mass gain rate and specific growth rate at 50% replacemen t.	>50% replacement	dietary inclusion of FPM, replacing 50% of dietary soybean meal.	Fan et al. (2023)
Barram undi (Lates calcarife r)	6.0 - 6.5	8	Fermented Peanut Meal (FPM)	No significant difference in growth, FCR, or survival compared to control diet at any level (15%, 30%, 60%).	No adverse effects on growth, health, or stress response were observed even at 60% replacement.	Up to 60% of fishmeal can be replaced.	Vo et al. (2020)
Rainbow trout (Oncorhy nchus mykiss)	58	62 (growth) + 20 (digestibi lity)	Lupin Protein Concentate (LPC) & Pea Protein Concentrate (PPC) at L/P ratios	No significant difference in weight gain or feed intake at 300 or 500 g/kg	Slight intestinal fold changes with highest PPC; Reduced starch digestibility	Up to 300 g plant protein/k g dietary protein for any LPC/PPC combinati	Zhang et al. (2012)

			of 3:0, 2:1, 1:2, 0:3	inclusion. FCR increased at 500 g/kg inclusion, especially with high LPC.	and higher FCR with high LPC at 500 g/kg.	on. For 500 g/kg, PPC is preferred over LPC.	
Europea n Sea Bass (Dicentra rchus labrax)	23.3 ± 1	11 weeks	Mixture of Pea Protein Concentrat e (PPC) & Wheat Gluten (WGM) 65:35 ratio, replacing 50% and 75% of fishmeal protein.	No impairment of growth; Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) improved at the 50% replacemen t level. Protein efficiency improved when replacing poorquality fishmeal.	Reduced feed intake (palatability) observed at all inclusion levels, but without negative impact on growth or digestible protein intake.	Up to 75% of fishmeal protein can be replaced, with essential amino acid (Met, Trp) suppleme ntation. Justified by maintaine d growth, improved feed efficiency , and reduced environm ental waste output.	Tulli et al (2007)
Sharpsn out Sea Bream (Diplodu s puntazzo)	14	125 days	Air- Processed Pea Protein Concentrat e (PPC) Included at 16%, 32%, and 48% of diet, replacing 20%, 40%, and 60% of	Significant linear reduction in final body weight, Specific Growth Rate (SGR), and Thermal Growth Coefficient (TGC) with	16% dietary inclusion (20% fishmeal protein replacement) . Negative effects on growth were observed at all tested levels.	Not Recomm ended. The air- processed PPC used caused growth depressio n and gut histo- morpholo gical alteration	Nogales- Mérida et al (2016)

			fishmeal	increasing		s at all	
			protein.	PPC levels.		inclusion levels, deeming it an unsuitabl e fishmeal substitute for this species.	
Europea n Sea Bass (Dicentra rchus labrax)	44 ± 1	104 days	Pea Protein Concentrat e (PPC) Replaced 30%, 60%, and 90% of fishmeal protein.	No negative effects on growth, FCR, or nutrient retention at 30% and 60% replacemen t. Significant impairment at 90% replacemen t.	90% fishmeal protein replacement, due to severely reduced palatability and feed intake.	Up to 60% of fishmeal protein can be replaced. Justified by maintaine d growth performa nce, feed efficiency , and nutrient retention equivalen t to the fishmeal control diet.	Tibaldi et al (2005)
Cyprinus carpio (Commo n Carp)	4.17 ± 0.02	56	Cottonseed Protein Concentrate (CPC) replacing Fishmeal	Significantl y improved growth performanc e, feed utilization, and intestinal health at 50% replacemen t.	75% replacement, leading to growth suppression and intestinal dysfunction.	Up to 50% of dietary fishmeal.	Fan et al.(2024)
Sander lucioperc a (Pike Perch)	3.55 ± 0.01	56	Cottonseed Protein Concentrate (CPC)	No significant difference in final mass,	>60% replacement (inferred), based on decreased	Up to 60% of dietary fishmeal.	Fan et al. (2025)

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	replacing	MGR, or	trypsin	
	Fishmeal	SGR up to	activity and	
		60%	negative	
		replacemen	microbiota	
		t; highest	shifts at	
		FER in the	lower levels.	
		60% group.		

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