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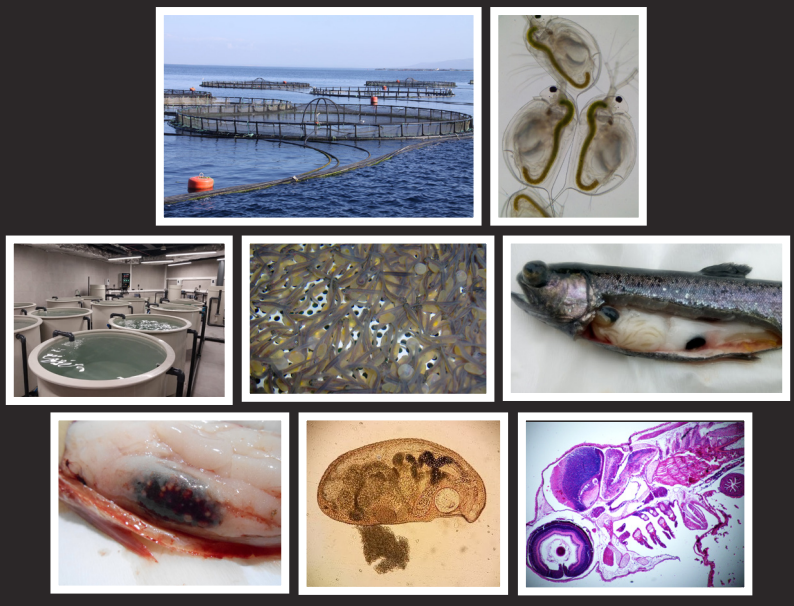
PRESENT-DAY TURKISH AQUACULTURE AND TRENDS IN INTERNATIONAL RESEARCH



Editors

Mustafa YILDIZ
Süheyla KARATAŞ

İSTANBUL UNIVERSITY PRESS

PRESENT-DAY TURKISH AQUACULTURE AND TRENDS IN INTERNATIONAL RESEARCH

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İSTANBUL UNIVERSITY PRESS

Published by
İstanbul University Press
İstanbul University Central Campus, 34452 Beyazıt/Fatih, İstanbul - Türkiye

https://iupress.istanbul.edu.tr

Present-Day Turkish Aquaculture and Trends in International Research
Editors: Mustafa Yıldız, Süheyla Karataş

E-ISBN: 978-605-07-1593-4

DOI: 10.26650/B/LS32LS24.2024.005

Istanbul University Publication No: 5339

Faculty of Aquatic Sciences Publication No: 12

Published Online in March 2024

It is recommended that a reference to the DOI is included when citing this work.

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This book was published as part of the Istanbul University Press' 100th Anniversary of the Republic book project.

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FOREWORD

Turkish aquaculture started in inland waters in 1970, has continued to grow with marine aquaculture since the mid-1980s and has grown in parallel with the developments in the world. Although aquaculture production started in many countries earlier than Türkiye, production success in these countries has been achieved by Turkish aquaculture industry thanks to the use of the latest technologies in this field. In Türkiye, 25 universities and 4 Research Institutes provide education in the field of aquaculture and various research are carried out by many scientists with different specializations. This book is considered important among the books to be published by Istanbul University on the 100th anniversary of the founding of the Turkish Republic, and therefore dedicated to the founding of the Turkish Republic by the authors.

PREFACE

The rapid population growth is causing notable challenges in global food supply. There has not been a proportional increase in the quantity of fish obtained through fisheries over the years due to factors such as climate change, water pollution and overfishing. Due to these factors, a significant reduction in the supply of wild fish is to be expected. On the contrary, a better understanding of the positive health benefits of seafood has led to an increase in fish consumption, making global aquaculture one of the fastest growing food production sectors in recent years. Therefore, research in aquaculture will play an increasingly important role in ensuring world food security. The rapid increase in mass fish production has triggered studies on the cultivation of new species specifically, and accelerated research on both the nutritional requirements of farmed fish species and the search for alternative protein sources as replacement ingredients in fish feed. Besides feed formulation, it has also created the need for more knowledge in areas such as disease prevention, aquaculture systems, environmental effects, marketing, processing technology, workforce and legislation. Despite the use of antibiotics and vaccination, infectious diseases still cause significant economic losses in aquaculture. Although antibiotics are accepted as effective agents in the control of bacterial diseases, their frequent use leads to resistance, which endanger their application. This situation has led to intensified research into alternative preventive and therapeutic methods. Several of these innovative solutions are discussed in this book. Within the scope of this book, the historical development of aquaculture in Türkiye and in the world is briefly mentioned, and the topics including production techniques, fish nutrition, fish health/welfare, business management, product quality and the contribution of aquaculture to the Turkish economy are evaluated in detail.

PART I

A BRIEF HISTORY AND THE PRESENT SITUATION

CHAPTER 1

DEVELOPMENTS OF TURKISH AQUACULTURE INDUSTRY

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DOI: 10.26650/B/LS32LS24.2024.005.001

1. Introduction

As a country with significant infrastructure and long history in fish farming, Turkish aquaculture is in a rapid growth period, ranking among major producers in the World and the largest producer among the non-EU and EU member countries, together with Norway, UK, and Russia. The Rainbow trout (*Oncorhynchus mykiss*), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are the main three species dominating aquaculture harvest from Turkish farms. According to the latest statistics supplied by the Food and Agricultural Organizations of the United Nations, production volume for these three key species was in the order of 165.683 tons, 155.151 tons, and 133.476 tons, respectively in 2021, representing 35%, 33%, and 28% of the total production in the Turkish aquaculture industry. Considering economic returns from for the country, European seabass production takes the lead with 41%, the highest share of the total income that was reported as 855.483 $, followed by gilthead seabream with a share of 31% and 637.187 $ contribution, and rainbow trout with 23% and 482.848 $ contribution in 2021. Fish species, other than the main key players represent 5% with an economic value of 112.798 $ for the Turkish aquaculture sector, that

has achieved a remarkable growth trend over the last two decades. The development of the Turkish fish farming industry was initiated with simple cage systems that has shifted to smart farm facilities with high-technologies over the years. However, there is still a way to go, and there is an urgent need to identify new production areas in response to shrinking carrying capacities, and to develop innovative methods and technologies for environmentally-sound and sustainable production in order to use the existing potential more efficiently. All these aspects have been evaluated in this chapter with insight to the strengths and prominent features that have brought Turkish aquaculture to an important position. Efforts and success of the Turkish aquaculture industry, as well as weak points that need considerations for future developments have been highlighted with comparative evaluation from past to present.

2. Importance of Food Security and Diversity

The aquaculture sector worldwide has recorded significant growth in recent years. The increasing food and water need of the world, which exceeds 8 billion people today, is one of the most important issues to be tackled for the future of human beings. The importance of the need for food and water becomes even more prominent in processes such as regional conflicts, epidemics, etc.

Because the break in the international trade flow that started with lock-downs and the closure of intercountry borders during the recent Covid-19 pandemic period, it has been noted that some countries faced severe difficulties in reaching healthy food (Yigit et al., 2023a). The regional crisis caused by the Ukraine-Russia war, which started with the growth of political disagreements in the north of the Black Sea, did not only affect the conditions in these two countries, but also became a global issue. In particular, the relative resolution of the grain crisis in the European continent, with the intervention of Turkish State, provided a fresh breath both for European countries and for many others struggling with hunger and poverty.

In times of global crisis, it has been observed that self-sufficient countries such as Türkiye, in terms of food security and diversity, could overcome these difficult periods more easily and with less damage, compared to those with foreign dependency (Yigit, 2023b). Therefore, ensuring food security along with food diversity is one of the most important issues for the future of nations, that it should be addressed together with energy resources, which are important for all kinds of industrial production and human life. Despite the fact that several countries have different production strategies, intercontinental collaboration on food safety management for the continuity of food flow between countries is important for food safety at global level.

3. Global Trends and Recent Developments in Turkish Aquaculture

Due to stagnations in capture-fisheries, natural resources are not enough to cover the increasing demand of the world population, hence the aquaculture became into foreground with a remarkable potential in meeting the increase of seafood consumption (López-Mas et al., 2021). The development and wider use of stabilization of fishing yields and the use of technological devises such eco-sounders in fisheries, challenged more pressure on natural populations. Global capture fisheries supply in 2021 was reported as 76.912.786,8 tons for finfish, which increased by only 1.6% over the previous year of 2020 and only 2.3% over the last 5 years from 2016 to 2021 (FAO, 2023a). Considering the total of all aquatic organisms from capture fisheries including fish, crustaceans and molluscs, aquatic plants, and other marine and freshwater animals, world capture-based fisheries supply was around 183.250.671,8 tons in 2021, with 1.6% growth from 2020 to 2021, but only 0.9% increase over the last 5 years from 2016 to 2020 (FAO, 2023a). The 1.5% increase of the wild harvest yields from 2020 to 2021 might be explained by the resurgence in 2021 of the serious decrease (-3.1%) in fishing activities during the pandemic period of 2020. In contrast, seafood supply from aquaculture activities is in a steady growth trend, with a harvest yield of 59.295.095,3 tons that gave around 3.1% increase from 2020 to 2021, and 16.3% increase over the past 5-years span from 2016 to 2021 (FAO, 2023b). This is a clear indication that the global aquaculture growth was around 8-times bigger than the capture fisheries that is in a stagnation due to the dependence on wild populations.

The total aquaculture production of 59.295.095,3 tons in 2021 is a sum of seafood harvest from 197 countries in total. The number of countries with a production volume of more than 100.000 tons is 32, while those producing over 200.000 tons of finfish is 19, whereas only 14 countries have an annual production of over 400.00 tons. Turkish aquaculture is within the top 14 countries with an annual harvest of 467.048 tons of finfish, that is mainly represented by the European seabass, gilthead seabream and rainbow trout, and comprises around 0.8% of the global production in 2021, according to the latest statistics (FAO, 2023b).

Considering the European finfish aquaculture, the total production yielded 2.953.601,9 tons in 2021, including the non-EU member states of Norway and Russia as the main key players in the European continents. When the Turkish production is included as another non-EU state, the total production rises to 3.420.650 tons, among which the percent share from Turkish farms would be around 13.7% of the total European finfish aquaculture (FAO, 2023b). In regards to the main key species in the Turkish aquaculture industry, Turkish aquaculture provides around 45% of the total harvest for seabass, seabream and rainbow trout in Europe.

Production yields over the last 40 years from 1980 to 2021 for the main producers have been illustrated in Figure 1. The total production of seabass, seabream and Rainbow trout for the main countries with over 40.000 tons of harvest in 2021 have been given in Figure 2, and the percent share of the volume among the main key players is shown in Figure 3.

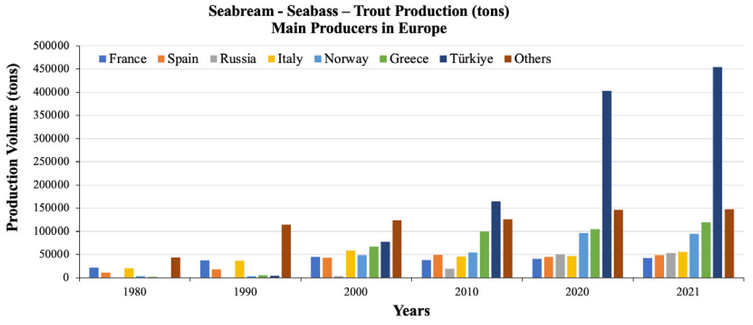

Figure 1. Production trends from 1980 to 2021 for the main producers with over 40.000 tons of harvest in 2021 (sum of seabass, seabream and rainbow trout, tons)

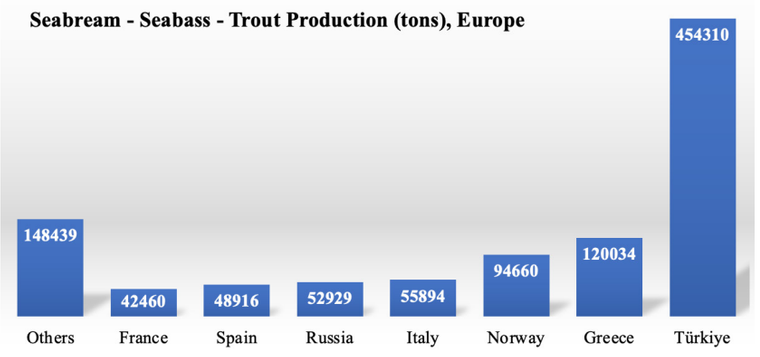

Figure 2. Production volume (tons) for the main countries with over 40.000 tons of seabass, seabream and rainbow trout harvest in 2021. Countries with less than 40.000 tons production are classified under "Others"

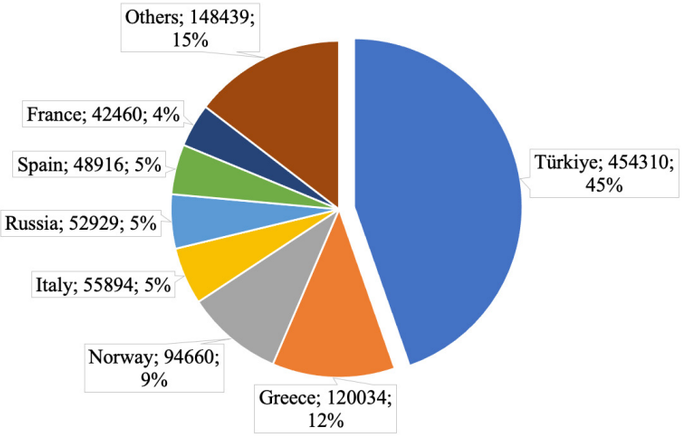

Figure 3. Percent share of the total production volume (tons) for seabass, seabream and rainbow trout among the main European countries with >40.000 tons harvest in 2021. Countries with <40.000 tons production are listed under "Others"

Turkish aquaculture industry, which occupies the first place as the largest producer of seabream and seabass in the world, ranks second in the world in trout production, after Iran. The quantitative share of the total production for seabass, seabream and trout by 2021, based on the latest statistics (FAO, 2023) has been presented in Figure 4, and the percent share of the production volume for the fish species dominating Turkish aquaculture is given in Figure 5.

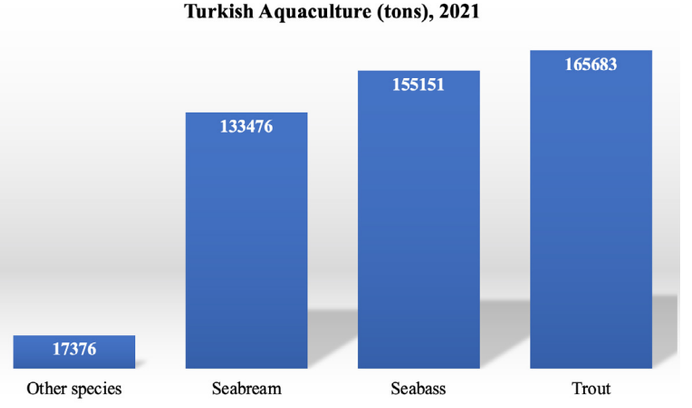

Figure 4. The quantitative (tons) share of seabass, seabream and trout production from Turkish farms in 2021

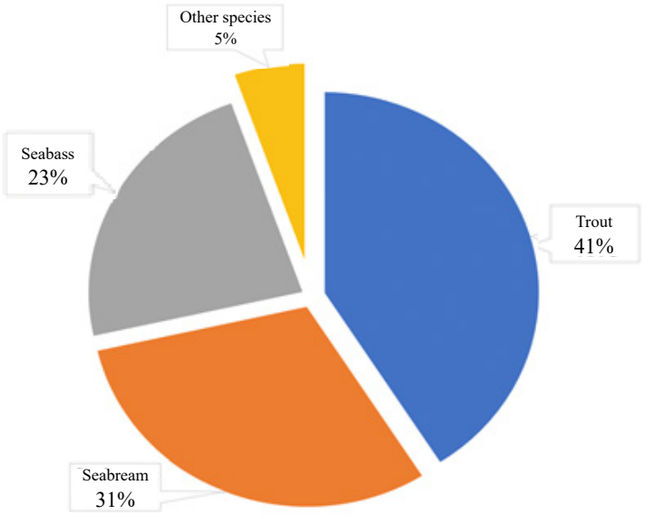

Figure 5. Percent share of the production volume for the main fish species harvested from Turkish farms in 2021

In regards to the rainbow trout, Turkish aquaculture ranked first in Europe and second in the World after Iran. Turkish rainbow trout production hit 165.683 tons in 2021 with a share of 17% of the global harvest, whereas Iran produced 193.852 tons comprising nearly 20% of the World total, with only 3% difference by share between the main two key players of Türkiye and Iran (FAO, 2023b).

Rainbow trout production in quantity (tons) among world countries with over 10.000 tons of production in 2021 has been given in Figure 6, whereas the percentage share of the total trout production for Türkiye and other countries is presented in Figure 7.

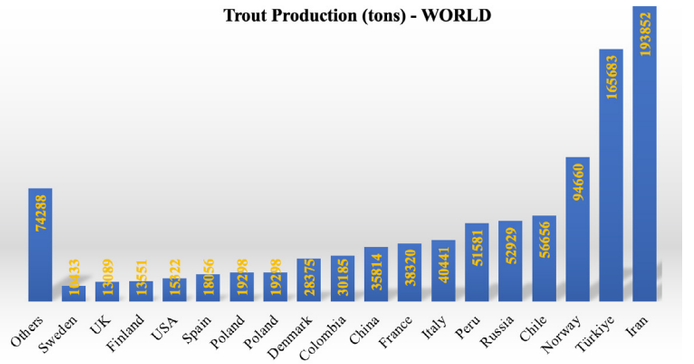

Figure 6. Rainbow trout production quantities (tons) among world countries with over 10.000 tons of harvest in 2021

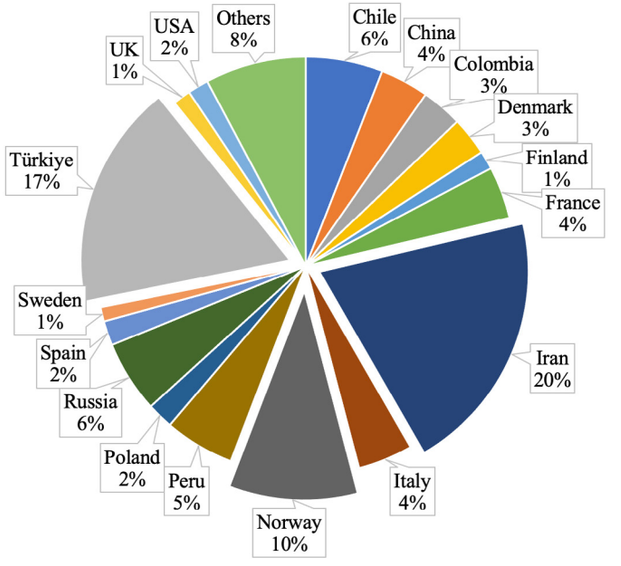

Figure 7. Percentage share of the total trout production among world countries with 10.000 tons of harvest in 2021

The production in quantity for Rainbow trout in Europe has been provided for the main countries with over 20.000 tons a year and presented in Figure 8, and the percent share among countries is given in Figure 9.

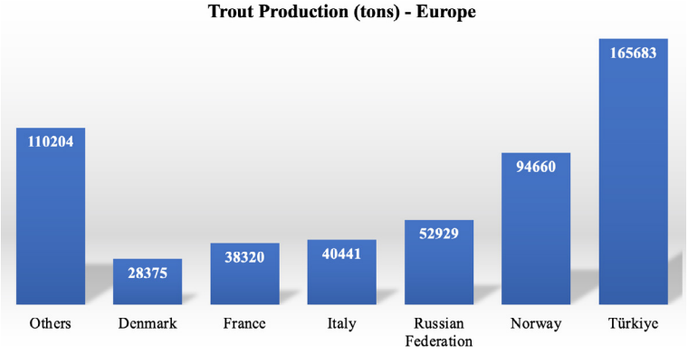

Figure 8. Rainbow trout production quantity (tons) in European countries with over 20.000 tons of harvest in 2021. Countries with less than 20.000 tons of annual production have been classified under "Others"

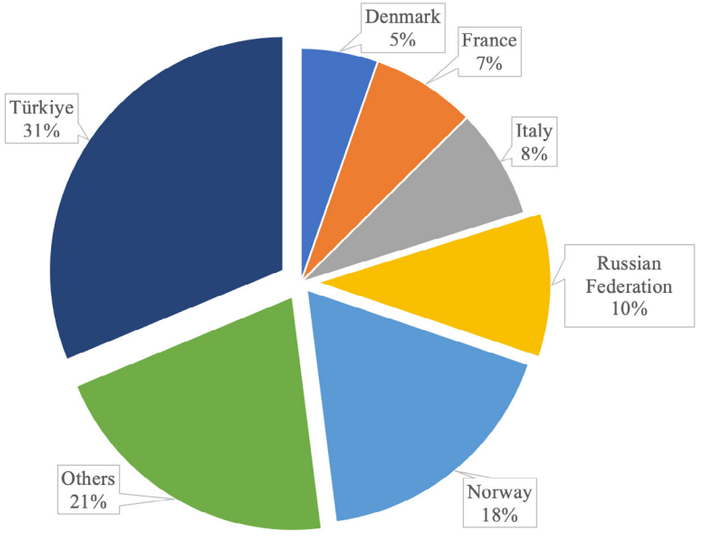

Figure 9. Percent share of rainbow trout production among European countries with over 20.000 tons of harvest in 2021. Countries with less than 20.000 tons of annual production have been listed under "Others"

For the seabream production in quantity (tons), data are illustrated in Figure 10, showing the World production yields for 2021, and percent share of the world production has been illustrated in Figure 11.

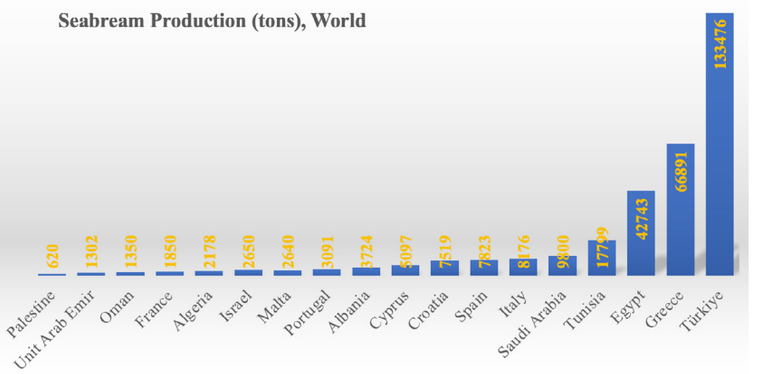

Figure 10. Seabream production in quantity (tons) among world countries by 2021

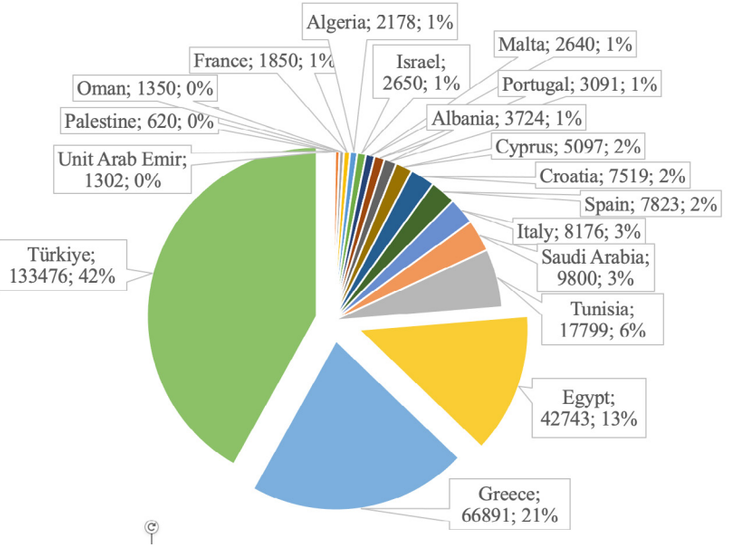

Figure 11. Percent share of quantity for seabream production in the world by 2021

The production in quantity (tons) for seabream in the European aquaculture has been provided for the main countries with over 1.000 tons a year and presented in Figure 12, while the percent share among countries has been illustrated in Figure 13.

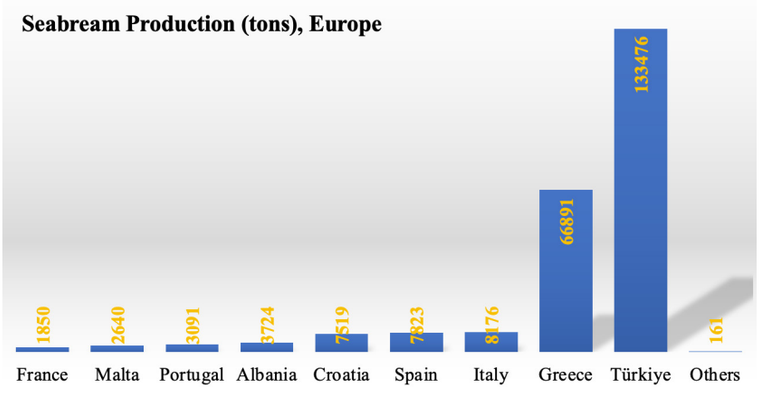

Figure 12. Rainbow trout production in quantity (tons) for the main countries in Europe with over 1.000 tons of production by 2021

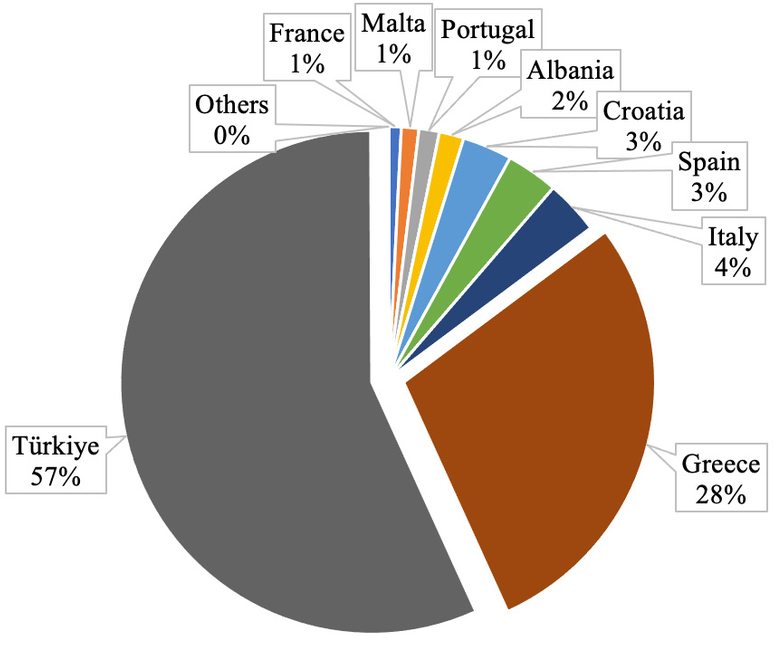

Figure 13. Percent share of seabream production in Europe by 2021

The world aquaculture production in quantity (tons) for seabass farming is given in Figure 14, and percent share of the world production among countries has been shown in Figure 15.

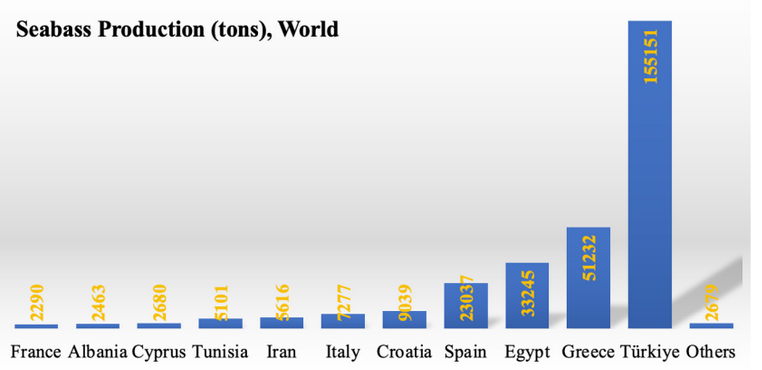

Figure 14. World aquaculture production for seabass (tons). Countries with less than 1.000 tons production in 2021 are listed under "Others"

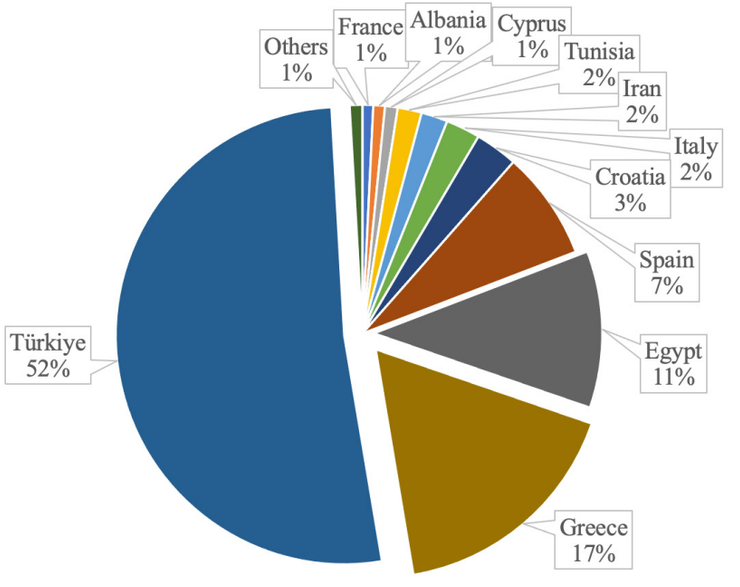

Figure 15. Percent share of the world production of seabass among countries with over 1.000 tons of annual harvest in 2021. Countries with less than 1.000 tons production are listed under "Others"

Seabass production in quantity (tons) for the main producers with over 1.000 tons a year in Europe has been given in Figure 16, and the percent share among countries is presented in Figure 17.

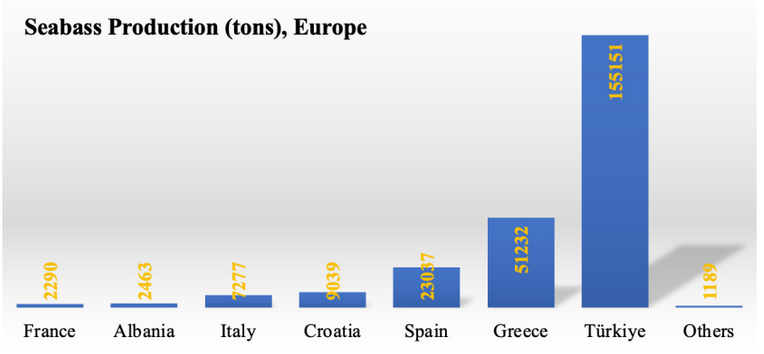

Figure 16. Seabass production in quantity (tons) for the main producers with over 1.000 tons of annual harvest in Europe. Countries with less than 1.000 tons production are listed under "Others"

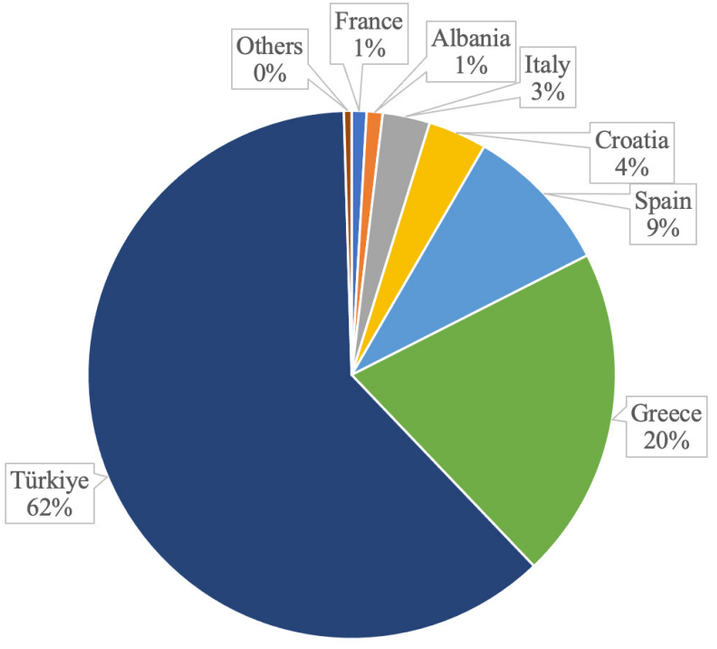

Figure 17. Percent share of seabass production among European countries with over 1.000 tons of annual harvest in 2021. Countries with less than 1.000 tons production are listed under "Others"

4. Early Stages and Progressive Development of the Turkish Aquaculture With Main Key Species Supplied to the World Markets

4.1. Carp

Although the beginning of fish farming in Turkiye dates back to the 1950s, those days the production was initiated with small-scale carp farms. The first carp harvest, recorded by the United Nations Food and Agriculture Organization, was 10 tons in 1954. Over the next 10 years, production increased by 15-fold to 150 tons of carp in 1964. In the following years, 390 tons of production was achieved in 1970 and 1180 tons in 1980, while the production reached its peak in 1988 with 2200 tons of carp harvest. However, after 1988, carp production decreased gradually, and immediately afterwards, it fell to 1033 tons in 1989 and 364 tons in 1991. Carp production, which showed a fluctuating course over the following years, decreased to 171 tons in 2021. The production trends of carp in the Turkish aquaculture from 1954 to 2021 have been given in Figure 18.

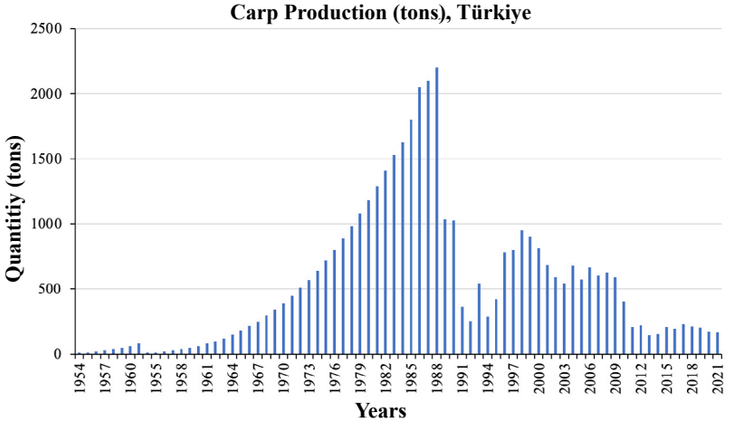

Figure 18. Production volume of carp in Türkiye from 1954 to 2021

4.2. Rainbow trout

The rainbow trout (*Oncorhynchus mykiss*) is among the main key species that shifts Turkish aquaculture to the foreground as the biggest producer in Europe and the second biggest in the world after Iran. First official records regarding the trout production in Türkiye were realized as 10 tons of Rainbow trout in 1968. According to the statistics provided by the Food and Agriculture Organization of the United Nations, 110 tons of trout production was

recorded in 1978, that reached to 1765 tons in 1988, which is the year when carp production peaked and then declined rapidly afterwards. Trout production reached 68.649 tons in 2008 and 112.427 tons in 2018. With the production volume of 165.683 tons in 2021, the Turkish aquaculture sector has now become the largest trout producer in Europe and the second largest in the world after Iran, comprising around 31% of the European, and nearly 17% of the world total production.

While rainbow trout farming in Türkiye was carried out in freshwater facilities until the 1990s, it become a major industry, destined for international markets followed by the investigations of Yigit (1996) and Yigit and Aral (1999) who underlined better growth performance for rainbow trout in seawater conditions of the Black Sea compared to the rainbow trout in freshwater facilities (Yigit et al., 2023a).

Today, the rainbow trout is most widely farmed salmonid worldwide (Candiotto et al., 2011; Stanković et al., 2015). The rainbow trout was formerly named as *Salmo gairdneri* classified under the genus "*Salmo*". However, this species was later reclassified and listed under the Pacific salmons following the research by Laird (2001) on genetic and native distribution of the species. Laird (2001) noted that the rainbow trout is among the main three farmed Pacific salmons with remarkable production volumes, along with the chinook salmon (*O. tshawytscha*), and the coho salmon (*O. kisutch*).

Nowadays, the harvest of 2.5 kg or bigger size rainbow trout (also known as Steelhead) is a new farm strategy of the Turkish aquaculture sector, providing large fish to the domestic and international market, that is sold as Turkish salmon (Yigit et al., 2023b). This has been reported as a new marketing strategy for the large steelhead harvested from the Black Sea (Yigit et al., 2023a,b), that increased profits and economic returns for the Turkish cage aquaculture business, which is in line with the report of Fernández-Sánchez et al. (2022), who underlined that fish farms with larger sized seabass harvest in the Mediterranean received better profits as result of consumer demands. Figure 19 shows the quantities (tons) of Rainbow trout harvested from Turkish farms between 1968 and 2021.

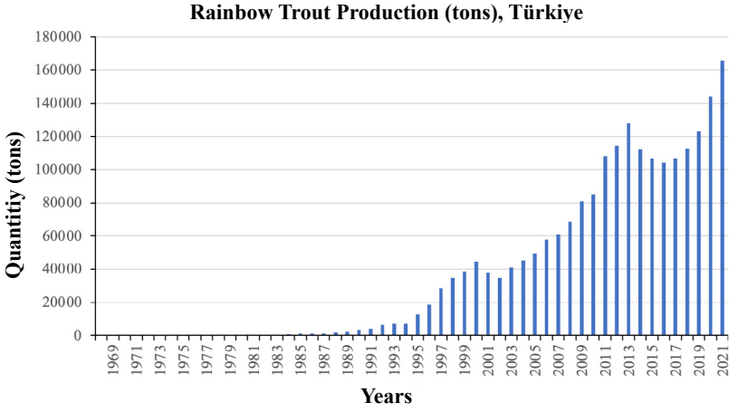

Figure 19. Production volume (tons) of rainbow trout in Turkish aquaculture from 1968 to 2021

4.3. Seabream and seabass

Marine aquaculture activities in Türkiye were initiated with seabream production in 1986, which was followed by seabass farming a year later in 1987. First, simple cage systems made of wood were used in those days. Cages with iron frames and buoyancy force created with drums set underneath were also used over the time. However, these cage frames were rectangular in shape and the fish nets attached to the frames had to be rectangular as well.

Over the years, the development of octagonal-cage frames made it possible to use circular-shaped net bags, which provided a healthier environment where fish could demonstrate schooling behavior. Introducing circular cage nettings was also the beginning for higher biomass per cubic meter, which moved farmers forward with increased production capacities per unit volume.

In the early 80's, both seabream and seabass juveniles were caught from wild populations and stocked into the cages, where feeding was conducted until market size. However, when it was understood that this type of production would not be sustainable over time, marine hatcheries were introduced, which was actually the beginning of the marine aquaculture industry in Türkiye. Production quantities (tons) of seabream and seabass harvested from Turkish farms from 1986 to 2021 have been illustrated in Figures 20 and 21, respectively.

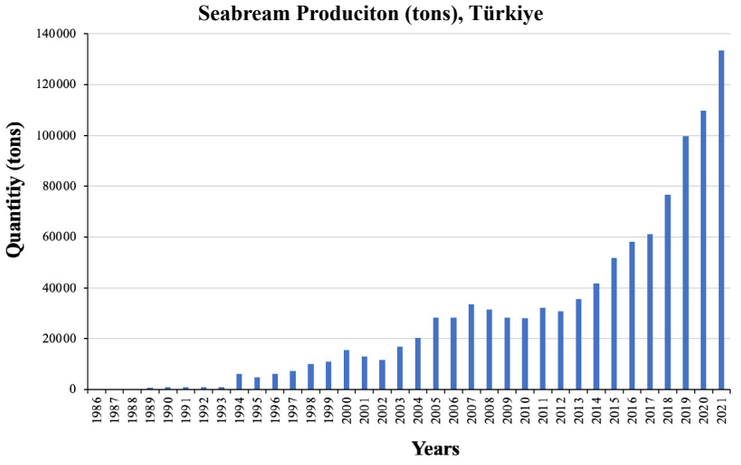

Figure 20. Production volume (tons) of seabream harvested from Turkish farms between 1986 and 2021

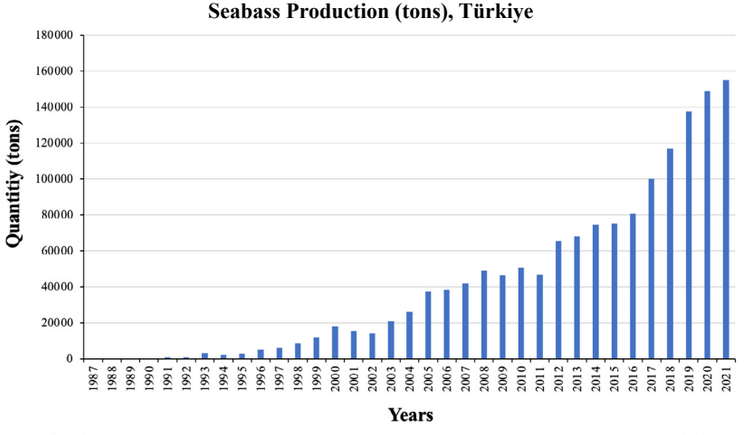

Figure 21. Production volume (tons) of seabass harvested from Turkish farms from 1987 to 2021

Today, the gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) production has become one of the main drivers for the Turkish aquaculture, ranking at the top among the main producers in Europe and the World.

The annual harvest of seabass and seabream from Turkish fish farms comprises 62 and 57% of the total harvest in Europe, whereas it contributes to 52 and 42% of the total product in the World. The harvest of 155.151 tons and revenue of 855.483$ for seabass and 133.475

tons with a sales income of 637.187$ for seabream from Turkish farms is followed by the second largest producer of Greece, which production hit around 51.232 tons for seabass and 66.891 tons for seabream, with around 20 and 28% contribution to the total production in Europe, and nearly 17 and 21% of the World production, respectively in 2021 (FAO, 2023b).

Spain is the third biggest producer of seabass in Europe with an annual harvest of 23.037 tons in 2021, followed by Croatia (9.039 tons), and Italy (7.27 tons), with shares of 9, 4, and 3%, respectively, to the total production in European waters. Among the World producers, Egypt reserved its third place in seabass production with 33.245 tons that equaled to 11% of the global production in 2021.

After the Turkish and Greek production, Italy and Spain shared the 3rd and 4th ranking in Europe with 8.176 and 7.823 tons, representing 4 and 3% of the total production. Egypt and Tunisia shared around 13 and 6% of the total world production of seabream with 42.743 and 17.799 tons, after Türkiye and Greece, based on 2021 data provided by FAO (2023b).

5. Conclusions and Future Challenges

The rapid development process experienced in the Turkish aquaculture sector and the increase in production capacities have led to a significant improvement in the competition of international trade. However, the increased production volume in the Turkish aquaculture industry is mainly concentrated on three key species of trout, seabream and seabass that indeed shrinks the product range in the market. Increasing production amounts may cause unbalanced price fluctuations from time to time. New marketing strategies can be developed with the supply of alternative products that in turns may increase the product diversity for the market. Therefore, the search for new candidate species is encouraged for further development of the Turkish aquaculture business, which is also an important step for the sustainable growth of fish farming activities in the country. In fact, attempts for the production of new finfish species other than seabream, seabass or rainbow trout have been noted between 1986 and 1993, with several quantities of annual harvests ranging from 5 to 100 tons of common two-banded seabream (*Diplodus vulgaris*) and mullets (*Mugil cephalus*). The culture of mullets were tested from 1991 to 1992, however it is likely that the consumer demands for mullets were quite low, as fish farms no longer supplied mullets after 1992, and no records were noted for the common two-banded seabream production after 1993. This was just after the disappointment that was experienced in the production of Atlantic salmon (*Salmo salar*) in offshore cages deployed off the Kefken Is-

land in the Western Black Sea between 1992 and 1994. The drastically loss of fish due to some technical failures such as using un-painted nets, which was torn of during a severe storm and all fish escaped into the cold waters of the Black Sea. This incidence may not be the only one, but a strong reason, among others, for the discouragement of farmers in the search of new species during that period. It is known that some companies continued small-scale operations for new candidate species, including Black Sea turbot (Kalkan), however, these were not reflected to the sales records until 2012. The price imbalances in the market put farmers into financial distress and bottlenecks were experienced in cash flow by the early 2010s. The decrease in prices probably due to the increased production volumes of the main key species, triggered the search for alternative species in order to provide product diversity in the market. Finally, the production of alternative species has been revived in the Aegean Sea by 2012, with meagre (*Argyrosomus regius*), sciaena sp., pink dentex (*Dentex gibbosus*)), shi drum (*Umbrina cirrosa*), red porgy (*Pagrus pagrus*), common dentex (*Dentex dentex*), blue spotted seabream (*Pagrus coeruleostictus*), red-banded seabream (*Pagrus auriga*) and sharpsnout seabream (*Diplodus puntazzo*). Around 500 tons of porgies and 1.000 tons of sciaena sp. were harvested as alternative species in 2012, which was followed by over 100 tons of Red porgy and Common dentex in 2014, Pink dentex, Shi drum and Bluespotted seabream in 2017. The production volumes for the Redbanded seabream and Sharsnout seabream remained below 100 tons, ranging from 1 to 66 tons between 2017-2018, and from 2 to 59 tons between 2014 and 2016, respectively. Apart from these, the Meagre shifted forward with increasing production volumes from around 3.000 tons to nearly 6.000 tons between the years 2014 and 2021, according to the statistical data provided by the Food and Agriculture Organization of the United Nations. With the introduction of Atlantic Bluefin tuna in 2004, the annual harvest increased gradually from around 400 tons to over 1.000 tons in 2014, and to around 5.000 tons in 2021.

In conclusion, the aquaculture activities that was initiated with carp farming in the 1950s in the Turkish fish farming business, achieved a significant success as the biggest producer for seabream, seabass and trout in Europe, and among top producers in the world. However, fluctuations in market prices or changes of consumer demands forces farmers to expand their product range for both domestic and international markets. Introducing new candidate species could be a novel marketing strategy with diverse products, alternative to the already-available fish species.

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CHAPTER 2

NEW SPECIES IN AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.002

1. Introduction

The world population will be estimated to be increased to 9.7 billion in 2050 and 10.4 billion by 2100. With the increasing human population, more protein sources are needed for healthy generations. Compared to terrestrial proteins, the quality of marine protein sources is very valuable in terms of essential amino acids and fatty acids. Currently, aquaculture production is exceeded to capture fisheries production (FAO, 2022). However, to be sustainable in aquaculture production, the industry must develop the production of new marine and freshwater species. From this point of view, survival and growth performance are the most important parameters for successful larvae and juvenile fish culture.

Türkiye draws attention to its aquaculture production potential. Due to its different geographical features with a variety of salinity and temperature of water parameters with the Black Sea in the North, the Aegean in the West and the Mediterranean in the South, and contains many marine fish species with commercial interest. European Sea bass (*Dicentrarchus labrax*), Gilthead Sea Bream (*Sparus aurata*), Meagre (*Argyrosomus regius*), and Salmon (*Salmo salar*) are among the most produced and consumed marine fish in Europe. These species are offered to the consumers as fillets or smoked which their sale prices increase with processed form. Rainbow trout (*Oncorhynchus mykiss*) takes first place among freshwater fish and it is offered to consumers in the form of fillet, smoked, canned and fresh. However, the market is still open for new products with different tastes and textures.

Marine fish production is gradually increasing in Türkiye, and new species are paid attention to in addition to gilthead sea bream, European sea bass, and Meagre. Among these

species; White grouper (*Epinephelus aeneus*), Clownfish (*Amphipron ocellaris*), Red porgy (*Pagrus pagrus*), Blue-spotted sea bream (*Pagrus caeruleostictus*), Greater amberjack (*Seriola dumerili*), Common dentex (*Dentex dentex*), Mussel (*Mytilus galloprovincialis*), Whiteleg shrimp (*Litopenaus vannamei*), and Common octopus (*Octopus vulgaris*) can be listed (Table 1). The feeding procedure, disease, and growth studies on gilthead sea bream, European sea bass, and meagre have been conducted in high numbers in the last 20 years leading to progress in the optimum breeding methods related to the cultures of these species.

Table 1. Marine fish production in Türkiye (TUIK, 2022)

Species	2013	2014	2015	2016	2017	2018	2019	2020	2021
Rainbow trout (*Oncorhynchus mykiss)*	5,186.2	4,812.0	6,187.0	4,643.0	4,972.0	9,235.0	9,411.0	18,182.0	31,509.0
Gilthead Seabream *(Sparus aurata)*	35,701.1	41,873.0	51,844.0	58,254.0	61,090.0	76,680.0	99,730.0	109,749.0	133,476.0
European Seabass (*Dicentrarchus labrax)*	67,912.5	74,653.0	75,164.0	80,847.0	99,971.0	116,915.0	137,419.0	148,907.0	155,151.0
Red Seabream (*Pagrus major*)	-	106,0	143,0	225,0	20,0	2,0	5,0	1,0	4,0
Redbanded Seabream (*Pagrus auriga*)	-	-	-	-	66,0	1.,0	-	-	-
Shi drum (*Umbrina cirrosa*)	-	39,0	61,0	20,0	125,0	30,0	47,0	26,0	2,0
Meagre (*Argyrosomus regius*)	-	3,281.0	2,801.0	2,463.0	697.0	1,486.0	3,375.0	7,428.0	5,913.0
Common Dentex (*Dentex dentex*)	-	113,0	132,0	43,0	51,0	24,0	27,0	-	-
Sharpsnout Seabream (*Diplodus puntazzo*)	-	8,0	59,0	2,0	-	-	-	-	-
Bluespotted seabream (*Pagrus caeruleostictus*)	-	75,0	90,0	61,0	107,0	70,0	66,0	-	3,0
Tuna (*Thunnus thynnus*)	-	1,136.0	1,710.0	3,834.0	3,802.0	3,571.0	2,327.0	4,338.0	4,952.0

Inland aquaculture fish production, only rainbow trout production increased after the Covid-19 pandemic in 2020, while the production amount of other species decreased. Another reason for the increase in the production of rainbow trout is the high demand for black sea salmon (Turkish salmon) produced in the Black Sea region. The need for rainbow trout juvenile fish arose for this production in cages (Table 2).

Table 2. Freshwater fish production in Türkiye (TUIK, 2022)

Species	2013	2014	2015	2016	2017	2018	2019	2020	2021
Rainbow trout (*Oncorhynchus mykiss)*	122.873,3	107.533,0	100.411,0	99.712,0	101.761,0	103.192,0	113.678,00	126.101,0	134.147,0
Common Carp (*Cyprinus carpio)*	145,5	157,0	206,0	196,0	233,0	212,0	203,0	173,0	171,0
Sturgeon (Acipenseridae *sp.)*	-	17,0	28,0	6,0	13,0	2,0	-	14,0	-
Tilapia (Oreochromis *sp.*)	-	32,0	12,0	58,0	8,0	12,0	6,0	13,0	6,0
Catfish (*European catfish)*	-	-	-	-	8,0	5,0	121,0	92,0	84,0

The most important factor affecting the annual targeted production is the success of the broodstock management and the survival rate achieved in the subsequent larvae and juvenile production. The highest mortality rates are recorded in the larval and pre-growth periods. Therefore, maintaining a high survival rate at the larval period is directly affected the year-end production target. During the larval and prelarval periods, live prey selection and utilization at the right time and size are very important. Moreover, determining the nutritional needs of cultured fish is also vital for further cultural success (Eryalçın et al., 2013; Eryalçın et al., 2015; Eryalçın et al., 2020). During the growing period, micro-diet utilization and adaptation of that formulated artificial compounds lead to postlarvae in difficult digestion and assimilation.

In general, feed production companies are located mainly Black Sea and Aegean Region. However, the quality of micro-diets still needs to be improved. In our country, the production of new species has been increasing gradually in recent years, and the production of new species has been tried in the private sector, research universities, and institutes. Within this chapter, new marine species for aquaculture are summarized with their problems, current situations, studies, and future perspectives.

1.1. White grouper (*Epinephelus aeneus*)

Groupers are high-demand fish species around the world. There are 24 species belonging to the Serranidae family that have been cultured. According to FAO (2022), approximately 226.200 tonnes of groupers were cultured in 2020. Cultural efforts of grouper species mostly take place in Israel in the Mediterranean region (Hassin et al., 1997; Gorshkov, 2010) and in Southeast Asia countries such as Taiwan, Hong Kong, China, Japan, and Australia. This culture process is based on captured fingerlings of this species from nature due to low survival and growth out under cultivation. White grouper is candidate marine fish species for intensive aquaculture with excellent taste, high economic value, and fast growth rates (Gorshkov, 2010) (Figure 1). On the other hand, this fish is stated as near threatened according to IUCN red list (Pollard et al., 2018).



Figure 1. White grouper (*Epinephelus aeneus*)
(http://www.ictioterm.es/nombre_cientifico.php?nc=70)

The white grouper (*Epinephelus aeneus*) belongs to the Serranidae family and is distributed in the southern and eastern part of the Mediterranean Sea, the southern parts of the Atlantic coasts where along the coastline of Portugal and Spain, and the south along the Atlantic coast of West Africa as far as southern Angola and the islands of the Gulf of Guinea at the eastern Atlantic Ocean.

However, larval and broodstock management had improved in recent years by developing diets and husbandry of groupers (Song et al., 2005). There are several feeding materials mentioned previously in the cage and pond culture of this fish species. For instance; trash fish, squid, octopus, and tilapia have been used for feeding. At the larval stage, Toledo (2002) showed declining in survival between 35 and 55 dph of larval age by 29.8% to 3% value.

This low survival rate is attributed to a lack of knowledge of exogenous feeding during the larval period (Koven et al., 2016). Larval culture success has been related to the green-water technique where microalgae are applied into the larvae culture tanks. Recently, the nutritional needs of groupers have been reported in some research. Protein and energy requirements have been studied in order to optimize feed formulation and feeding regimes extensively (Lupatsch et al., 2003; Lupatsch and Kissil, 2005). According to those studies, the utilization of energy and protein for the growth of white grouper does not appear to be different than gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Gamsız and Muhtaroğlu (2016) have resulted in an efficient weaning period from the larval stage to the juvenile and portion size was as recorded 52 grams in 3.5 months and 437 grams in 9.5 months. This result was obtained at ordinary sea bass and sea bream tank culture procedure.

Protein and lipid contents are mainly depending on fish size due to different nutritional needs at different sizes. For instance, 2-10 gram fish could require 58% crude protein and 10.5% crude lipid while larger white grouper more than 500-gram fish need less protein (42.1%) and higher lipid (14.7%) (Williams, 2009). Essential nutrients such as fatty acids are important for grouper feeding. Wu et al. (2002) showed that especially longer chain fatty acids such as DHA and EPA are essential for Malabar grouper (*Epinephelus malabaricus*). Fish oil replacement by other terrestrial vegetable oils such as soybean, corn, peanut, and sunflower oils in diets has resulted in different muscle fatty acid compositions in grouper (*Epinephelus coioides*) (Lim et al., 2007).

Recirculating aquaculture systems (RAS) offer alternative culture methods compared to cage farms or pond production of grouper. Physical and chemical culture conditions are important for fish health and culture performance. Skeletal malformations are a major problem in many culture fish species leading to low market prices, affecting swimming ability, and feed intake and caused to low survival (Boglione et al., 2001). Elsadin et al. (2018) reported the clear effects of high dissolved CO_2 and environmental temperatures on growth and skeletal anomalies of white grouper (*E. aeneus*) which may affect the juvenile quality and advised keeping dissolved CO_2 levels below the 5.64 mg L^{-1} in rearing systems. White grouper juveniles can survive and grow under low saline brackish water conditions without any negative effect (Peduel and Ron, 2003). In agreement with this study, Cnaani et al. (2012) also stated that white grouper can adapt, grow and survive under low salinity waters. Moreover, it has been reported that a 3% salt added to the diet improved both growth and survival. The effect of rearing temperature has been studied extensively in groupers. Temperature mainly affects the growth, digestion activities, and physiological status of groupers. Among aquacultured groupers, the optimum rearing temperature is reported 26 ºC depending on their life stages (Das et al., 2021). A similar culture temperature

was successfully applied in white grouper larval and juvenile stage in Türkiye by Gamsız and Muhtaroğlu (2016) with 38 ppt salinity at the Aegean Sea.

As a result, in order to increase the amount of commercial production of grouper species on a large scale it requires optimization of formulated diets on growing, juvenile, and portion-size fish. Moreover, culture procedure and culture water parameters should be similar to the natural habitat of groupers. Therefore, cage culture should be set up in the warm and subtropic areas of the Southern Aegean and Mediterranean Seas.

1.2. Clownfish (*Amphipron ocellaris*, Cuvier 1830)

Ocellaris Clownfish (*Amphiprion ocellaris*) belongs to the family Pomacentridae. They live in the warm waters of the Pacific Ocean, mostly from Southeast Asia (Sumatra and Philippines) to Great Barrier Reef at the rocky bottom with anemone structures (Santini and Polacco, 2006). The selection of hosts by clownfish species is different. For instance; some species such as *A. percula* and *A. ocellaris* choose three anemone host species: *H. magnifica, Heteractis crispa*, and *Stichodactyla gigantea* forthe first then *H. magnifica*, *S. mertensii*, and *S. gigantea* for the last whereas *A. clarkii* prefer ten anemone species to live in (Olivotto and Geffroy, 2017). These species become much more popular for marine ornamental fish after the movie ‘’Finding Nemo’’ (Yong et al., 2011). The marine ornamental fish trade has a high economic value of around US15$ billion (Rhyne et al., 2017). There is still fisheries pressure on the wild stock of marine ornamental fishes. Therefore, production efforts on this high-demand clownfish species are crucial in order to sustain enough number of fish (Olivotto and Geffroy, 2017). Despite the development trend of clownfish production, there are still several issues left to be solved such as broodstock management, hatching, larval rearing, survival, growth, and diets at different life stages (Vargas-Abúndez et al., 2019) (Figure 2).



Figure 2. Clownfish (*Amphipron ocellaris*) (Akvatek Aquaculture Inc.)

There are highly demanded ornamental fish species around the world. The economic value of clownfish is differed depending on their pattern, color, and brightness. Recently, this species become more important due to ideal experimental fish that can easily keep under laboratory conditions. In spite of the culture procedure of broodstock management, larvae, and juvenile production being well established, there is remained a couple of issues to be solved such as desired pigmentation. Compared to other cultured marine fish diets, there is a huge gap in live prey utilization and enrichments and formulated diets of ornamental fish species for large-scale production (Calado et al., 2017). There are several studies on the replacement of both fishmeal and fishoil with alternative ingredients in diets. However, energy content, essential amino acids, and fatty acids should be considered when new ingredients are candidates for common protein and lipid sources which is the case fishmeal and fishoil. In terms of clownfish diets, black soldier fly (*Hermetia illucens*) meal has been evaluated with different ratios and the results have no negative effects on growth, stress response, and survival of juvenile clownfish (*Amphiprion ocellaris*, Pomacentridae) (Vargas-Abúndez et al., 2019). There are also plant-based protein replacement trials in the same species. Basic growth parameters such as specific growth rate, final body weight, feed conversion ratio, and protein efficiency ratio values were increased with substitution by 15% *Spirulina platensis* meal in growth-out diets. Moreover, in the same study, coloration was succeeding by adding this microalgae meal due to the natural carotenoid contents (Güroy et al., 2022). Exogenous feeding of this species showed that gastric contents of adult fish mostly feed on algae, zooplankton, larval fish, and crustacean. From this point, plant-based protein and HUFA are the main requirements of the formulated diets. Therefore, the protein: lipid ratio of formulated diets is essential for maintaining a high rate of survival and growth under controlled laboratory conditions (Diaz-Jimenez et al., 2020).

Skin colouration is the main problem in clownfish culture similar to other colourful fish species under culture conditions. Fish can synthesize pigment sources in their body where they mostly get from their diets (Gupta et al., 2007). The colourful ornamental fish species can lose their bright colours under captivity conditions if they can not be able to reach pigments from diets and as a result, they start to get pale under these circumstances. In captivity conditions, their colour is also affected by different parameters such as water quality and light intensity (Yasir and Qin, 2010). Different pigment groups are responsible for different colours. For instance, group of Lutein, tunaxantin, doradexantin play in yellow colours, β-carotene, canthaxanthin, zeaxanthin play in the formation of orange and astaxanthine is responsible for red colour in aquatic animals (Hieu and Tam, 2009). Carotenoids are the most important

pigment for clownfish and accumulation of this pigment mainly depends on fish species, water quality, age, dietary level, feed intake and quality (Gupta et al., 2007). There are two types of pigment sources; synthetic and natural. For instance; most common synthetic caratenoids are astaxanthin, canthaxanthin, lycopene, β-carotene, carophyll pink. On the other hand, natural pigment sources are widely integrated in fish tissue compare to synthetic ones. Moreover, availability and production prices are cheaper in natural pigments. There are several studies on natural pigment production from red yeast *Xanthophyllomyces dendrorhous* (Xu et al., 2006), Alfalfa, *Medicago sativa* (Yanar et al., 2008) and marigold, *Tagetes erecta* (Villar-Martinez et al., 2013) microalgae species such as *Spirulina platensis* (Teimouri et al., 2013), *Porphyridium cruentum* (Hekimoglu et al., 2017), *Haematococcus pluvialis* (Pan and Chien, 2009) and *Chlorella vulgaris* (Yu et al., 2022). Ebeneezar et al. (2020) have focused on other pigment sources such as oleoresins where obtained from paprika, turmeric and chlorophyll. The paprika oleosresins and combination of pigments had higher results on growth of clownfish. Nhan et al. (2019) used natural β-carotene source as sweet potato and gut weet in false clownfish (*Amphiprion ocellaris*) and showed that skin coloration was enhanced by the utilization of this organic source.

Survival and growth are important issues that should be successfully managed at larval and juvenile stages of culture period. High survival rate at this period lead to affect total production target at the of the year. Therefore, several parameters should be optimized for high survival. Among them, optimum water parameters, feed intake, feed quality (essential nutrients such as PUFA, EAA, minerals and vitamins) and stock density are important topics. Live prey is the first food for mouth-opening larvae which should contain similar nutrient composition of egg yolk. Rotifer (*Brachionus plicatilis*) should be used from hatching to 8-9 days post hatched (dph) larvae with a level of 10 individual/ml in culture vessel. Artemia is also introduced from 6 dph with a level of 1 individual mL^{-1}. then linearlly increased. At larval feeding period, nutritional composition of live prey rotifer and artemia play an important role for survival. Highly unsaturated fatty acids such as docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6) are important in clownfish nutrition. For instance; rotifer and artemia enriched with microalgae oil showed improvement at larval and juvenile stage of *A.percula* and this result is attributed that high HUFA content of those live prey and as a result improved growth performance and shorten larval stages (Dhaneesh and Kumar, 2017). Metamorphosis of larvae starts around 12-15 dph and at this point formulated particule diets (200-400 µm) are given to larvae. Broodstock nutrition is important for culture performance and formulation should be similar in their natural foods. They mostly consume copepods, algae, polychaetes, crustaceans, tunicates and amphipods in natural feeding behaviour (Olivotto and Geffroy, 2017). Feeding rate is important in clownfish culture and feeds should be introduced several times a day with a small portion just like in their natural feeding habit. However, diets must be balanced with protein and lipid ratios with essential nutrients. Most importantly, organic pigment sources should be in their diet in order to sustain orange colour.

1.3. Red porgy (*Pagrus pagrus*)

The red porgy (*Pagrus pagrus*) is protogynous hermaphrodite fish species widely found in moderate marine water temperatures around Mediterranean and Atlantic regions. In Türkiye, red porgy is found from north of Eagean sea coastline to east Iskenderun Bay. This species distributed from 20 m to 300 m depth at water column with rocky, sand and gravel sediment in natural habitat. Red porgy is carnivorous fish species mostly fed on crustaceans such as crabs, shrimps and molluscs like other sparid fish (Figure 3). Broodstock of red porgy can reach to 20 kg in weight. Ayyildiz et al. (2020) had reported that there is no restriction on amount of captured fishing for red porgy. In other studies, also reported that population of red porgy has decreased in due to overfishing (Russell et al., 2014). Therefore, regulations and legislations are needed for protection of natural stocks of this species. Natural stocks of this species should be controlled for sustainable production for future aquaculture purposes.



Figure 3. Red porgy (*Pagrus pagrus*) (http://www.ictioterm.es/nombre_cientifico.php?nc=108)

Broodstock management has been successfully sustained since 1995 around Mediterranean countries. Egg collection from broodstock is generally occurred from January to May under natural photoperiod. Culture of red porgy has been started commercially early 1990s (Kentoyri et al., 1995) in Mediterranean region and Argentina around 1994 (Machinandiarena et al., 2003; Radonic et al., 2005). Total production of Red porgy mainly occurred in Argentina (34.1%), Brasil (22.0%), Greece (11.7%) and Türkiye (10.8%) (Costa et al., 2021).

Several studies have focused on the culture of this fish due to fast growth performance and good adaptation to further culture periods such as juvenile and cage production steps. Larval culture of red porgy is also well established based on other sparid species gilthead sea bream (*Sparus aurata*) larvae culture experiences. Larvae culture success is mainly affected by fish husbandry and culture procedure such as microalgae addition (microalgae species and density), live prey selection (depending on mouth size after hatching), requirement of essential nutrients, culture tank shape, colour and volume, water quality, light intensity and photoperiod. For example, DHA requirement of red porgy larvae is highly essential for skelethal development (Roo et al., 2009). At larval stage, essential fatty acids, amino acids, vitamins and minerals are mainly delivered by enrichment of live prey such as rotifer and artemia. Availability of live prey in larval culture tanks is an important issue in order to get enough nutrients by larvae. In this case, culture system plays vital role in red porgy larval production. Mesocosm system is comparing to intensive system lead to much more interaction between live prey and larvae (Roo et al., 2010a).

The benefits of using synthetic pigments are much more digestible and more effective. On the other hand, those chemical pigment sources should be introduced continuously by feeding in order to keeping desired colour till the harvesting. Moreover, synthetic pigments are estimated to be removed from both market and production process of cultured fish. Comparing to synthetic pigments, natural ones have positive effect on taste of flesh. In addition, sustainable production should be managed by natural-based pigment sources such as microalgae and other terrestrial plants (Makri et al., 2021).

One of the other study topics is the replacement of fish oil and fish meal by alternative terrestrial or marine based diet ingredients for sustainable aquaculture production. Recent feeding trials revealed that FM protein can be replaced by up to 54% poultry-based meal protein in the diet for juvenile Red Porgy diet (Hill et al., 2019). The other problems of culture of this species are occurance of skelethal deformities at larval and juvenile stage. In marine fish larvae, successful osteological development is related to several factors such as light intensity (Cobcroft et al., 2004), salinity (Cobcroft et al., 2004), temperature (Sfakianakis et al., 2004), stock density, culture systems (Roo et al., 2010b), water inlet current, broodstock management and genetics, nutritional deficiencies at larval and broodstock feeding. Roo et al. (2010c) has reported that intensive rearing system had a moderate effect on the development of particular skeletal deformities such as cranial malformations and kyphosis and the rest of the deformities and bone developments disorders are not affected by the type of rearing system.

Economic value of produced fish is increased by their flesh and skin colour. Especially, pink-red skin pigmentation plays important role in consumer's first impression. Pigmentation

is also important in flesh-quality cultured fish species such as salmonid fish (rainbow trout and Atlantic salmon). In terms of red porgy, main drawbacks of the culture process are skin and meat colour is pale comparing to natural individuals have shiny red-silver colour. In order to solve this problem, synthetic and natural pigment sources have been evaluated for juvenile feeding studies. For instance, dietary astaxhantine supplementation with several time applications such as 50-80 mg kg^{-1} for 6 months or 50 mg kg^{-1} 3 months and 80 mg kg^{-1} 3 months have resulted better skin color (Nogueria et al., 2021). Skin colour, flesh taste and texture are still open for development of cultured fish comparing to wild individuals. Therefore, alternative diets should be studied with essential nutrients needed for this species, for instance, amino acid requirements studies are scarce.

1.4. Bluespotted seabream (*Pagrus caeruleostictus*)

Bluespotted seabream (*Pagrus caeruleostictus* Valenciennes, 1830) belongs to class Actinopterygii, order Perciformes of the family Sparidae and genus Pagrus. This fish is a demersal marine fish distinctive pink colour with blue spotted points at dorsal and lateral layers (Figure 4). They live between 50-200 meters at rocky covered substrates. It is commonly found in Mediterranean Sea and Eastern Atlantic region.



Figure 4. Blue spotted sea bream (*Pagrus caeruleostictus*) (http://www.ictioterm.es/nombre_cientifico.php?nc=107)

Bluespotted seabream is a highly valuable commercial fish, however, aquaculture effort on this fish has not been improved yet. On the other hand, due to the similarity to other sparid fish, it is possible to culture and develop methods. *P. caeruleostictusis* is a protogynous hermaphrodite (Chakroun-Marzouk and Kartas, 1987) that becomes sexually mature at two years old. Larval and broodstock husbandry, live prey utilization, nutritional needs of both larval, juvenile, portion and broodstock stages should be determined. The optimization of

those factors is crucial for successful cultural performance. Bluespotted seabream fed on mostly crustaceans, small fish, polychetes, echinoderms and molluscs (Hamida et al., 2009). Some parasite occasions have been reported at gill (Bouguerche et al., 2019) and arterial bulb (Khlifa et al., 2012) in bluespotted seabream. Therefore, in order to be sure that broodstock candidate is parasite free, external and internal sanitation with appropriate chemicals should be applied. Determination of female and male individuals during the maturation period is also important. For that purpose, histology seems like the most preferred method for gender determination, reproduction stage and hermaphroditism verification (Ismail et al., 2018).

Especially, larval survival should be increased by utilization of enriched live prey and formulated diets for further culture period such as on-growing and adaption period. Therefore, new feed formulations with probiotics and prebiotics should be improved and commercially produced.

1.5. Greater amberjack (*Seriola dumerili,* Risso 1810)

The greater amberjack (*Seriola dumerili,* Risso 1810) belongs to Carangidae family and is a marine pelagic carnivorous fish which is distributed mainly in Atlantic Ocean, Mediterranean See and all over the world. This fish is highly characteristic with large mouth, blue-greenish dorsal skin colour and strong functional tail (Figure 5).



Figure 5. Greater amberjack (*Seriola dumerili*)
(http://www.ictioterm.es/nombre_cientifico.php?nc=83)

Reproductive season of greater amberjack is between late spring to early summer (Corriero et al., 2021). This fish is characterized with high flesh quality and very fast growth rate which are both very important criteria for the aquaculture industry (Nakada, 1999). Japan has high interest in Seriola sp. Production, where *S. dumerili* has accounted for one-third of the total, produced Seriola biomass (Sawada et al., 2006). Therefore, it is still captured juvenile from natural stocks in order to answer this high demand of this fish (Japanese Ministry of Agriculture Forestry and Fisheries (www.maff.go.jp/j/tokei/kouhyou/kaimen_gyosei).

Mazzola et al. (2000) mentioned that this fish can grow up to 6 kg in 2.5 years. Its culture studies were based on collecting juveniles from the natural habitat of this species. Recent studies have promising results on larval and juvenile production due to investigations on diet requirements, physical and chemical conditions of larval and juvenile stages. Temperature is one of the most important parameter for optimum growth and survival. Abbink et al. (2012) has determined optimum temperature 26.5 ºC for 4-gram yellowtail juvenile during 32 days of culture and resulted in good growth. In addition, Fernandez-Montero et al. (2018) have investigated optimum culture temperature during on-growing period of greater amberjack. They also recorded 26 ºC as the optimum temperature for growth and feed utilization for 20-gram juvenile fish.

Another problem of offshore culture of greater amberjack is monogenean ectoparasites occasion. In order to solve this problem, some efforts have been done such as increasing the depth of cages and adaptation and development recirculating aquaculture systems (Abbink et al., 2012). Wild-caught fish showed deteriorated gametogenesis reared in captivity, this result could be nutritional deficiency during broodstock management (Zupa et al., 2017). In fact, feeding larvae and juvenile with high quality nutritional diets are important in order to have high productive broodstocks. Therefore, determination of nutritional needs of larvae and juvenile is a critcal issue in order to be successful culture performance. It has been reported that inedequate larval feeds may lead to low survival and skelethal anomalies. Recently, some efforts to determination of larval nutritional requirements of greater amberjack have been done. For instance, Matsunari et al. (2013) evaluated effects of rotifers enriched taurine on survival and growth of greater amberjack larvae. One of the main problems at larval period is contact of the larvae to tank bottom and this is attributed to high mortality. Therefore, live prey and formulated diets should have enough energy against to swim up to the vertical movement at water column. Roo et al. (2019) has determined optimum n-3 HUFAs levels in *Artemia* as a result that level should be 12-17% of the total fatty acid contents. Comparative studies on fatty acid compositions of wild and cultured female broodstock revealed that dietary fatty acid profile needs higher levels of oleic acid, arachidonic acid and contrary lower levels of linoleic and eicosapentaenoic acids (Rodriguez-Barreto et al., 2012).

Rearing method is another important factor that achieves high survival and larval growth. Mesocosm, the semi-intensive technology has been reported as the best culture method for Seriola species. Within this method, infrastructures, environmental factors in rearing, food sources and feeding methods differ. Overall, formulation of both larval and juvenile diets, stock density and broodstock management should be studied in this fish in order to enhance

high production rates. Research and development departments of commercial hatcheries and universities should work and propose projects in order to get large-scale production success.

1.6. Common dentex (*Dentex dentex*)

Dentex dentex is a carnivorous Sparidae fish of high desirable market value with excellent taste. They are abundant in shallow rocky waters between 50-100 m deep and live on various subtrates such as *Posidonia oceanica* meadows, coastal detritic areas, coralligenous community, sandy bottoms with *Caulerpa* and *Cymodocea* (Marengo et al., 2014). *Dentex dentex* is classified as "vulnerable" in the Red List of Threatened Species in the Mediterranean Sea by the International Union for the Conservation of Nature (IUCN) (Abdul Malak et al., 2011) (Figure 6).



Figure 6. Dentex dentex (*Dentex dentex*) (http://www.ictioterm.es/nombre_cientifico.php?nc=103)

Aquaculture and fisheries potential of this fish have been widely reviewed (Rueda and Martinez, 2001; Gimenez and Estevez, 2008). Most studies are related to their larval rearing, physiology, nutrition, osteological ontogeny, morphologic, immunology, and malpigmentation and skelethal deformaties. There are a couple of issues still remained in production. Especially, the survival rate is quite low (1.5-35%) comparing to other cultured marine fish at larval and weaning period. It is due to their aggressive behavior, low stress tolerance and disease occurance at these stages. In fact, Dentex has high tolerant to handling compare to other cultured fish species during larval and on-growing periods and the handling issue is not major factor responsible for the low survival (Morales et al., 2005).

Different culture methods affect the larval performance of Dentex. Mesocosm culture technique had better survival and growth during the larval period due to similar to their natural habitat (Gimenez and Estevez, 2008). Skelethal anomalies such as saddleback syndrome are one of the most deformaties in this fish (Koumoundouros et al., 2001). Rearing conditions were found to be related to those anomalies. In the natural environment, appearance of these

deformations is low frequency and mostly they occur due to chemical pollution. However, under intensive cultivation of this fish, anomalies are major problems caused by stock density, water parameters and other culture parameters.

The low (higher than 35% mortality 3 dph) and high-quality (lower than 10% mortality at 3 dph) batches have been investigated base on egg nutritional value (Gimenez et al., 2006). According to this study, low hatching eggs contained higher carbonhydrate levels and activity of some enzymes such as alkaline phosphatase and pyruvate kinase. High survival rate is very important at larval and juvenile production. Nutritional needs of larvae, fingerlings (2-3 gram) and juveniles (10-30 gram) can be changed due to their high growth rate and physiological needs. Early studies of *Dentex dentex* fingerlings showed that optimum dietary protein and lipid levels were 45% and 17%, respectively. (Espinos et al., 2003). Skalli et al. (2004) stated that *Dentex dentex* can efficiently use lipid contents of formulated diets, as high as 24% lipid and dietary lipid had a protein-sparing effect on this species at different growth stages.

Fish meal and fish oil are the main sources of protein and lipid in formulated diets. Those ingredients have high price due to increasing demand and fishing pressure. Therefore, fish-meal replacement by different plant sources are being evaluated for fish species. For instance, Chatzifotis et al. (2008) replaced fish meal by soy protein meal at level of 25% did affect feed intake and growth, moreover adding 2 g/kg Taurine improved growth in dentex juveniles. Moreover, *Dentex dentex* is not able to metabolize high levels of carbonhydrates in their diet. For that reason, carbonhydrate levels should not exceed 28% levels (Pérez-Jiménez et al., 2009). Same author also stated that protein-sparing was achieved by utilization less complex carbohydrates (maltodextrin) and feed utilization was promoted by optimum 18% of carbonhydrate (Pérez-Jiménez et al., 2015) and oxidative protection (Pérez-Jiménez et al., 2017). In addition, replacement fishmeal by soymeal with a level of 25 and 40 had effect on celluler response such as Heatshock response and apoptotic pathway of the digestive tract in dentex dentex (Antonopoulou et al., 2017).

1.7. Mussel (*Mytilus galloprovincialis*)

Mussel (*Mytilus galloprovincialis*) is common bivalve species in the Mediterranean, Aegean, Marmara, and Black Sea regions. They create large sea bed communities where the water column is rich in phytoplankton. They are rich in essential nutrients such as protein, lipids, iron, and vitamin B12. However, they may have contained some heavy metals and pollutants based on where they grow due to their filter-feeding ability. Therefore, the cultivation of black mussels with vertical or horizontal long-line systems offshore can keep those values at low levels.



Figure 7. Black mussel individual (http://www.ictioterm.es/nombre_cientifico.php?nc=358)

There is an increasing demand for the culture of this bivalve species due to high meat gain, fast growth performance, low cost for investment and high nutritional values. Cultivation place is mainly depending on the primer productivity of the water column and this productivity is changed by months of the year. In this case, natural spat (mussel larvae) biomass is affected by these seasonal changes (Karayucel et al., 2010). Therefore, culture attempts of bivalve species are very important in order to sustain enough spats for culture performance. There are some reports on different materials of long-line cultivation of black mussel in Sinop, Türkiye. Karayucel et al. (2003) mentioned that nylon socks are better for spat settlement compared to cotton ones and harvesting time is only two years since spats are settled. Currently, Marmara Sea has several investments on mussel farming around south of Marmara Islands. In this region, mussels are cultured by long line systems between 15 to 30 meters in depth. This method is based on releasing ropes to the water column and letting it be spat settled from nature. Harvesting time is depending on water temperature and phytoplankton abundance. This period can take two years from spat settlements and harvesting time is decided according to mussel growth.

Due to the increasing human population, natural stocks seem to be not enough for future demand. Therefore, spats should be continuously produced and sustained by govermental and private companies in the cultural areas. Within this protocol, spats can be settled to long lines throughout the year and this may increase total mussel production.

1.8. Whiteleg shrimp (*Litopenaus vannamei,* Boone 1931)

White leg shrimp (*Litopenaus vannamei*) is the most common cultured shrimp species around the world. The global production of whiteleg shrimp exceeded 154.5 thousand tons in 2000 to 5.812,5 tousand tonnes in 2020 (FAO, 2022). Shrimp culture has high potential due to its fast growth and biomass gain. However, there is still some limitation because of the feed formulation, disease problem and high mortality at the larval stage. Fluctuation in environmental conditions mostly affects the health of cultured shrimp and its production.



Figure 8. Whiteleg shrimp (*Litopenaus vannamei*) (https://ssafc.tiikm.com/project/ignorant-factors-about-litopenaeus-vannamei/)

Microalgae are the main feed of shrimp and the nutritional composition of microalgae plays an important role in growth and survival (Pina et al., 2006). There are several attempts on developing shrimp dies. The antioxidant contents of microalgae are attributed to have beneficial effects on shrimp health via directly used in diets or fresh utilization in water column. For instance, Rahman et al. (2017) mentioned that supplementation of microalgae *Tetraselmis chuii* with a 50% in diets had positive effect on both survival and water column. That result is attributed to antioxidant contents of microalgae that supply vitamin E and pigments. In another study, microalgae *Thalassiosira weissflogii* have been evaluated in diet of whiteleg shrimp with improving survival, growth and reduced the metamorphosis time due to their HUFA contents (Tam et al., 2021). Not only microalgae but also macroalgae can be utilized in diets of whiteleg shrimp. Schleder et al. (2017) showed macroalgae *Undaria pinnatifida* have positive effect of immunology resistance to *Vibrio* spp. and other brown seaweed *Sargassum filipendula* enhances culture temperature-caused stress resistance of this species. Similar positive results were gained by addition of other macroalgae species such as *Ulva lactuca* and *Gracilaria parvispora*. Rodríguez-González et al. (2014) showed the possible usage of 5% addition of *Ulva lactuca* and three levels (5%, 10% and 15%) of *Gracilaria parvispora* replacement by fishmeal without any negative effect on growth.

Most studies are related to feed formulation for better growth and survival gain. For instance, Terrazas-Fierro et al. (2010) evaluated different meal sources instead of fish meal such as shrimp head meal, squid meal and scallop by-product meal and these meals were reported as good sources of amino acids for juvenile whiteleg shrimp. More interestingly, effect of different feed additives such as garlic utilization had improved survival and 6% addition of garlic improved weight gain of whiteleg shrimp at the end of the 60 days of feeding trial (Labrador

et al., 2016). Growth is mostly related to quality and quantity of protein contents in diets. Shahkar et al. (2014) evaluated optimum protein level for juvenile whiteleg shrimp. According to this study, optimum protein level should be at least 25% and not higher than 33.4% of diet and more than that level has been reported to increase ammonium level in rearing water.

Shrimp culture in biofloc technology has many advantages in both water column and culture animal health and growth (Yun et al., 2017). Within this culture method, inorganic material can be converted into organic biomass and this lead to additional nutritional and immunological effect on shrimp. Yun et al. (2017) stated that when biofloc system is applied in culture water, up to 33% fishmeal replacement by soybean meal in diet of juvenile whiteleg shrimp resulted in better physiology and health condition. In other fish meal replacement has been performed by Hamidoghli et al. (2019) that studied single-cell protein sources in diet of whiteleg shrimp. It has been shown that fish meal in diet can be replaced by single-cell bacteria *Corynebacterium ammoniagenes* with a level of between 10% and 20% without any additional amino acids. Overall, biofloc system has appeared to be the best production methods in terms of improved growth performance, immune response and disease resistance even when low-protein feeds are used in production (Panigrahi et al., 2019). This positive effect is mainly related to beneficial microorganisms that naturally occur in rearing water. Moreover, Sadat Hoseini Madani et al. (2018) mentioned that specific dietary addition Bacilli probiotic species (*Bacillus subtilis* and *Bacillus licheniformis*) had positive effects on growth, feed utilization and immune parameters.

In another culture technology is integrated multi-trophic aquaculture (IMTA) systems which allow different species can have cultivated at the same time by utilization their organic waste for each other. For instance, improvement of water quality and reduction of feed supply by 75% have been obtained by integrated systems *Litopenaus vannamei* and *Caulerpa lentillifera* (Anh et al., 2021). Moreover, survival and growth were increased in integrated system compared to monoculture. Recent studies focused on effect of different nursery systems of whiteleg shrimp culture. Photo-heterotrophic and biofloc technologies are still promising techniques with high growth performance whereas clean-water recirculating aquaculture system is announced with lower survival (Rodríguez-Olague et al., 2021). This is due to the lack of beneficial probiotic bacterias in this system. As a result, it seems to be RAS system is the most potential system that leads to produce large-scale whiteleg shrimp with biofloc application at a low production price. Therefore, RAS system should be built and feed the system with carbonhydrate sources such as corn starch (Tinh et al., 2021) or mannan oligosaccharides (Kaya et al., 2019) can enhance the microorganism biomass for shrimp postlarvae and juvenile for future production success.

1.9. Common octopus (*Octopus vulgaris*)

Türkiye has great potential for the common octopus (*Octopus vulgaris*) production due to their natural habitat occurring in Eegaen Sea. The common octopus has several positive advantages for cultivation such as rapid growth, valuable market price, high fecundity and food conversion rate. However, in order to commercialize, larval and juvenile production should be managed like other cultured animals. Therefore, most of studies are focused on paralarval and broodstock managements. Octopus paralarvae hatch with about 1.0-1.5 mm of mantle length and they are planktonic, actively swim (Figure 9). At this stage, they require high amount of nutrients due to their high metabolic rate. This period ends depending on rearing temperature such as 47-54 days at 21.2 ºC and 30-35 days at 23 ºC (Imamura, 1990; Villanueva, 1995). Igleasias et al. (2016) have also mentioned about effect of salinity levels on *O. vulgaris* individiuals. The authors suggested that 20 psu salinity is lethal for *O. vulgaris* and at 25 psu salinity, daily food ingestion was decreased. Their nutritional needs differ in development stages; larval, paralarval, juvenile, and portion size and broodstock individuals. Proteins are the main organic materials in Octopus species.



Figure 9. Common octopus (*Octopus vulgaris*)
(http://www.ictioterm.es/nombre_cientifico.php?nc=226)

Garcia and Gimenez (2002) have stated that Protein: Energy ratio is a very important factor ongrowing period of common octopus. In addition, importance of fatty acids is also mentioned paralarvae of common octopus. Especially during this period, based on their fatty acid composition of hatching, octopus needs a high amount of polyunsaturated fatty acids, mostly docosahexaenoic acid with a level of 20-30% of total fatty acids (Navarro and Villanueva, 2003). Recent studies on fatty acid requierements of *O. vulgaris* paralarvae and broodstocks revealed that especially, n-3 HUFAs, oleic acid and Arachidonic acids are necessary for both reproduction and paralarval production performance (Estefanell et al., 2017).

Paralarval production is mainly established in Italy (De Wolf et al., 2011) and Spain (Iglesias et al., 2004; Roo et al., 2017). At this period of *O. vulgaris*, artemia is the most suitable

live prey for paralarvae culture and this live prey should be enriched in order to contain high amount of essential fatty acids and amino acids. Enrichment procedures differ in both products (mainly lipid emulsions, fresh microalgae and powders) and application time. For instance, Seixas et al. (2008) enriched juvenile Artemia at different sizes with several microalgae but it has been not met for *Octopus vulgaris* paralarvae due to low levels of essential fatty acids comparing to natural feeds. However, microalgae *Rhodomonas lens* seemed to be best for artemia enrichment with high protein, low carbonhydrate and moderate lipid levels. Villanueva et al. (2004) evaluated methionine-enriched *Artemia* for paralarval feeding of *O. vulgaris* that resulting increased survival and accumulation of essential amino acids in octopus. Crustacean zoeae are the main diet of paralarvae in natural habitat. Determination nutritional needs are very difficult at this stage, therefore, several diets based on natural ones are still being evaluated for better growth and survival. For instance, Iglesias et al. (2014) have recently focused on effect of crab zoeae (*Maja brachydactyla*) on growth and biochemical composition of *O. vulgaris* paralarvae and mentioned that crab zoeae had a positive impact on growth and biochemical composition of *O. vulgaris* paralarvae. Moreover, importance of prey size, prey density and feeding frequency are also crucial factors for successful paralarvae culture performance (Iglesias et al., 2006). The best growth and survival rates have been achieved by utilizing both crustacean zoeae used co-feeding with Artemia (Iglesias and Fuentes, 2014).

The natural diets of octopuses are mainly crabs, fish and other cephalopods. However, crab biomass is not enough for both directly human consumption and octopus feeding. Therefore, several attempts have been done to replace the natural diets of octopuses during ongrowing period. For instance, Prato et al. (2010) have evaluated different orgin diets combination or solely usage of mussel (*Mytilus galloprovincialis*), fish (*Diplodus vulgaris* and *B. boops*) and crab (*Carcinus mediterraneus M. crispata*). According to this study, DHA and EPA rich diets, in this case, fish and crab (*M. crispata* and *D. vulgaris*) combination showed the best growth rates. In another study, Domingues et al. (2010) studied alternative prey crayfish (*Procambarus clarki*) and hake (*Merlucius gayi*) for *O. vulgaris* resulting in hake being a promising diet for Octopus feeding whereas crayfish is not.

Physical parameters are also important as chemical properties of diets. Stock density and shelter type play important roles in stress tolerance and growth of *Octopus vulgaris* under captivity. Juvenile individuals can be cultured in floating cages attached with internal shelter (Rodriguez et al., 2006). However, octopus should be kept individual shelters due to group culture can induce high cannibalism (Estefanell et al., 2012). Domingues et al. (2010) studied three culture densities for ongrowing period (4 kg/m^3, 8 kg/m^3 and 15 kg/m^3) and the highest survival was obtained in the low-density culture. They also concluded that maximum stock density should not exceed 20 kg/m^3. Kwon et al. (2019) evaluated three different types of shelters (tube, pipe and no-shelter) for on-growing *O. vulgaris* together with an assessment of low and high temperature resulted that tube shelters have positive effects on growth and survival. Moreover, different light type has been announced that affect the survival of *O. vulgaris* paralarvae. Garrido et al. (2016) mentioned that red-orange light had a better effect than white light on the survival of paralarvae.

In order to succeed at commercial scale production, nutritional needs of paralarvae and juvenile of *O. vulgaris* should be determined. Alternative live prey such as water flea (*Daphnia magna*), fairy shrimp, and moina and nutritional improvement of formulated diets should be evaluated. Currently, there are several microalgae rich in DHA and EPA such as *Crypthecodinium cohnii* and *Schizochytrium sp.* which are commercially produced and packed. Enrichment blended with those microalgae via live prey or alternative ingredients in diets can be solved the nutritional needs of paralarvae in terms of both amino and fatty acids compositions. Worms have quite high protein content which can be used as a paste or dried food for paralarval nutrition. There are several studies on mealworm (*Tenebrio molitor*) and superworm (*Zophobas morio*) production and the nutritional contents of these worms. Future lay on alternative protein and lipid sources in order to sustainable feed production and this reality positively affects culture performance together with research.

2. Conclusion

Overall all-new species require special conditions for their growth and survival. Physical and chemical water parameters should be optimized depend on new species. Feed properties are also important for delivering essential nutrients to the cultured animals. Essential nutrient requirements can be changed depending on fish species and their natural feeding habits. For instance, colorful fish need additional pigment sources in diets and those should be organic made. In this case, microalgae can be a nice option as an organic pigment source. Additionally, broodstock management and stock density of new species should be carefully managed in order to successful larvae and juvenile culture production. To sum up, when new species are considered to be cultured, not only one parameter but also the whole culture period should be optimized. For that purpose, commercial and academic efforts should be given equally to the same target species. Research and development strategies and human sources must be increased; international connections should be arranged between both commercial and academic areas for the common interest.

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CHAPTER 3

CURRENT BACTERIAL AND VIRAL DISEASE ISSUES IN MARINE AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.003

1. Introduction

Aquaculture is one of the fastest growing industries among the industries producing food animals, and it has an important role in meeting the need for animal-based protein for the ever-increasing world population, and it is predicted that it will maintain this position in the near future. As can be seen when looking at the statistics of FAO (Food and Agriculture Organization), aquaculture production throughout the world is in a continuous increase trend, excluding the pandemic period, and according to the data of 2020, the total aquaculture production is 87.5 million tons, of which 33.1 million tons are obtained from marine fish farming (FAO, 2022). Similar to the world statistics, a continuous increase is observed in aquaculture production in our country over the years; according to TUIK (Turkish Statistical Institute), the amount of aquaculture production in Türkiye is 514,983 tons in 2022, of which 368,742 tons is marine aquaculture (TUIK, 2022).

In aquaculture, especially in recent years, with the contribution of both scientific and technological advances, both species diversity and production volume have increased significantly and continue to increase. However, this situation causes the environmental stress on living things to increase, making them more susceptible to diseases and sometimes causing disease outbreaks with devastating effects. The best method in the fight against diseases is the use of a proactive approach in which various preventive measures are taken before the disease has appeared yet.

In order to minimize the losses caused by diseases in aquaculture; various prophylactic measures are taken, such as the use of supplements, the use of immunostimulants, probiotics, vitamins, vaccine, as well as reducing the stock density and improving water quality. After disease outbreaks, various chemotherapeutics, chemicals, some living organisms and phages are used in fish farming for treatment purposes. Among them, antibiotics, which have been used for many years as the only option in the treatment of bacterial diseases, cause antibiotic resistance with the contribution of their frequent and misuse, and as a result, there are great difficulties in the treatment of some diseases. However, despite all the precautions taken; bacterial, viral, fungal and parasitic fish diseases still cause significant economic losses in aquaculture.

Once disease occurs, the etiology should be diagnosed as soon as possible and the treatment process should be started. For this, rapid, reliable and highly sensitive diagnostic tests are needed. In the diagnosis of fish and shellfish diseases; Post-mortem necropsy and sampling, isolation and culture of the agent (if applicable), identification of the agent with the help of various staining and/or imaging techniques (histopathology, immunohistochemistry, serology, molecular diagnostics, MALDI-TOF etc.) are the primary methods.

During the scientific literature search, two previously known problems were encountered again while writing this book chapter. First, the status of a previously reported disease in subsequent years is often unknown because similar types of studies are not conducted due to the assumption that little academic interest will be received. For this reason, the implementation and promotion of monitoring and surveillance as a state policy, especially as in Scandinavian countries, will make invaluable contributions to both sustainable aquaculture and environmental protection. Another problem encountered is that some important information such as anamnesis, mortality rate, pathogen and/or host-specific features, environmental parameters, that may be very important in the evaluation of the disease has been given incompletely in case reports.

In this chapter, detailed information about bacterial and viral infectious diseases having only high impact and/or incidence rate seen in the Turkish marine aquaculture industry are given, it is not intended to create an inventory. Apart from these main diseases, some of the less severe or rare diseases are also briefly mentioned.

2. Bacterial and Viral Diseases

2.1. Vibriosis

Vibriosis is a common name for disease where dozens of different species belonging to the *Vibrio* genus, which infect a wide range of brackish/marine animals including fish, crustaceans and molluscs, have been reported as etiological agents. Whereas some of the disease-causing *Vibrio* species are well-known for many years, some are emerging species that have been reported in recent years and in lesser numbers. Due to the fact that some members of the *Vibrio* genus are still in a relatively undefined position taxonomically, and it is difficult to make a differential diagnosis with biochemical tests and even molecular biological diagnostic tools. Some researchers have thus concluded that caution should be exercised in defining the names of species in this second group as pathogens (Austin, 2011; Thompson et al., 2013).

2.1.1. *Vibrio anguillarum*

Vibrio anguillarum is a Gram-negative, halophilic, motile by means of a polar flagellum, facultative anaerobic, non-spore forming curved rod belonging to the family Vibrionaceae. Members of the species reduce nitrates, produce acid from D-glucose, D-mannose, L-sorbitol, sucrose, are oxidase and Voges–Proskauer positive as well as able to growth in the presence of 0.2% to 6% sodium chloride (Garrity, 2007). *Vibrio anguillarum*, like many other marine bacteria, requires salt to grow. The bacterium is easily grown on many general-purpose media containing 1-2% sodium chloride such as trypticase soy agar after 24-48 hours of incubation time at 20-25°C. At the end of the incubation period, it forms cream coloured (TSA) or greyish-white coloured (Blood Agar), raised and entire bright colonies on the agar surface. Although this species has a very wide temperature and salinity tolerance, more than 7% salinity and temperatures over 41°C have a lethal effect on the bacterium (Garrity, 2007).

Vibrio anguillarum is one of the earliest known fish pathogens and the bacterium has been found to be responsible to cause diseases in many fish including Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus* spp.), rainbow trout (*Oncorhynchus* mykiss), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), turbot (*Scophthalmus maximus*), striped bass (*Morone saxatilis*), cod (*Gadus morhua*), Japanese eel (*Anguilla ja-*

ponica), European eel (*Anguilla anguilla*), ayu (*Plecoglossus altivelis*), sole (*Solea* spp.), and mullet (*Mugil* spp.) (Toranzo et al., 2005; Hickey and Lee, 2018).

Studies have shown that *V. anguillarum* is found in the marine environment as well in the aquaculture environment including feed, equipment, tanks, pipes and live feed (Garrity, 2007; Austin et al., 2012; Turgay et al., 2019). Although there is no consensus on exactly how the infection occurs, the most common opinion is that the bacteria first cling to the skin of the fish and penetrate into the tissues or are taken directly into the gastrointestinal tract with water or live feed. According to this view, after the bacteria colonize the intestine, they enter the bloodstream and causes septicemia (Grisez et al., 1996; Svendsen and Bøgwald, 1997; Prol-García et al., 2010). Environmental factors also play a role during infection, especially in aquaculture systems, and factors such as worsening of water quality parameters (physical and chemical) of the cultivated water, increase in pollution and organic load, increase in stock density, sudden changes in water temperature promote disease emergence (Frans et al., 2011).

Various virulence factors of bacteria such as chemotaxis and motility regulating genes, protease and haemolysin activity, iron uptake system, OMPs (outer membrane proteins), quorum sensing and related virulence genes such as flagellin gene (*flaA*) have been described as partakers in pathogenesis (Hickey and Lee, 2018). In vertebrates, iron in the fish blood is bound to certain specific molecules, such as transferrin in serum. *Vibrio anguillarum* has a highly efficient iron uptake system encoded in the pJM1 plasmid as one of its most important virulence features. This uptake system includes anguibactin, a siderophore produced by cells of *V. anguillarum* and transport components. Thanks to this plasmid, the bacterium becomes able to use the iron of the infected host, which is crucial for bacterial growth (Tolmasky and Crosa,1991).

The disease is a typical highly lethal generalized hemorrhagic septicemia seen in many fish diseases and can cause mortality rates of up to 100%. In cases where the infection progresses acutely, an intense mortality can be observed without any obvious symptoms. Petechiae on the body surface, discoloration and ulcers on the skin, erythema on the bottom of the fins and in mouth, exophthalmos in the eyes as external findings in diseased fish; as internal findings, swelling in the intestines and fluid in the intestinal lumen, necrotic lesions and haemorrhage in the muscles and other internal organs such as liver and kidney can be observed (Frans et al., 2011; Austin et al., 2012). Although O1, O2 and O3 serotypes are determined to be highly virulent among the serotypes defined as disease-causing, O4 and O5 serotypes are less frequently isolated from disease outbreaks. O1 serotype, which is one of the serotypes directly associated with fish diseases, is homogeneous in itself, while O2 and O3 serotypes

are subdivided within themselves, and accordingly, O2a is associated with both salmonid and non-salmonid species, O2b is associated only with the sea, O3a is associated with diseased fish, while O3b is only environmental/non-disease type. (Toranzo et al., 2005; Austin et al., 2012). *Vibrio anguillarum* was first reported in Türkiye by Candan (1993) as a disease agent in cultured gilthead sea bream (*Sparus aurata*), since then the bacterium has been isolated from many marine species and continues to be isolated occasionally including European seabass (Çağırgan, 1993), Atlantic salmon (Candan, 2000), red porgy (*Pagrus pagrus*) (Korun and Gökoğlu, 2007), rainbow trout (marine cages) (Timur et al., 2011) and meagre (*Argyrosomus regius*) (Dinçtürk and Tanrıkul, 2018).

The disease can be diagnosed by many methods. Conventionally, general-purpose media such as Blood Agar, Marine Agar or 1-2% sodium chloride added Trypticase soy agar (TSA), Nutrient Agar, Brain Heart Infusion (BHI) agar are sufficient for laboratory culture of bacteria. If a selective medium is preferred, Thiosulfate Citrate Bile Sucrose (TCBS) agar or Chromagar, or VAM medium designed for predictive diagnosis can be used. Bacterial isolation is done from internal organs (kidney, spleen, liver) or lesions of infected fish or eye tissue by inoculation into media using standard methods and incubating at 25 ± 2°C for 2-4 days. Following basic tests (Gram stain, oxidase, O/129) on purely obtained isolates: serological tests (ELISA, agglutination etc.), molecular biological tests targeting various genes (16S rRNA, *empA*, *rpoS*) or their sequence (PCR, qPCR, MLSA, etc.) are recommended to be diagnosed/confirmed with more specific methods (Austin et al., 2012; Zrncic, 2020). Additionally, multigene analyzes can be used as an important tool for the differentiation of *V. ordalii* strains, which are genetically closely related to *V. anguillarum*. For example, Steinum et al. (2016) conducted an MLSA-based study on a large number of isolates, including Turkish isolates, and partially sequenced eight housekeeping genes. It was concluded that most of the environmental strains previously identified as *V. ordalii* are actually *V. anguillarum* species and that fish pathogenic *V. ordalii* strains have very close genetic similarity to *V. anguillarum*.

Various levels of success have been reported in the prevention of the disease using alternative methods according to literature, including probiotics (various *Aeromonas*, *Vibrio*, *Pediococcus pentosaceus*, *Vagococcus*, *Kocuria*, *Rhodococcus* species), immunostimulants (bovine lactoferrin, peptidoglycan), use of quorum sensing inhibitors, ozone treatment and the use of various Antimicrobial peptides (Frans et al., 2011; Austin et al., 2012). Vibriosis is one of the first diseases for which a commercial vaccine was developed. In addition, many experimental studies have been conducted according to literature on vaccine production techniques (live, attenue, whole cell-killed etc.) and application methods (injection, immersion

and oral with feed). In those studies, it was seen that the effectiveness of the vaccine was the highest with injection administration and the least via oral route delivery. Globally it is seen that vaccine companies produce many vaccines with monovalent or multivalent ingredients for use against *V. anguillarum*: AQUAVAC® Vibrio Oral, AQUAVAC® Vibrio Pasteurella, AQUAVAC® Vibrio (Salmon and Trout), Norvax Minova 6, Alpha Ject 2000, Alpha Ject 3000, Alpha Ject 4000, Alpha Ject 5-3, Alpha Ject 6-2, Alpha Ject Micro 6, Alpha Marine Vibject, Alpha Marine Vibrio, Lipogen Duo, Aquavac 6 vet, Aquavac PD7 vet. In the case of Türkiye: Alpha Ject 2000 (for European seabass, with Pasteurellosis), Alpha DIP Vibrio (for European seabass, monovalent), Alpha DIP 2000 (for European seabass, with Pasteurellosis). It requires multiple vaccinations both in the hatchery stage (immersion-based) and in cage systems (injection-based) to ensure full protection until harvest.

Despite all efforts and practices to prevent this disease, the use of antibiotics is inevitable when disease outbreaks occur. Although *V. anguillarum* has natural (amoxicillin and ampicillin) or acquired resistance against some antibiotics, those containing the *tet* gene contribute to tetracycline antibiotic or the cat gene chloramphenicol, *gyrA* and *parC* genes contribute to quinolone resistance; In general, it can be treated with antibiotics (oxytetracycline, potentiated sulphonamides, flumequine, florfenicol) used in aquaculture (Hickey and Lee, 2018; Zrncic, 2020). However, in Vibriosis disease, treatment with antibiotic containing feed may be inefficient, as fish lose their appetite and interest in eating shortly after the onset of the disease.

2.1.2. *Vibrio harveyi*-related species

Vibrio harveyi (synonyms *V. carchariae* and *V. trachuri*) is a Gram-negative, pleomorphic, rod-shaped, motile by means of polar and/or lateral flagella, fermentative, nitrate-reducing marine bacterium. The bacterium has bioluminescence ability, which is regulated by quorum sensing. *Vibrio harveyi* is oxidase and catalase positive, ornithine decarboxylase positive but arginine dihydrolase negative. It needs salinity for its growth, but cannot grow at 10% concentration. While sensitive to 150 mg of vibriostatic agent, many strains are resistant to 10 mg of the agent. Bioluminescence can be observed in some colonies. The bacterium produces yellow colonies on TCBS medium, hemolytic activity (both alpha and beta) in some strains is observed on blood agar (Pretto, 2020). As suggested by recent MLSA analyses, *Vibrio harveyi* constitutes the “Harveyi clade”, which is one of 14 different clades, that consists of species with high genetic and phenotypic similarity under its own name within the *Vibrio* genus (Sawabe et al., 2013). Although there is no definitive list of the species that make up the members of the clade, according to studies that performed MLSA analysis using several housekeeping genes, the species suggested by the researchers are *V. alginolyticus*, *V. azureus*, *V. campbellii*, *V. communis*, *V. harveyi*, *V. jasicida*, *V. mytili*, *V. natriegens*, *V. owensii*, *V. sagamiensis*, *V. parahaemolyticus*, and *V. rotiferianus* (Cano-Gómez et al., 2010; Yoshizawa et al., 2010; Yoshizawa et al., 2012; Sawabe et al., 2013). Clade members are found in sea water, marine animals, salt marsh mud however some other species within this clade have also been isolated from coral mucus. Another group of clade members includes pathogenic species (opportunistic or primary) as well as commensal species. A member of genus *Vibrio* known to be present in the mouth of sharks (*Vibrio carchariae*, synonym of *V. harveyi* in the current taxonomy) has also been reported in a human infection from a shark bite-associated wound (Pavia et al., 1989). *V. harveyi*, *V. campbellii*, *V. rotiferianus* and *V. owensii* species in the clade cause diseases with high mortality in fish and shellfish. However, although it has been shown that *V. harveyi* and *V. campbellii* species in the clade are closely related to each other genotypically and phenotypically, some researchers have reported that it is more accurate to define some *V. harveyi s*pecies as *V. campbellii* (Cano-Gomez et al., 2009).

Vibrio harveyi has been isolated and identified from a wide variety of aquatic animals so far. The main fish species reported so far are sharks (*Carcharhinus plumbeus*, *Negaprion brevirostris*, *Centropomus undecimalis*, *Caranx hippos*, *Mola mola*), Asian seabass (*Lates calcarifer*), cultured groupers (*Epinephelus coioides*) with heavy mortalities, seahorse (*Hippocampus* sp.), cultured flounder (*Paralichthys dentatus*), cultured red drum (*Sciaenops gastroitisertus*) with gastroenteritis, cultured tiger puffer (*Takifugu rubripes*), Golden pompano (*Trachinotus ovatus*) and important species in European/Mediterranean aquaculture, European seabass (*Dicentrarchus labrax*) and sparids (gilthead sea bream, common dentex), sole *(Solea senegalensis*), Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) and non-fish animals including shrimps, lobsters, abalones, oysters, cucumbers and corals (Pujalte, 2003; Zorrilla et al., 2003; Haldar et al., 2010; Austin et al., 2012; Montánchez and Kaberdin, 2020).

Many virulence mechanisms of *Vibrio harveyi* have been reported so far: Flagellar motility (both polar and peritrichous types) for the attachment and colonization (Yang and Defoirdt, 2015), extracellular enzymes/substances such as haemolysins, proteases and lipases, chitinases (Svitil et al., 1997; Teo et al., 2003; Sun et al., 2007); iron acquisition by siderophores synthesis (Owens et al., 1996) and quorum sensing and biofilm formation (Karunasagar et al., 1996; Henke and Bassler, 2004). Although how the virulence mechanisms are regulated in *Vibrio harveyi* has not been fully elucidated, studies have shown that these changes during infection are affected by the “quorum sensing” system (which is regulated by the “a membrane-localized regulatory protein” toxR and the “two master transcription” factor AphA

and LuxR) (Van Kessel et al., 2013). *V. harveyi* usually cause pathogenesis that ends with septicaemia in infected animals. While lethargy, loss of appetite/no feeding, haemorrhage at the bottom of the fins, dermal ulcers and necrotic subcutaneous cysts were observed in infected individuals; internal examination vasculitis, encephalitis, meningitis, kidney necrosis, liver and spleen damage, gastroenteritis have been reported. Ophthalmic lesions such as keratitis and corneal opacities can be observed in infected fish (Austin et al., 2012). *Vibrio harveyi* isolated from disease outbreaks in marine fish farming in Türkiye has been reported by various researchers. Korun and Akaylı (2004) reported that they isolated it together with *Ceratothoa oestroides*, a parasitic isopod in cultured European seabass. Korun and Timur (2008) identified *V. harveyi* in farmed European seabass, weighing 2-5 g and having clinical signs including lethargy, exophthalmia, ulcerative skin lesions, haemorrhages in the visceral organs, pale kidney and liver, ascites. In another study, Çanak and Akaylı (2011) reported that they found *V. harveyi* as a mixed infection with *V. anguillarum* from diseased gilthead sea bream. Turgay and Karataş (2016) reported the first disease caused by *V. harveyi* in cultured dentex (*Dentex dentex*).

Vibrio harveyi requires salt for its bacteriological culture in laboratory like many marine bacteria. The bacterium can be cultured in general purpose media supplemented with 1.5-2% sodium chloride such as TSA, BHI agar, Nutrient Agar, Blood Agar (BA), Marine Agar, MacConkey agar and it grows easily within 24-48 hours at 20-25°C. Apart from routine internal organs (kidney, spleen, liver), brain and, if any, ocular and cutaneous lesions of diseased fish can be sampled for the isolation of the agent. TCBS is a suitable for a selective media (Pretto, 2020). Isolates of the bacterium may have different colony morphology but it usually forms 2 mm diameter, round, soft-surfaced convex, cream-colored colonies on TSA medium. In Marine Agar the bacterium produces bright, whitish colonies on the medium, while colonies appear as yellow coloured on TCBS agar. In the differentiation of *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* species which are arginine-negative, lysine-positive species in the Harveyi clade; VP test, growth at 8% and 10% salinity; fermentation of sucrose, salicin, cellobiose, lactose, L-arabinose, and inhibition zone sizes in the presence of colistin, ampicillin, carbenicillin can be used (Garrity, 2007).

In addition to conventional bacteriological culture, API 20E (bioMérieux), Biolog GN and similar rapid diagnostic kits can also be used in the diagnosis of bacteria, although these species are not usually found in their databases. As in the diagnosis of all other pathogens, PCR (polymerase chain reaction) is very useful and reliable for *V. harveyi*. For this, diagnosis can be made by amplification and subsequent sequencing of the *pyrH* (uridylate kinase) gene,

which is a housekeeping gene (Cano-Gomez et al., 2011). Another molecular biological diagnostic option is to amplify the *toxR* gene with species-specific primers (Pang et al., 2006). Mougin et al. (2020) stated in their study that 16S rRNA gene sequencing in the Harveyi clade does not allow much differential diagnosis, on the contrary, MALDI-TOF MS profiling is much more reliable than 16S rRNA gene sequencing in the clade.

There is a wide variety of studies in the scientific literature for the control of the disease induced by *V. harveyi* including the use of probiotic bacteria (Vaseeharan and Ramasamy, 2003; Amenyogbe et al., 2021), quorum sensing system inhibitory substances/compounds (Bai et al., 2008; Peng et al., 2009; Aqawi et al., 2020) and bacteriophages (Stalin and Srinivasan, 2017). Although many experimental vaccine studies have been undertaken, there is no commercially available vaccine for fish as of today. Once disease occurs, it can be treated with various antimicrobials. In general, many antibiotic options are available for treatment including florfenicol, tetracycline, flumequine and potentiated sulfonamides, although some researchers have reported that bacteria are resistant to ampicillin, penicillin G, and colistin antibiotics (Pretto, 2020).

2.1.3. *Vibrio alginolyticus*

Vibrio alginolyticus, belonging to the Harveyi clade, is a Gram-negative, motile, fermentative, oxidase-positive, nitrate-reducing bacterium. Lysine decarboxylase is positive but arginine dihydrolase is negative. Fermentation of cellobiose, maltose, d-mannitol, d-mannose, sucrose and trehalose are observed. No acid is produced from sugars L-arabinose, D-arabitol, myo-Inositol, lactose, L-rhamnose, L-sorbitol and D-xylose (Garrity, 2007).

The bacterium with a single polar flagellum in broth media may exhibit the formation of multiple lateral flagella when on solid agar. The bacterium is a natural member of the marine environment and some researchers are of the opinion that the disease caused by *V. alginolyticus* is opportunistic in fish, causing diseases in immunocompromised or stressed fish or fish that are already injured, or as a secondary infection alongside a primary disease agent (Austin et al., 2012).

The bacterium causing soft tissue infections, wound and ear infections and, less commonly, eye infections in human medicine, have been isolated from a wide variety of marine animals until today. These include both non-fish animals such as corals, shellfish, crustacean; non-food fish such as sharks and ornamental fish and various cultured fish species including gilthead sea bream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), grouper (*Epinephelus malabaricus*), silver sea bream (*Sparus sarba*), flathead gray mullet (*Mugil*

cephalus) (Austin et al., 2012; Emam et al., 2019; Xie et al., 2020). Clinical findings in diseased fish have been reported consistent with septicemia: mostly lethargic and anemic, darkening of the skin, loss of scales and ulceration, necrotic skin lesions can be observed as external findings; internally, congestion in the liver, spleen and intestinal wall, and fluid-filled swelling in the intestine and gallbladder have been reported (Burke and Rodgers, 1981; Colorni et al., 1981; Austin et al., 1993; Lee, 1995; Balebona et al., 1998; Li et al., 1999; Austin et al., 2012). Some of the identified virulence factors of *V. alginolyticus* are biofilm forming ability, *rpoS* gene regulating bacterial adhesion, hemolysins, polar and lateral flagellum and presence of flagella related genes (*flrA*, *flrB* and *flrC*), enzymes with proteolytic activity such as serine proteases, LuxS quorum sensing system (Ye et al., 2008; Rui et al., 2009; Darshanee Ruwandeepika et al., 2012; Luo et al., 2016). The first report of *V. alginolyticus* in Turkish aquaculture was made by Çağırgan (1993) in gilthead sea bream and European seabass. In the following years, notifications continued to be made by various researchers. Korun et al. (2013) isolated and identified *V. alginolyticus* from diseased portion-sized European seabass individuals cultured in Türkiye, with clinical findings such as hemorrhage in the fins, exophthalmos, enlarged spleen, pale liver and kidney. Duman et al. (2023) performed Multi Locus Sequence Analysis (MLSA) and Multi Locus Sequence Typing (MLST) on *Vibrio* species isolated from European seabass and gilthead sea bream farmed in various provinces of Türkiye and they concluded that *V. alginolyticus* is one of the predominant species together with *V. anguillarum* and that there is genetic heterogeneity between *Vibrio* species.

Vibrio alginolyticus grows in simple culture media with sodium chloride added or prepared with seawater. For the isolation of the bacterium, the internal organs of the diseased fish and also blood tissue can be used. The bacterium produces round, whitish, convex colonies on the media at the end of an incubation period of 2-7 days at 15-25°C. Different results are obtained at the end of the incubation period on various media: in TCBS medium, it produces yellow colonies; on CHROMagar™ Vibrio-selective agar, colonies appear cream coloured; the bacterium developed yellowish-green colonies on *Vibrio alginolyticus* agar (VAL), a media with a high salinity concentration using bile salts, formulized by Chang et al. (2011). In addition to classical morphological and biochemical identification, molecular biological methods targeting various gene regions can be used in diagnosis. Multiplex PCR (Wei et al., 2014) and LAMP (loop-mediated isothermal amplification) targeting the *gyrB* gene (Cai et al., 2010), real-time LAMP (Zhou et al., 2007), and highly specific ssDNA aptamers (Yu et al., 2019) have been developed for the diagnosis *V. alginolyticus*.

There is no commercial vaccine developed against *V. alginolyticus* yet. However, especially in the Far East, there are autogenous vaccines with varying degrees of successful results.

2.1.4. *Vibrio ordalii*

Vibrio ordalii is a Gram negative, curved-rod, fermentative, oxidase positive, motile bacterium that needs at least 0.5% salinity to grow and can tolerate up to 6%. Arginine dihydrolase, lysine and ornithine decarboxylase tests are negative. Only a few carbohydrates are utilized including glucose, maltose and sucrose are positive but most others such as L-arabinose, D-galactose, myo-Inositol, lactose, D-mannitol, D-mannose, L-rhamnose, sorbitol are not utilized. Methyl red and Voges-Proskauer tests are negative. The bacterium is sensitive to Vibriostatic agent, 0/129.

The bacterium was formerly classified as *Vibrio anguillarum* biotype II. Although *V. ordalii* is phenotypically very different from *V. anguillarum*, they have many genetic similarities. On the other hand, Tiainen et al. (1995) reported in their ribotyping study that all *V. ordalii* strains have three different ribotypes and they are clearly different from *V. anguillarum* ribotypes. In addition, *V. ordalii*, unlike *V. anguillarum*, is a serologically homogeneous group and does not contain different serotypes/serovars. Steinum et al. (2016) conducted an MLSA-based study on a large number of isolates obtained from various geographical regions including Türkiye. It was found that many environmental isolates previously identified as *V. ordalii* actually are *V. anguillarum* and also that *V. ordalii* isolates from diseased fish have a very close genetic relationship to *V. anguillarum*.

The bacterium grows on general purpose media supplemented containing sodium chloride, but is a slow-growing bacterium, so it is recommended to incubate at 15–22°C for up to 7 days, no growth at 37°C. At the end of the incubation period, they produce 1-2 mm diameter, smooth/round, convex colonies on Marine Agar. No growth is seen on TCBS selective medium (Garrity, 2007).

Vibrio ordalii causes a systemic infection in fish, both wild and farmed marine species including Atlantic salmon (*Salmo salar*), Pacific salmons (*Oncorhynchus kisutch*, *O. tshawytscha*, *O. nerka*), rainbow trout (*O. mykiss*), Ayu (*Plectoglossus altivelis*), Atlantic cod (*Gadus morhua*) and in Mediterranean marine species, European seabass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). However, hemorrhagic septicemia develops much later in *V. ordalii* infections compared to infections caused by *V. anguillarum* (Schiewe et al., 1981; Muroga et al., 1986; Wards et al., 1991; Bohle et al., 2007; Austin et al., 2012). Some researchers have reported that the pathogen attacks preferably skeletal and cardiac muscle, gills, the anterior and posterior gastrointestinal tract, then causing generalized septicemia (Ransom et al., 1984). The first *Vibrio ordalii* infection in marine fish cultured in Türkiye was

reported by Akaylı (2001). Later, Korun (2004) identified the *Vibrio ordalii* with some other *Vibrio* species in a study investigating the cause of a disease outbreak with a high mortality rate in a commercial European seabass farm. In that study, it was reported that the diseased fish exhibited clinical signs such as lethargy, loss of appetite, loss of scales, hemorrhages, ulcerative skin lesions on the body surface, pale liver and kidney, enlarged spleen, and fluid accumulation in the abdominal cavity. In another study examining the effectiveness of API rapid diagnosis kits, bacteriological analysis was performed on samples taken from kidneys of diseased European seabass showing classical vibriosis signs, Tanrıkul et al. (2004) identified *V. ordalii* together with *V. anguillarum* as the causative agents.

The plasmid pJMI in *V. anguillarum* is not found in *V. ordalii*, but all strains of *V. ordalii* have a plasmid called pMJ101. At present, despite several studies, the plasmid pMJ101 has not been characterized fully and its role in the life cycle or virulence of *V. ordalii* is still unknown (Bidinost et al., 1999).

In natural infections of salmonid fish, the bacterium is mostly localized in the muscles and skin, it has been observed that the presence of micro-colonies in the spleen and liver in the internal organs. In addition, low concentrations of bacteria are also found in the bloodstream. Clinical external findings are skin lesions and hemorrhagic ulcers; internally pericarditis, peritonitis, necrotic foci in the liver can be observed. A similar situation was observed in experimentally induced infections in salmonid fish; infection created parenterally and then systemic infection developed and the bacteria were re-isolated from the spleen, liver and blood in a short time (Schiewe et al., 1981; Crosa et al., 2006).

Diagnosis can be made by conventional biochemical and morphological characterization and also rapid diagnosis kits can be used to identify bacteria. Molecular diagnostics methods based on 16S rRNA gene sequencing or amplification of some other specific genes can be used in diagnosis; Avendaño-Herrera et al. (2014) concluded that the use of the *vohB* (hemolysin) gene to detect *V. ordalii*-infected tissues is rapid, specific and sensitive.

A variety of multivalent commercial vaccines are available to use in different fish species with different routes of administration: AQUAVAC® Vibrio Oral, for rainbow trout (*Oncorhynkus mykiss*) and European seabass (*Dicentrarchus labrax*), contains inactivated *V. anguillarum* and *V. ordalii* strains for oral route; AQUAVAC® Vibrio Pasteurella, for European seabass (*Dicentrarchus labrax*) and contains *Vibrio anguillarum* serotype 01 and O2α (*V. ordalii*) and *Photobacterium damselae* subsp. *piscicida*; VIBRI-FISHVAX, for *Dicentrarchus labrax* and *Sparus aurata*, contains inactivated *V. anguillarum* and *V. ordalii* strain, for immersion and intraperitoneal route.

2.1.5. *Vibrio vulnificus*

Vibrio vulnificus members are commonly found in temperate and tropical aquatic ecosystems and both in marine and brackish water environments but are also isolated from human clinical specimens, causing wound infections and septicaemia in humans. *V. vulnificus* serovar A and E, which cause disease in fish, enter the fish body mostly from the anus and gill regions (Austin et al., 2012; Fouz et al., 2010). *V. vulnificus* strains are divided into three biogroups with different phenotypes, ecological characteristics and host range. In biogroup determination; citrate test, indole production, ONPG test, ornithine decarboxylase and cellobiose, lactose, D-mannitol, salicin and D-sorbitol fermentation test results are considered. The bacterium is sensitive to vibriostatic agent. Characteristics that are common in all three biogroups including Gram-negative, motile with a single polar flagellum, oxidase positive, nitrate reducing, Lysine decarboxylase positive, acid is produced from D-glucose, D-galactose, maltose and trehalose and a salinity tolerance range is 1-6% (Garrity, 2007). Some researchers, on the other hand, adopted the view that it would be more accurate to classify as serovar instead of biogroup/biotype classification, and they created two separate groups as serovar A and E under biotype 2. While biotype 2 serovar A is the group that infects only fish, biotype serovar E is the group that infects both fish and humans (Arias et al., 1997; Fouz et al., 2006; Fouz et al., 2007; Fouz et al., 2010).

Eels (*Anguilla* spp.) are most susceptible to the disease caused by this bacterium, however tilapia (*Oreochromis* sp.), rainbow trout (*Oncorhynchus mykiss*), pompano (*Trachinotus ovatus*) and shrimp (*Penaeus vannamei*) are affected. The bacterium has been isolated from gilthead sea bream and European seabass in Mediterranean countries. The disease can progress with acute hemorrhagic septicemia and cause high mortality, especially in farmed eels. On external examination, petechial hemorrhages in the abdomen and redness on the body surface can be seen at the beginning of the infection. In more advanced disease, moribund fish exhibits dark coloration and lethargy, haemorrhages on the external surface of the body, especially at the base of the fins and also in the viscera including the liver and the intestine (Chen et al., 2006; Li et al., 2006; Pedersen et al., 2008; Austin et al., 2012).

The isolation of the bacterium is done by sampling from the internal organs of the diseased fish or from the lesions. The bacterium grows in 24-48 hours at 20-25°C in general purpose media (TSA, NA, BHI) supplemented with sodium chloride (1-2%) or prepared with sea water and the incubation period can be extended up to 7 days. Diagnosis can be made by conventional biochemical and morphological tests, however, as it is also an important species in human medicine, many commercial companies have developed biochemical identification kits or qPCR-based diagnostic kits.

To our knowledge, the only commercially available vaccine against *Vibrio vulnificus* is Vulnivaccine, which is developed for use in eels. As in many other diseases, it is very important to improve the aquaculture conditions for controlling the disease, and to carry out daily controls, especially in periods when the temperature change difference is high.

2.2. Photobacteriosis (Pasteurellosis)

Photobacteriosis, a very dangerous septicemic disease in aquaculture species due to its wide host range and ubiquitous spread, was formerly called fish pasteurellosis or pseudotuberculosis, however, as a result of molecular genetic studies, the causative bacterium that gave its name to the disease has now been placed under a different genus, so the name "photobacteriosis" is widely used now (Gauthier et al., 1995). *Photobacterium damselae* is a species in the family Vibrionaceae, and as a result of various molecular biological typing analyses, it has been determined that it has two subspecies, both of which cause disease in marine fish: *Photobacterium damselae* subsp. *damselae* and *Photobacterium damselae* subsp. *piscicida*. Both subspecies are marine species and require salt for growth. Various biochemical tests (utilization of specific sugars, gas production from sugars, nitrate reducing ability etc.) or molecular diagnostic methods can be used to distinguish subspecies of this species. *Photobacterium damselae* subsp. *piscicida* strains are present in commercial vaccines for cultured marine fish, either monovalent or polyvalent with other pathogens.

2.2.1. *Photobacterium damselae* subsp. *piscicida*

Photobacterium damselae subsp. *piscicida* is a Gram-negative, oxidase and catalase positive, non-flagellated rods with having translocating capability over surfaces thanks to a flagella-independent mechanism, showing bipolar staining but cell morphology can become pleomorphic according to culture conditions. Arginine dihydrolase is positive while lysine and ornithine decarboxylase are negative. Utilization of D-fructose, D-galactose, maltose, ribose are positive but glycerol, myo-Inositol, D-lactose, D-mannitol, sucrose, sorbitol are not utilized, gas is not produced. Growth occurs at 20°C and 30°C, but not at 4°C and 40°C. The bacterium needs salt for growth (Garrity, 2007). The presence of a wide variety of plasmids, in terms of number and size, has been determined in *P. damselae* subsp. *piscicida*. It has been determined that these transferable multiple-drug resistance plasmids encode resistance against many antibiotics used in aquaculture such as kanamycin, chloramphenicol, tetracycline, trimethoprim, sulfonamide (Kim et al., 2008).

The bacterium is highly pathogenic and appears to have a very wide host range including yellowtail *(Seriola quinqueradiata*), ayu (*Plecoglosus altivelis*), blackhead seabream *(Acanthopagrus schlegeli*), red grouper (*Epinephelus akaara*), oval fish (*Navodon modestus*), Bastard halibut (*Paralichthys olivaceus*), and fish species cultivated in the Mediterranean region including gilthead sea bream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), sole (*Solea senegalensis* and *Solea solea*), meagre (*Argyrosomus regius*), and some other less common fish (Romalde, 2002; Austin et al., 2007; Costa et al., 2017).

Disease outbreaks generally occur at temperatures from 18-20°C and above, below these temperatures the infection can remain subclinical for a long time. In acute infections, mortality can be observed with very few symptoms. As an external finding, erratic swimming may be seen, as well as anorexia, lethargy and skin darkening. The presence of white nodules in the internal organs, especially in the spleen and kidney, is characteristic of the disease, and for this reason, the disease was called pseudotuberculosis in the past. The anus may be red, protruded and abdomen distended and may contain purulent fluid in the abdominal cavity. Congested spleen and petechial haemorrhages on liver may be observed (Buller, 2004; Toranzo et al., 2005). The first case of Pasteurellosis (Photobacteriosis) in marine aquaculture in Türkiye was reported in cultured gilthead sea bream (Çağırgan, 1993). In the following years, Candan et al. (1996) reported firstly this bacterium as a disease agent in cultured European seabass. More recently, *Photobacterium demselae* subsp. *piscicida* was isolated from sturgeon (*Acipenser gueldenstaedtii*) (n=5, 60 g) cultured in sea water (Türe and Alp, 2016).

The virulence of the bacterium in different fish and the prognosis of the disease may differ. For example, the disease is observed mostly in larvae and juveniles in gilthead sea bream. On the other hand, it has been determined that fish over 50 grams are resistant to the disease. In European seabass, disease outbreaks are more common in cages (Toranzo et al., 2005). Possession of R-plasmids, ability to adhere to and invade the host, phospholipase, cytotoxic and hemolytic activities of extracellular products (ECPs) secreted by bacteria; siderophore-mediated iron-uptake system and many other virulence mechanisms make bacteria dangerous and deadly for marine aquaculture (Romalde, 2002).

2.2.2. *Photobacterium damselae* subsp. *damselae*

The bacterium can be isolated from internal organs such as kidney and spleen by inoculation on Marine Agar, Nutrient Agar or Blood Agar, and incubation at 25°C for 48-72 hours. At the end of the incubation period, the bacterium produces shiny, grayish-yellow, entire, convex, opaque colonies. Diagnosis of the disease can be made by conventional biochemical and morphological characterization. In addition, although profile databases do not contain this bacterium, rapid identification kits (API, BIONOR, etc.) can be used (Austin et al., 2007).

There are also commercialized products on the market such as agglutination kits (Bionor AS), monoclonal antibodies (Creative Diagnostics) and qPCR kits (Genesig/Primerdesign) that can be used in pathogen identification. In addition to conventional tests, diagnosis can be made based on 16S rRNA gene-based sequencing, as well as subspecies verification by using it as multiplex PCR with the *ureC* gene (Osorio et al., 2000).

There are commercial vaccines against Photobacteriosis on the market manufactured by different companies for use in different fish species such as AlphaJect 2000™, ALPHA DIP® 2000 from PHARMAQ, ZOETIS; AquaVac Photobac Prime™, Aquavac Photobac Boost (Oral), AquaVac™ Vibrio-Pasteurella from MSD ANIMAL HEALTH; ICTHIOVAC® PD, ICTHIOVAC® VR/PD. Apart from these, autogenous vaccines are also produced by various companies.

In addition to the intracellular location of the bacteria in infected phagocytes, the presence of R-plasmids makes treatment with antimicrobials difficult or weak. The best prevention of this devastating disease requires combining multiple efforts such as vaccination, use of glucans in the diet, and water temperature control (Romalde, 2014).

The bacterium is Gram-negative, motile, oxidase and catalase positive, nitrate reducer rods. Arginine dihydrolase positive, lysine or ornithine decarboxylase negative. Growth occurs at 20°C and 30°C, but not at 40°C. Methyl red and Voges-Proskauer tests are positive. Acid is produced from cellobiose, D-fructose, D-galactose, glycogen, glycerol, maltose, D-mannose, D-ribose sugars but not from L-arabinose, myo-inositol, D-lactose, D-mannitol, rhamnose, D-sorbitol and sucrose. The bacterium produces round to slightly irregular, off-white, opaque, flat and shiny colonies. Requires salt for the growth; although the salinity tolerance range is 1-6% (optimum 3%). The bacterium is sensitive to vibriostatic agent, O/129 (Garrity, 2007).

The disease was first isolated in *Chromis punctipinnis* (blacksmith) belonging to the Pomacentridae (Damselfishes) family, from which it is named. Thereafter, the disease has been described in various fish species such as sharks (*Carcharhinus plumbeus*), turbot (*Scophthalmus maximus*), redbanded sea bream (*Pagrus auriga*), barramundi (*Lates calcarifer*), rainbow trout (in the sea), eel (*Anguilla reinhardtii*), gilthead sea bream (*Sparus aurata*).

Lethargy, distended abdomen, haemorrhages in the fins and tail, pale liver may be observed in diseased fish. Ulcers and similar skin lesions are characteristic to disease, and these skin lesions are often accompanied by lysis of the underlying muscle tissue. In histopathology, these areas appear as granulomatous ulcerative dermatitis (Buller, 2004; Austin et al., 2007).

Terceti et al. (2016) performed genetic and pathobiological characterizations on 14 strains of *Photobacterium damselae* subsp. *damselae* isolated from farmed European seabass with high mortality in the southeastern Black Sea region of Türkiye and it was concluded that the strains of *Photobacterium damselae* subsp. *damselae* in this region have a multiclonal origin.

Isolation of the bacterium can be done from skin lesions or internal organs of diseased fish, by inoculating in a general-purpose culture medium supplemented with sodium chloride and incubating at 25°C for 2-5 days. Diagnosis of the disease can be made by isolation of the bacterium followed by conventional biochemical and morphological characterization. In addition to conventional tests, diagnosis can be made based on 16S rRNA gene-based sequencing, as well as subspecies verification by using it as multiplex PCR with the *ureC* gene (Osorio et al., 2000).

Reports on disease outbreaks caused by *P. damselae* subsp. *damselae* and its treatment show that very different results are obtained in different fish species; for instance, antibiotics reported to be effective in some species were determined as ineffective in a report of disease affecting another fish. In addition, the presence of antibiotic resistance genes in *P. damselae* species makes us believe that antibiotic use may not be beneficial in the long term. Currently, while commercial vaccines are available against strains of subspecies “piscicida”, there is no commercial vaccine for isolates of the “damselae” subspecies. In addition, a vaccine produced for one subspecies, found that to be ineffective for the other one. Therefore, intensive studies are carried out on the use of probiotics/prebiotics, phages, and herbal-origin substances and many others in disease control (Romalde, 2002; Gouife et al., 2022).

2.3. Tenacibaculosis

The etiological agent of this disease is *Tenacibaculum maritimum*, a Gram-negative, rod-shaped, non-flagellated but motile by gliding, aerobic, catalase and oxidase positive marine bacterium. Cell morphology may appear as filaments, but as the culture ages the cells become more rounded. Indole and H_2S production is not observed, nitrates are reduced, spores and gas vesicles are not formed. Bacterial cells produce yellow colored pigment called zeaxanthin, but flexirubin-type pigment not exist. The methyl red test is negative. Casein and gelatin are utilised, but not aesculin, cellulose, chitin, starch, arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose, xylose and urea. Bacteria can grow at a temperature range of 15-34°C and a pH range of 5.9–8.6, however, it requires potassium chloride and sodium chloride to grow (Rainey et al., 2015).

Tenacibaculum maritimum is found in marine organisms such as macroalgae, fish and invertebrates. *Tenacibaculum* soleae has been isolated from diseased sole (*Solea senegalensis*)

and European seabass (*Dicentarchus labrax*), *Tenacibaculum* gallaicum from turbot (*Psetta maxima*) and sole, *Tenacibaculum dicentrarchi* from European seabass and *Tenacibaculum discolor* from cultured sole (Pineiro-Vidal et al., 2008a; Pineiro-Vidal et al., 2008b; Pineiro-Vidal et al., 2012; Castro et al., 2014).

Members of this species cause very serious losses in marine aquaculture worldwide. The bacterium is capable of causing disease in many marine species including red sea bream (*Pagrus major*), farmed tub gurnard (*Chelidonichthys lucerna*), lumpfish (*Cyclopterus lumpus*), Korean olive flounder (*Panaeolus olivaceus*), Sockeye salmon (*Oncorhynchus nerka*), black sea bream (*Acanthopagrus schlegeli*), rock bream (*Oplegnathus fasciatus*), sole (*Solea* spp.), turbot (*Scophthalmus maximus*), European seabass (*Dicentrarchus labrax*) and gilthead sea bream *(Sparus aurata*) (Austin et al., 2007; Mabrok et al., 2023). With the latest studies, it has been determined that there are four different serotypes of the bacterium (O1-O4), but there is no clear relationship between serotypes and host disease progression/symptoms (Fernández-Álvarez and Santos, 2018). In juvenile fish affected by the disease, stomatitis in the mouth, erosion in the gills and loss of the tail are observed, while ulcerative lesions starting with gray-white lesions on the body surface are observed in older fish. Local depigmentation of the skin, loss of scales and, in some cases, ulcerative lesions progressing to the muscle can also be seen in the eyes and gills. In Türkiye, *Tenacibaculum maritimum* was first isolated from farmed gilthead sea bream and European seabass (Türk, 2006). Later, this bacterium was isolated from farmed (Black Sea) rainbow trout (marine cultured) by Timur et al. (2007) as mixed infections. Yardımcı and Timur (2015) identified *T. maritimum* as an etiological agent in a disease outbreak seen in European seabass farmed in Türkiye by bacteriological and molecular methods. In diseased fish, erythemic and erosive jaw and operculum, skin lesions from superficial to deep ulcers on the head and body surface, exophthalmia and hemorrhagic fins. The authors observed bloody fluid in the peritoneal cavity as well as hyperemia and hemorrhage in the visceral organs in internal examination. In addition, histopathological examination revealed degeneration and liquefying necrosis in the liver, kidney and spleen.

Cytophaga agar (prepared with 70% sea water), FMM (*Flexibacter maritimus* medium) and TCY (tryptone-casamino acids-yeast) medium can be used to isolate the disease agent. After the pure culture is obtained, the bacterium can be diagnosed by conventional biochemical and morphological tests. Fluorescent antibody test (FAT), which is one of the serological methods in diagnosis, is very useful and fast, especially for detection in fish tissues (Baxa et al., 1988a). 16S rRNA gene-based diagnosis is successful and practical. Also, species-specific primers (MAR1-MAR2) by Avendaño-Herrera et al. (2004a) are useful for the diagnosis. For

detection in tissues, nested-PCR has been developed to detect much lower amounts of bacteria (Avendaño-Herrera et al., 2004b). Recently, Steinum et al. (2021) stated that CRISPRcas loci-based "spoligotyping", which they suggested as a typing method other than existing ones, can be used as a fast, easy and inexpensive subtyping tool for *Tenacibaculum* strains and they reported that its discriminative power is comparable to MLST.

The only commercially available vaccine against *Tenacibaculum maritimum* today is ICTHIOVAC® TM (HIPRA) for turbot administered by intraperitoneal injection. Akinbowale et al. (2006) reported that the antibiotic enrofloxacin, a second-generation fluoroquinolone, generally exhibits promising antimicrobial activity against *Flavobacterium* spp. In addition, florfenicol is already used for the treatment for the genus members (Irgang et al., 2021).

2.4. *Aeromonas* infections

Members of the genus *Aeromonas* are traditionally divided into two main groups as motile and non-motile species. Taken together, more than 20 species have been reported having widespread distribution in aquatic environments and causing disease in both freshwater and marine fish. *Aeromonas* species are mostly considered as opportunistic or secondary pathogens. When the disease reports are evaluated, it is revealed that more than one factor plays a role in the formation of the disease caused by *Aeromonas* species (Austin et al., 2007; Fernández-Bravo and Figueras, 2020). Among these species, *Aeromonas hydrophila*, *A. sobria* and *A. veronii* as motile *Aeromonas* species and *A. salmonicida*, have been widely observed in Mediterranean aquaculture in recent years. The same species have been isolated and identified from diseased aquatic animals in our country (Smyrli and Katharios, 2020).

Some shared characteristics of the all motile *Aeromonas* species (MAS) are as follows: Gram-negative, straight, having bacillus/coccobacillus cell morphology with rounded ends, non-spore-forming, oxidase and catalase positive rods. All members of the family are facultatively anaerobic and chemoorganotrophic and nitrate reducers (Martin-Carnahan and Joseph, 2005). The majority of the members of the genus, as well as those that cause disease in aquatic organisms, are motile species. Various biochemical tests can be used to differentiate between fish pathogen *Aeromonas* species. These tests include arginine dihydrolase and lysine and ornithine decarboxylase, esculin hydrolysis, Voges Proskauer test, gas is produced from D-glucose, acid is produced from L-arabinose, susceptibility to carbenicillin (30 ug), and cephalothin (30 ug), Pyrazinamidase test, hemolysis type. Apart from this, methods targeting various housekeeping genes (such as *gyrB*, *rpoD*) have been developed to diagnose *Aeromonas* at the species level (Soler et al., 2004).

The virulence factors of *Aeromonas* members have a complex structure including adhesins, lipopolysaccharide (LPS), outer membrane proteins (OMPs), extracellular products such as haemolysins, proteases, phospholipases, DNases, cytotoxic enterotoxins, having iron acquisition mechanisms and biofilm forming ability and quorum sensing systems in a complex structure that interacts by themselves or with each other (Beaz-Hidalgo and Figueras, 2013).

2.4.1. *Aeromonas hydrophila*

A. hydrophila is Gram-negative, motile, catalase and oxidase positive, nitrate reducer rods. Indol, Voges-Proskauer, ONPG tests are positive but isolates are urea negative. Lysine decarboxylase and arginine dihydrolase are positive while ornithine decarboxylase is negative. It produces acid from D-mannitol and sucrose sugars but not from D-rhamnose and D-sorbitol, gas is produced from D-glucose. Members of this species can grow at temperatures up to 41°C and hydrolyze esculin at 37°C, hemolysis is observed. *A. hydrophila* is resistant to ampicillin, carbenicillin and cephalothin antibiotics and pyrazinamidase positive.

The bacterium has been isolated from diseased freshwater and marine fish as well as aquatic animals other than fish, molluscs, terrestrial animals and humans. Disease findings are generally compatible with hemorrhagic septicemia. Scale loss and ulcerative lesions on the skin may occur in diseased fish. Haemorrhage can be observed in the gills, at the base of the fins and anal opening. Internally, the abdomen may be swollen and filled with ascitic fluid and damage to the kidney and liver may occur (Noga, 2010). The first study in which motile *Aeromonas* species namely *A. hydrophila* was reported from diseased fish in Türkiye was in cultured eel (*Anguilla anguilla*) (Timur, 1983). In diseased eels, external lesions of varying degrees ranging from scale losses to large ulcers and underlying muscle tissue, as well as hemorrhages on the fins were observed, while internally petechial hemorrhages were observed in the intestinal canal walls. The same bacterium was reported as a disease agent in gilthead sea bream and European seabass in the Aegean Region (Turk, 2002) and in European seabass in the Black Sea region (Savaş et al., 2006).

Aeromonas hydrophila can be easily isolated from the internal organs of the diseased fish by inoculating on general-purpose medium such as TSA, Nutrient Agar and after 24-48 hours of incubation at 20-25°C. For a selective medium, Rimler-Shotts medium supplemented with an antibiotic can be used (Shotts and Rimler, 1973) and after incubation, cream-colored, round, convex colonies of 2-3 mm in diameter are observed.

Diagnosis can be made by standard biochemical tests and determination of morphological features and rapid diagnosis kits such as API, BIOLOG and similar can also be used, but it

should be taken into account that this species may not be present in manufacturers' profile databases so it may return a wrong identification. Molecular diagnostic methods can also be used with high accuracy or confirmation in the identification of the agent. Sequence-based standard 16 rRNA genes can be used for this as well as; multiplex-PCR developed by Chu and Lu (2005) can be used, which targets both the specific 16S rRNA gene and a virulence-associated *Aeromonas* cytolytic aerolysin gene (*Aero*). Apart from this, methods targeting various housekeeping genes (such as *gyrB*, *rpoD*) have been developed to identify *Aeromonas* at the species level (Soler et al., 2004).

2.4.2. *Aeromonas sobria*

Member of this species are Gram negative, motile, oxidase positive, catalase negative, nitrate reducer rods. The bacterium produces acid and gas from glucose, acid from mannitol, sucrose, maltose and trehalose but does not produce acid from L-arabinose, L-rhamnose, lactose. It does not hydrolyze esculin and not produce haemolysis. Indole, ONPG and citrate tests are positive. *A. sobria* was occasionally isolated from diseased fish both in wild and farm; including tilapia (*Oreochromis niloticus*), perch (*Perca fluviatilis*), red garra (*Garra rufa*), American gizzard shad (*Dorosoma cepedianum*) (Toranzo et al., 1989; Wahli et al., 2005; Li and Cai, 2011; Majtán, 2012). *Aeromonas sobria*, was reported as a secondary pathogen during Pasteurellosis disease in gilthead sea bream and European seabass in Türkiye by Avsever et al. (2012).

2.4.3. *Aeromonas veronii*

These bacteria are Gram-negative, motile, oxidase and catalase positive, nitrate reducer, fermentative rods and lysine decarboxylase positive, indole and ONPG tests are positive but isolates are urea negative. The bacterium utilizes maltose, sucrose, D-trehalose and D-mannitol but not L-rhamnose, D-xylose, D-sorbitol.

A. veronii is divided into two separate biovars, namely "veronii" and "sobria". Both biovars have been isolated from the aquatic environment and diseased fish as well as serious clinical diseases in human medicine, including diarrhoea, pulmonary complications (biovar veronii) and wounds, septicaemia, peritonitis, meningitis (biovar sobria). Esculin hydrolysis, ornithine decarboxylase, arginine dihydrolase and arbutin hydrolysis tests can be used for the differential diagnosis of two biovars.

A. veronii infection has caused serious problems in Mediterranean aquaculture especially in recent years. In the Mediterranean region, it has been reported that the disease occurs in

European seabass in warm months when the water temperature rises above 21°C. If untreated, the cumulative mortality can reach up to 80%. Lethargy, loss of appetite, erythema, haemorrhage and superficial ulcerative lesions on the skin, exophthalmia, distended abdomen (may contain bloody exudate); internally, nodules can be observed in organs such as liver, spleen and kidney. *Aeromonas veronii*, another MAS member, has been isolated from diseased fish more frequently in recent years. Uzun and Ogut (2015) examined the frequency of bacteria observed in diseases in European seabass cultured in the Southeastern Black Sea region, 477 diseased fish were examined in total, and it was reported that *Aeromonas veronii* biovar sobria was the most common bacterial species isolated from these fish with 65.2%. In another report about European seabass, Dinçtürk (2021) reported that disease outbreaks were seen in three different farms and hemorrhages on the body surface, enlarged spleen and liver, and hemorrhages in the internal organs in diseased fish. In the mentioned study, the disease agent was defined as *A. veronii*. However, a biovar designation was not performed. In a very recent study on the detection of the agent responsible for a disease outbreak with a low mortality rate in European seabass farmed in the Aegean Region, superficial ulcerative lesions on the skin, pale gills, kidneys, liver, and petechial hemorrhages in the tongue, maxilla and operculum were observed in fish affected by the disease. In some fish, a bloody exudate in the abdominal cavity and ecchymosis in the liver have been reported. Biovar designation was also done in the referred study and the causative bacterium identified as *Aeromonas veronii* biovar veronii (Karataş et al., 2023).

The bacterium can be isolated from the internal organs of diseased fish or from lesions on the skin by incubation at 22-25°C for 24-48 hours after inoculating in general-purpose media (such as TSA, BHI) or selective *Aeromonas* Medium containing ampicillin. Standard biochemical and morphological tests can be used in the diagnosis, as well as molecular diagnostic methods based on 16S rRNA sequence-based or amplification of various gene regions, such as *fstA* (ferric siderophore receptor) (Beaz-Hidalgo et al., 2008).

2.4.4. *Aeromonas salmonicida*

A. salmonicida includes five subspecies, among them the “salmonicida” subspecies is mainly the causative agent of furunculosis in salmonid fish, and referred to as the typical strain. The other subspecies “achromogenes”, “masoucida”, “pectinolytica” and “smithia” are considered atypical. Typical *A. salmonicida* subsp. *salmonicida* is a psychrophilic, non-motile species and produce strain-specific brown diffusible pigment. In contrast, the atypical subspecies are mesophilic and usually cause ulcerative lesions in non-salmonid fish, with or without septicaemia (Austin et al., 2007).

It is possible to distinguish the typical *Aeromonas salmonicida* species provisionally from other fish pathogens with some simple tests. Typical *A. salmonicida* subsp. *salmonicida* is Gram-negative small rods in cell morphology, usually non-motile, mostly no growth at 37°C, fermentative metabolism, positive catalase and oxidase tests and acid production from sucrose (mostly negative) and xylose (negative). Atypical strains often give different results to these tests.

Clinical findings in infections of atypical origin were reported as petechial haemorrhages, enlargement of the spleen, ulcerative lesions of the skin progressing down to the muscles; pale gills, darkening in colour, haemorrhage in the mouth and gills of gilthead sea bream (Karatas et al., 2005; Real et al., 1994; Fernández-Álvarez et al., 2016). Timur et al. (2008) isolated *A. salmonicida* subsp. *salmonicida* along with some other bacterial and viral pathogens cultured European seabass (*Dicentrarchus labrax*) in Black Sea region in Türkiye. Karataş et al. (2005) reported *A. salmonicida* subsp. *achromogenes* infection with a cumulative mortality of 20% in European seabass (*Dicentrarchus labrax*) farmed in the Black Sea region.

2.5. Mycobacterosis

Members of the *Mycobacterium* genus under the Mycobacteriaceae family are found in many ecosystems and spread worldwide. There are currently 195 validly published species under the genus *Mycobacterium*, about 20 of them (*M. abscessus*, *M. avium*, *Mycobacterium avium* subsp. *avium*, *M. chelonae*, *M. fortuitum*, *M. marinum*, *M. neonarum*, *M. poriferae*, *M. scrofulaceum*, *M. simiae*, *M. smegmatis*, *M. stephanolepidis*, *M. triplex-like*, *M. triviale*, *M. gordonae*, *M. montefiorense, M. peregrinum, M. pseudoshottsii, M. salmoniphilum, M. shottsii*) have been isolated as disease agents from fish (Toranzo et al., 2005; Austin et al., 2017; Delghandi et al., 2020). However, the most common non-tuberculosis mycobacterium (NTB) species in marine fish is *Mycobacterium marinum*. *M. fortuitum* and *M. chelonae* species are less frequently isolated from diseased fish (Toranzo et al., 2005; Garrity, 2007).

Mycobacterium species are non-motile and do not produce spores and their cell walls are rich in mycolic acids. Members of the genus are difficult to stain with Gram staining and are resistant to acid-alcohol, therefore acid-fast stain or Ziehl-Neelsen staining is performed. Cell growth is generally slow, so incubation times are long. Members of the genus are adapted to growth on basic media, however, clinically important species grow better on egg-based media such as Löwenstein–Jensen or Middlebrook media (Garrity, 2007).

Mycobacterium marinum is non-motile, strictly aerobic, acid-fast pleomorphic bacilli. After incubation on Löwenstein–Jensen medium and Middlebrook agar at 30°C for periods

ranging from 7 days to several weeks. *M. marinum* colonies are photochromogenic, when grown in light the bacterium produces colonies in bright yellow colour. The β-Galactosidase test is negative and resistant to hydroxylamine (500 ng/L). Nitrates are not reduced. No growth occurs at 42°C. It is a zoonotic agent and can infect people who come into contact with contaminated water and fish. It causes cutaneous granulomas and ulcers on the skin in humans, often requiring drug therapy (Garrity, 2007).

The transmission route of the agent is not fully understood. Horizontal transmission, the "oral-enteral route" includes the ingestion of contaminated food or environmental debris, or cannibalism of infected fish. Otherwise, it can be transmission through water and faecal products, by feeding on infected fish tissue, via environmental protozoans or by infected live feed such as tubificid worms (*Tubifex tubifex*), water fleas (*Daphnia* spp.) and infected mosquito larvae (Hedrick et al., 1987; Nenoff and Uhlemann, 2006; Peterson et al., 2013; Chang et al., 2019). Infection via the intra-ovarian route is controversial; although vertical transmission has been shown in some studies, some researchers do not consider it plausible (Austin et al., 2017; Delghandi et al., 2020)

The main species that causes disease in fish is *Mycobacterium marinum*. *M. marinum* causes a chronic progressive disease. Loss of appetite, erratic swimming, abdominal bloating, skin ulceration and open lesions, and in cases where the disease is advanced, haemorrhages, loss of scales, loss of fins and tails and exophthalmia can be seen as external clinical findings. On internal examination, characteristic nodules (granulomas) in the spleen, liver and kidney and other internal organs (Toranzo et al., 2005; Austin et al., 2017). Mycobacteriosis, which has been reported in many fish species, poses a threat to fish species cultured in the Mediterranean, such as European seabass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and turbot (*Scophthalmus maximus*).

Isolation of the agent is difficult compared to many fish pathogenic bacteria. *Mycobacterium marinum* is a slow growing bacterium than the other two most commonly isolated species from fish, and can be covered by other fast-growing non-mycobacterial species on non-selective media. For isolation, standard mycobacterial media such as Löwenstein-Jensen agar, Middlebrook 7H10 or 7H11 Agar and Dorset egg media, as well as Blood Agar or BHI (Brain Heart Infusion) media with varying degrees of success, can be cultivated from the internal organs of diseased fish. Although the bacteria can grow in the temperature range of 15-30°C, the optimum temperature is around 25°C. At this temperature, the first colony development is observed after 2-28 days in an aerobic environment. It can be distinguished from the closely related species, *Mycobacterium fortuitum*, by nicotinamidase, pyrazinamidase production

and nitrate reduction tests (Austin et al., 2007; Garrity, 2007). *M. marinum* cannot grow on a medium containing thiacetazone.

There are various methods described in scientific literature for diagnosis of the disease. The bacterium can be visualized in tissue sections or smears of kidney and spleen. Various kits are also available for rapid staining such as (Quick TB Kit Cold Staining, RAL Diagnostics). Histopathological sectioning followed by an immunocytochemical method using the avidin-biotin complex (Gómez et al., 1993) was demonstrated. In addition, a FRET probe assay has been developed in more than one step but with high detection power (Salati et al., 2010). Another option is diagnosis by morphological and biochemical characterization, but this option can be unsuccessful and takes quite a bit of time as it requires difficult MALDI Biotyper, a simplified MALDI-TOF mass spectrometry instrument, the diagnosis is based on proteomic fingerprinting using high-throughput (Shitikov et al., 2012). Molecular tools can also be used and the simplest and the most common loci are 16S rRNA gene, ITS (internal transcribed spacer) and hsp65. In the scientific literature, there are analysis systems using various gene regions such as *dnaJ1, gyrB, recA, rpoB, secA1, smpB, sodA, ssrA, tuf* and their multiple combinations that have been reported by researchers (Yamada-Noda et al., 2007; Dai et al., 2011). In addition, Salati et al. (2010) described a FRET (fluorescent resonance energy transfer) probe assay that includes an initial PCR followed by a secondary amplification using FRET chemistry in real-time PCR for the identification of *M. marinum* in fish tissue samples. The authors concluded that the FRET probes allowed the detection of the pathogen in all infected samples and showed a high specificity that distinguishes the pathogen from other closely related fish *Mycobacterium* species.

Although there is some information in the literature about antibiotics used in various disease outbreaks, as of today, there is no commonly accepted treatment for Mycobacteriosis in fish. However, in line with the experience gained from human medicine, many antibiotics are used in combination over a period of months or even years in the treatment of tuberculosis caused by *M. tuberculosis*, a species closely related to *M. marinum* (Horsburgh et al., 2015).

Prevention of mycobacteriosis in any culture system (intensive, extensive, recirculated systems) seems very difficult, even almost impossible, due to the slow-growing nature of the causative bacterium and its intracellular survival ability, and the chronic course of the disease (Gauthier and Rhodes, 2009). For these reasons, the best strategy for controlling mycobacteriosis is prevention. Proper stocking density and control of water quality parameters (where applicable), a balanced/appropriate diet, minimal handling and similar improved fish health management practices are suggested as the best options (Gauthier and Rhodes, 2009; Jacobs et al., 2009).

In Türkiye, *Mycobacterium marinum* infection was first reported in cultured meagre (*Argyrosomus regius*) in a commercial farm, showing clinical signs including lack of appetite, lethargy, abdominal swelling and skin lesions, nodular lesions in the visceral organs (Avsever et al., 2014). Later, the agent was identified in diseased farmed gilthead sea bream and European seabass, exhibiting various degrees of skin lesions, cachexia and tubercles on their internal organs (Avsever et al., 2016). Researchers isolated the causative agent and identified them using a commercially available DNA-strip assay based on reverse hybridization, GenoType Mycobacterium CM/AS in both studies.

2.6. Lymphocystis

Lymphocystis disease (LCD) is caused by the lymphocystis disease virus (LCDV), a dsDNA virus consisting of a bilaminar capsid and a core with icosahedral structure that is relatively large and can reach several hundred nanometers, which is under the genus *Lymphocystivirus* in the family Iridoviridae. The pathogen is widely distributed all over the world and the Mediterranean basin is among regions where the disease is endemic. The virions are heat labile but resistant to ultrasonic treatment, however inactivation is possible by using UV treatment or by chemicals such as ether and glycerol (Anders, 1989; Borrego et al., 2017).

It is thought that the route of transmission of this virus can be both horizontal and vertical through gills, skin and eggs from diseased individuals or from water. However, there is no definitive study on how long the agent can remain infective in water and sediment. It has also been shown that live feed may act as a vector to transmit the agent (Cano et al., 2009; Carballo et al., 2019; Cano et al., 2013).

Lymphocystis disease (LCD) is a chronic, self-limiting disease that affects more than one hundred fish species in both freshwater and marine environments worldwide, including wild, ornamental and cultured fish species (Borrego et al., 2017). LCD is characterized by the hypertrophy of dermal fibroblastic cells in the connective tissue (Samalecos, 1986). As a consequence of this, small nodules are observed on the fins and skin of the diseased fish in the early stages as a typical sign of the disease and with the progression of the disease, these small nodules may merge and have an appearance similar to neoplastic cells. These nodules can also be seen in visceral organs such as spleen, liver, kidney and heart (Noga, 2010; Volpatti and Ciulli, 2022).

Although the disease actually causes low mortality, it greatly deteriorates the appearance of the fish's bodies, rendering them unsaleable, and as a result causes serious economic losses (Borrego et al., 2017). In some fish, the disease occurs in certain temperature ranges (for example 22 to 27°C for gilthead sea bream) and skin lesions who survived, can heal after a certain period of time, leaving little or no scar tissue (Colorni and Padrós, 2011). To date, the only lymphocystis disease in Türkiye was reported by Candan (1991) in farmed gilthead sea bream.

As in the diagnosis of other viral diseases of fish, the definitive method for LCD disease is made by isolating the virus using cell lines and then by any confirmation method (serological, molecular). For this purpose, a suitable cell line such as BF-2 (Bluegill fry, a fibroblast-like cell) or SAF-1 (*Sparus aurata* Fibroblast-1) or HINAE (HIrame Natural Embryo) is used to culture the virus (Borrego et al., 2015). In scientific literature, several serological methods such as indirect immunofluorescence, immunoblot, ELISA have been used. Several molecular diagnostics tools including specific primer sets, multiplex PCR, loop-mediated isothermal amplification (LAMP), have been successfully used (Kitamura et al., 2006; Cano et al., 2007; Li et al., 2010).

There is no known cure for lymphocystis disease. For controlling the disease, it is recommended to optimize the aquaculture conditions, eliminate stressor factors, and perform surface disinfection of the eggs in the rearing conditions (Cano et al., 2013).

2.7. Other bacterial and viral pathogens

Apart from the main diseases, low-impact and/or low-incidence disease outbreaks are occasionally seen in marine fish farming in Türkiye. The common features of this second group of diseases are; they are rare and/or do not pose a big problem for today's marine fish farming systems in Türkiye.

Vibrio parahaemolyticus is a Gram-negative, motile, halophilic, abundantly found in the marine/estuarine aquatic environments. It is attached to the surface of many organisms living in the aquatic environment such as zooplanktons, crustaceans, fish. The bacterium produces round, opaque, green/blue colonies of 2-3 mm in diameter on TCBS agar, the selective medium for *Vibrio* species. This species causes diseases in both humans and animals. In human medicine, it causes a serious disease from gastroenteritis to life-threatening septicemia. *Vibrio parahaemolyticus* was isolated and identified by Balta and Yılmaz (2019) in European seabass cultured in floating cages in the Eastern Black Sea Region of Türkiye. The symptoms of diseased fish were nonspecific and reported as darkening in color, exophthalmia, scale loss, degeneration and ulceration of skin, pale gills, hemorrhages on the operculum, and at the bases of the pectoral and pelvic fins.

Yardımcı (2020) reported *Vibrio pomeroyi* and *Vibrio crassostreae* along the well known fish pathogen *V. anguillarum* in cultured European seabass (150-250 g) in the Aegean Region of Türkiye. The outbreak was evaluated as acute vibriosis and fin rot and lesions ranging from small patches to superficial hemorrhagic ulcers on the skin were observed in the moribund fish.

Aeromonas caviae, a motile *Aeromonas* species, was reported in Atlantic salmon (*Salmo salar*) cultured in the Black Sea by Candan et al. (1995). Hemorrhagic areas on the body surface of diseased fish, especially in the mouth, eyes and anus, exophthalmos, petechiae on the intestinal walls and almost completely liquefied kidney were reported.

Among the Gram-positive bacteria, Kubilay and Uluköy (2004) isolated *Staphylococcus epidermidis* in juvenile gilthead sea breams (3-5 g) cultured in marine cages. The outbreak was occurred in the middle of the spring season, after the sudden increase in water temperature, daily fish loses was reported as up to 12%. The main clinical findings in diseased fish were haemorrhages in the fins and gills, anemic liver and distended abdomen with ascitic fluid. The causative agent was isolated from the visceral organs anterior kidney, spleen and liver. In the following years, the same agent (*S. epidermidis*) was also reported with a viral disease and various ectoparasites, in European seabass cultured in the Black Sea by Timur et al. (2008).

Akaylı et al. (2011) isolated and identified *Staphylococcus cohnii* subsp. *cohnii* together with some *Vibrio* species, from diseased common dentex (100-130 g) sampled from sea cages of a commercial company. Identification of the bacterium was based solely on biochemical and morphological tests. As clinical findings in diseased; darkening in color, swollen abdomen, loss of scales, hemorrhage on the gills; internally, haemorrhage in the visceral organs, reduced size of the spleen, yellow colored fluid accumulation in the intestine were noted.

The first observation of *Rickettsia*-like organisms (RLOs) in Turkish aquaculture was reported in European seabass cultured in the Black Sea by Timur et al. (2005). In the mentioned study, not only RLOs but also *Vibrio anguillarum* and *Photobacterium damselae* subsp. *piscicida* were isolated from moribund fish. High mortality was recorded during disease outbreaks. Moribund fish (90-250 g) were observed swimming near the surface of the water or edges of the cages, they were anorexic and lethargic; internally most fish exhibited yellow colored nodules in their kidneys and spleens.

Viral Hemorrhagic Septicemia (VHS) is caused by a negative-sense ssRNA virus with bullet-shaped virions belonging to the genus Novirhabdovirus of the Rhabdoviridae family.

Although viral hemorrhagic septicemia virus (VHSV) causes devastating disease in rainbow trout, its main host, many marine and freshwater species are known to be susceptible to the disease (Skall et al., 2005). The disease was reported in turbot (*Scophthalmus maximus*) fry and broodstocks cultured in the Black Sea region of Türkiye and the etiological agent was isolated and identified (Kalayci et al., 2006). Bilateral exophthalmia, darkening of the skin, pale gills, hemorrhage in the head region, at the base of the fins and in the intestinal tract were reported by these authors.

Viral Erythrocytic Necrosis (VEN) is a disease observed in marine and anadromic fish species, caused by the recently identified erythrocytic necrosis virus (ENV), a new member of the family Iridoviridae (Purcell et al., 2016). In electron microscopy studies in affected fish species, it was observed that the etiologic agent has variable morphology in different hosts, suggesting that several strains play a role in the etiology. The disease affects the host's red blood cells, causing severe anemia, making the host more vulnerable to other infections (Winton and Hershberger, 2014). The only occurrence of viral erythrocytic infection in Türkiye was reported by Timur et al. (2008) in cultured European seabass (*Dicentrarchus labrax*) along with pathogenic bacteria and some ectoparasites. These researchers reported moderate to high levels of mortality during disease outbreaks.

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CHAPTER 4

CURRENT PARASITIC AND FUNGAL DISEASE ISSUES IN MARINE AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.004

1. Introduction

Parasites, which develop complex strategies for living and exploiting their hosts, form the largest group that causes disease in marine fish, including species with direct or indirect life cycles, species-specific as well as those with low host specificity that can infect more than one host. Among the parasites with a wide taxonomy from unicellular (protozoan) to multicellular (metazoan), the most common group in cultured fish is protozoan ectoparasites. In a review study in which the parasitic diseases of the three fish species (sea bass, sea bream, meagre) that are most intensively farmed in Mediterranean aquaculture were evaluated according to the criteria of causing high mortality rates and incidence of epidemics; *Cryptocaryon irritans* from ciliates, *Amyloodinium ocellatum* from dinoflagellates, *Enteromyxum leii* from myxozoa, *Sparicotyle chrysophrii* from monogeneans, *Ceratothoa oestroides* from isopods were stated as the main parasitic pathogens (Cascarano et al., 2021). In the same study, *Trichodina* spp. and *Philasterides dicentrarchi* from the ciliates; flagellate *Ichthyobodo* spp.; apicomplexa group coccidiosis agents *Cryptosporidium molnari*, *Eimeria* and *Goussia* species; myxozoa

Ceratomyxa and *Kudoa* species (*Kudoa dicentrarchi*), *Sphaerospora testicularis*, *Sphaerospora sparis*; monogeneans *Diplectanum aequans, Diplectanum sciaenae, Encotyllabe spari, Lamellodiscus* spp., *Lamellodiscus echeneis*, *Polylabris* sp.; nematodes *Anisakis* sp. and *Hysterothylacium* sp., copepods *Lernanthropus kroyeri* and *Caligus* species are mentioned as minor parasitic pathogens (Cascarano et al., 2021).

Many of the parasite species that cause diseases in Europe have also been reported in Türkiye. With the increase of invasive species due to climate change and the potential created by parasites carried with them, a differentiating picture emerges day by day. In addition, *Enteromyxum leii*, one of the myxozoa that has not been reported in Türkiye yet but has been reported in other countries, is considered as a potential future threat by Özer (2020). The life cycle of this parasite can be affected by very small temperature fluctuations, which has significant effects on the dynamics of parasitism. Lõhmus and Björklund (2015) stated that even fluctuations as low as 0.5-1°C can cause extinction of the parasite. Thus, especially for parasites with multiple intermediate hosts, this progressive increase in temperature due to climate change can cause death in a definitive or intermediate host and from this point of view, may be beneficial in reducing the spread and density of the parasite. On the other hand, Pasternak et al. (2007) reported in their study, the increase in temperature caused by climate change favors the invasion of the Mediterranean Sea by the parasite *Polylabris mamaevi* (Microcotylidae, Monogenea) associated with the marbled spinefoot (*Siganus rivulatus*). It is emphasized that parasites originating from the Red Sea pose a potential threat to marine fish farmed in the Mediterranean if they adapt (Cascarano et al., 2021).

Most fungal agents are grouped into two taxonomic groups as those who lead a saprophytic or a parasitic lifestyle (Candan and Karataş, 2010). The Oomycetes group, which is saprophytic, is an important group of fish pathogens, while the Microsporidia group, which has a parasitic life, primarily parasitizes aquatic invertebrates and fish, it is known that the Basidiomycota group causes respiratory diseases, especially in marine mammals (Pang et al., 2021). Also, fungi in *Eurotiomycetes* are pathogens of fish. This group includes species commonly found in soil, as well as the most frequently reported pathogens in marine fish *Exophiala angulospora, E. salmonis* and *E. pisciphila* that cause *Exophiala* infections of the *Ascomycete* (de Hoog et al., 2011). Aquaculture practices, climate change and marine pollution can increase fungal diseases in aquatic organisms (sponge, coral, etc.). Although fungal diseases have been detected intensively in freshwater fish until today, marine fish, especially their eggs in hatcheries, are susceptible to these diseases. Although fungal diseases are generally defined as secondary infectious agents, they cause changes in the prognosis of the disease but are occasionally defined as primary infectious agents, some of which can be aggressive.

In this chapter, the main parasitic and fungal pathogens that cause economic losses in important cultured fish species in Türkiye and those with relatively lower mortality have been reported, but other commonly found parasitic and fungal pathogens have been summarized. In addition, treatment strategies and experimental vaccines against fish parasites are briefly discussed.

2. Parasitic Diseases in Turkish Mariculture

2.1. Main parasitic pathogens

2.1.1. Cryptocaryonosis (Marine White Spot Diseases/ Marine Ich)

Cryptocaryon irritans is a unicellular ciliate protozoan, which an obligate and has a low host specificity parasite of marine fish. This parasite has become a significant issue in mariculture causing high mortality rates. It has been reported in gilthead sea bream and European seabass farmed both in soil ponds and cage systems in Türkiye (Yavuzcan and Otgucuoğlu, 2018). This species is responsible for the marine white spot disease, cryptocaryonosis, which is ubiquitous in aquaculture worldwide. Clinical signs of the disease include visible white nodules on the skin and fins of infected host fish, increased respiration rate, anorexia, excessive mucus secretion and pallor. It causes death in juvenile fish and may reduce growth performance in adult hosts. It has also been reported in the cornea, as well as in the gill and skin epithelium. The average lifespan of the parasite, which has a direct life cycle, varies between 1-2 weeks, depending on the temperature and salinity of the water. The parasitic stage that infects the host is called the trophont, the pro-tomont living under the epidermal skin layer of the host, the cyst-forming tomont where reproduction takes place, and the theront, which actively swims and seeks a host to infect. The dimensions of the trophonts attached to the host can vary from about 48 x 27 microns to 452 x 360 microns. These trophonts turn into pro-tomonts after 3-7 days. The pro-tomont that empties the host tissue and falls into the benthos continues to settle here for a short time, forming tomonts/cysts in the substrate or in crevices between small stones or sand. Theronts protruding from the cyst are oval to pear-shaped. Theronts contain four round and fused horseshoe-shaped nuclei and are motile. Approximately 6-8 hours after separation from the cyst, its infectivity is greatly reduced, but a non-infective theront can act for up to 48 hours (Colorni and Burgess, 1997; Dan et al., 2009). They usually actively seek fish and invade the host skin within 5 minutes (Dickerson, 2006). Although white spots, nodules can be easily seen in fish, other diseases can cause similar symptoms. Microscopic evaluation of skin, fin and gill samples and identification of the trophont or one of its other life stages is required to confirm infection. Polymerase chain reaction

(PCR), which is a universal approach widely used in various clinical diagnosis, is also used for the detection of *C. irritans*. Chen et al. (2008) developed a specific PCR method based on internal transcribed spacer (ITS) of ribosomal DNA. Imajoh et al. (2016) created a TaqMan real-time PCR technique that can determine the presence of *C. irritans* in water and mud substrate samples quantitatively. They discovered that although the frequency of tomonts in the mud substrate was highest at a place where a cryptocaryoniasis outbreak had occurred, there were many theronts in the water there. In another study, in which specific primer pairs could not confirm the presence of *C. irritans*, an updated PCR method-specific primer sequence within the highly variable region of the 18S rRNA gene was developed by Xie et al. (2021).

2.1.2. Amyloodiniosis (Marine Velvet Diseases)

Dinoflagellates are endosymbionts or parasites of many invertebrates as well as primary producers and common in aquatic systems. These dinoflagellates, of which five genera (*Amyloodinium*, *Piscinoodinium*, *Crepidoodinium*, *Ichthyodinium* and *Oodinioides*) are reported to be parasitic in fish, also cause mass mortalities with the ichthyotoxins they produce (Rensel and Whyte, 2003). Among them, the most common and important parasitic pathogen in marine fish is *Amyloodinium ocellatum*. Since this parasite, which causes amyloodiniosis, was first diagnosed in gilthead sea bream in 1994, it has been reported to cause serious economic problems in many marine fish and has been reported in gilthead sea bream and European seabass cultured in Türkiye (Çağırgan and Tokşen, 1996; Akbas, 2011). This parasite also causes economic losses in important shrimp species (Aravindan et al., 2007) and bivalve species such as Pacific oyster (Soussa, 2015) causing intense tissue reaction, and has been reported as a hyperparasite with *Neobenedenia melleni* (Monogenea: Capsalidae), a monogenean capsalide in gilthead sea bream (Colorni, 1994). Amyloodiniosis causes typical symptoms of apathy, dyspnea, increased respiratory rate with difficulty breathing, and gathering of water surface. Fish may exhibit behaviors such as hitting and/or rubbing against surfaces. There is a lack of interest in feeding, anorexia and a decrease in weight gain is observed. In high concentrations, it causes breathing difficulties with the obstruction of the vessels in the gills. Although trophonts scattered on the skin can give a dusty appearance, which is called sea velvet disease due to its appearance, mortality is seen in some fish species without any common findings. Generally, the life cycle is completed between 5-7 days and is divided into three phases. The parasitic stage, the trophont, has a spherical to oval (pear)-shaped, brownish-colored appendix disc, 150 x 300 µm in size, filled with granules. A special tentacle-like mobile organ (stomopode) extends along with the peduncle of the attachment disc from the basal end. The cytoplasm has no chloroplasts, but the cytoplasm contains large digestive vacuoles with

particulate food and starch grains. In some trophonts, a long red eye spot can be seen that is difficult to observe from the cell contents due to density. Predominantly found in gills and skin epithelium (attachment site); it was also reported that trophons are attached to the oropharyngeal cavity in European seabass (Beraldo et al., 2017). The free-living cystic reproductive stage, which can reproduce asexually, is called tomont. The third stage is the dinospore stage, which includes the free swimming stage coming out of the tomont, 256 dinospores emerge in each tomont and the transmission from fish to fish occurs directly by the dinospores. The diagnosis of the parasite that is deeply embedded in the epithelial cells of the host, is made by microscopic examination of wet mounts prepared from gills and skin. Staining with Lugol makes the trophonts more distinctive. Electron microscopy as well as molecular methods are used for diagnosis. Levy et al. (2007) developed a sensitive and specific PCR method based on ribosomal DNA amplification for its identification, and Picon-Camacho et al. (2013) developed a LAMP (loop-mediated isothermal amplification) method with a more sensitive and specific approach for the determination of the life cycle.

2.1.3. Sparicotylosis

Sparicotyle chrysophrii (Microcotylidae, Monogenea) (formerly *Microcotyle chrysophrii*) is a monogenean parasite belonging to the subclass Polypisthocotylea. This species, which is less motile than other monogeneans, feeds on the blood of the host fish and causes tissue damage in the gills, where it feeds. Cachexia and anemia, excessive mucus secretion, lethargy and hypoxia are observed in infected fish. This parasite is responsible for major economic losses in gilthead sea bream (Faisal and Imam, 1990; Villar-Torres et al., 2018). De Vico et al. (2008) reported that the size and number of melanomacrophage centers dramatically increased in the infected sea breams' spleen, indicating an increase in hemosiderin (which causes erythrocyte destruction) and lipofuscin (which causes tissue catabolism and chronic degenerative disorders). This parasite, which has a wide salinity range, is a species specific to gilthead sea bream and was reported in Türkiye (Akmirza, 2010). It was also reported both in wild and in cultured sharpsnout seabream (*Diplodus puntazzo*) (Athanasopoulou et al., 2005). Reversat et al. (1992) stated that parasites can be found in sea breams in all seasons, but they are observed mostly in spring and at least in autumn. Poor aquaculture conditions, such as high stocking density in floating cages, infrequent net changes or lack of adequate water change rates due to not cleaning the nets, have an increased effect on disease emergence. Mortality rates are higher in small fish depending on their density. A case report from southern Spain stated that the amount of the parasite increased at a stable water temperature (13-14°C) (Sans, 1992). Similarly, in a study conducted in Corsica, the highest abundance of *S. chrysophrii* was recorded when the

water temperature was 13°C in winter (Antonelli et al., 2010). *S. chrysophrii*'s adults produce approximately 20 eggs per day, usually released into the environment as egg bundles. Eggs are oval shaped, 110 x 27-30 µm in size, and two long tendril-like filaments at the opposite polar ends, one of which is armed with small hooklets at the edge (Mladineo et al., 2023). The optimal temperature ranges for oncomiracidium hatching from the egg is 14-22°C. It has been determined by *in vivo* and *in vitro* experiments that the hatching rate decreases above 30°C (Repullés-Albelda et al., 2012). Oncomiracidia grow slowly while attempting to attach to gill lamellae, then the growth progress to a fast phase until they reach maturity. Paired buccal suckers help with the feeding of the adults, becoming pharynx is visible since the early stage and visible in the post-larval stage with three pairs of clamps. The intestine is firstly sac-like, becoming bifurcated later. The growth slows down again till the maximum number of clamps has been incorporated within the opisthaptor (Mladineo et al., 2023). It causes epizootics in fish throughout the year, regardless of temperature, so parasite spread is mainly affected by the size of the fish. The most critical factor is the low density of infected old fish donors, the infection progresses differently in different year classes overlapping within the fish farm with different age categories at the same time. Adult parasites can be easily detected with the naked eye and stereomicroscope. Microscopic examinations are sufficient for parasite identification and post-larval detection. A comprehensive molecular characterization was also performed with 28S rRNA and cox1 genes (Lablack et al., 2022).

2.1.4. Ceratothoasis

Parasitic crustaceans from the Isopoda order are generally found in the gills, skin and oral cavity of fish (Vagianou et al., 2004). Rarely, they cause post-hemorrhagic anemia in the host, with low growth rates and increased mortality (Horton and Okamura, 2003). *Ceratothoa oestroides* (Cymothoidae, Malacostraca), is an ubiquiter and the most common protandric hermaphrodite isopod species in the Mediterranean with low host specificity that can infect and multiple hosts, is also indicated as the main pathogen that creates local and systemic immune response (Piazzon et al., 2021). It has been reported that it causes economic losses in cage cultured European seabass, gilthead sea bream and meagre (Papapanagiotou and Trilles, 2001; Horton and Okamura, 2001, 2003; Čolak et al., 2018). *C. oestroides* also caused an epizootic with mortalities in a marine fish farm in Chios, Greece, between August and November in 2000, with temperatures varying between 21-23°C (Papapanagiotou and Trilles, 2001). In Türkiye, the highest prevalence was recorded in European seabass in hotter months (Horton and Okamura, 2001). The female parasite, which has a pair of eyes with multiple eye spots and is sexed separately, has an egg sac called a marcipium in its ventral region between its

feet. This sac is protected by a special layer called oostegites and the embryonic development is completed there. Eggs develop into Pullus larvae. Pullus I (primus) changes to a Pullus II (secundus/manca) with 6 pairs of thoracic legs inside the marsupium. After this stage, the parasites leaving their marsupium undergo a second change and pass the third stage, nauplii. In this stage, 7 pairs of pleopods are formed, the rudimentary pleopods contracted towards the sternal surface. Pullus II larva emerging from the marsupium descends to the ground, where some of the larval stages (nauplii) take place. After this stage, they undergo one more shell change and pass to the post larval (PL) stage. Sexual separation can only be made after the larva (Pullus II) leaves the marsupium. The parasite, which is male at first, then turns into a female individual. In post larval fish, which have seven free segments on their thorax and each of them has thoracic legs, it attaches to the mouth, gills and the whole body surface. The nauplii larvae that develop from the eggs in the brood sac of the adult female with pathogenic characteristics infect the host and settle in the oral cavity and then lose their swimming capacity. Feeding on host blood, this isopod absorbs blood from their enteric caeca. Since it is an intraoral species, it causes morphological changes in the host skull. Adult forms of *C. oestroides* in pairs were reported by Korun and Akaylı (2004) in the cheek cavities of cultured sea bass with vibriosis. In bogue (*Boops boops*), a tissue hyperplasia protruding ventrally as well as tongue narrowing and shortening in the mouth due to *Ceratothoa oestroides* has been reported (Özdemir et al., 2016). The same species can settle in the gills instead of the mouth. It causes degeneration in the gill tissue. Changes are also observed in the pharynx and gill cavity. Anemia, increase in erythroblasts, enlargement of the spleen, pressure on the heart and pericardial space are present, and respiration is affected. Feeding by the blood or tissues of the host fish, this isopod causes a decrease in growth and weight, weight loss and cachexia in infested fish (Mladineo et al., 2020), so juvenile fish are more susceptible than adults (Papapanagiotou and Trilles, 2001). In addition to the increase in temperature, wild fish populations around the cages also pose an additional risk for the spread of the parasite. For this reason, it is recommended to reduce the feeding load of the cages, especially in periods when the temperature is higher. A recent study used a RAD-Seq (restriction-site-associated DNA sequencing) to test whether *C. oestroides* transfers between wild and farmed fish. In the study, parasite transfer between isopods collected from aquaculture and wild fish population was found to be continued but there is no clear genetic differentiation between parasites; and the group of wild fish isopods was found more genetically heterogeneous than farmed parasites and therefore it was concluded that may serve as a source of infection for farmed fish (Mladineo et al., 2021). Various species of *Nerocila* (Cymothidae; Isopoda) were reported on many marine fish species around the world. Although less important than *Ceratothoa* species,

Nerocila species found in the skin, fins, cheek or gill cavities, and sometimes in the pouch of fish from Cymothoid isopods have been reported in Türkiye. Some species are extremely host-specific, even during the manca stage. It was reported that *Nerocila orbignyi*, which is normally endemic to fish of the Mugilidae family, infects cultured bass, causing a reduction in weight and condition factor and death in infected fish (Bargoni et al., 1984). *N. orbignyi* was reported by Kayış and Ceylan (2011) in between the operculum and pectoral fin of the sole, in Türkiye.

2.2. Less important parasitic pathogens

Trichodinids and scuticociliates are ciliate protozoans and opportunistic or secondary parasitic pathogens in marine fish farming. Trichodinids (Trichodinidae, Oligohymenophorea) are common in many fish species and are usually found on the skin and gill surfaces where they feed. These parasites, which cause weakening of the fish, destroy the epithelial tissue under stressful conditions. Several Trichodina species have been reported in cultured European seabass and gilthead sea bream, and water temperature and organic load are important factors influencing infection intensity. Scuticociliatia members are known to cause high mortality rates in a few cases. *Philasterides dicentrarchi* (Philasteridae, Oligohymenophorea), a histophagous ciliate of this group, causes the disease called scuticociliatosis and has been reported to cause high mortality in farmed European seabass by experimentally (Dragesco et al., 1995). This species has also been reported in turbot reared in northern Spain (Iglesias et al., 2001). It has been reported that *P. dicentrarchi* showed better growth rates *in vitro* at 23°C than at 18°C, and their reproduction stopped at 13°C (Iglesias et al., 2003). It has been defined as an aggressive, invasive disease agent that survives under all conditions and does not lose its ability to reproduce even under adverse conditions. On the free form of this parasite in the skin and gills of the host fish, Iglesias et al. (2002) reported that chemical substances were effective, but they were not successful against their form in the tissue, which was detected internally in the brain, spinal cord and digestive system, as well as in muscle tissue. Although it is predicted that feeding with immunostimulants stimulates the general immunity of the fish and supports against the effects of the parasite, the mortality rate in juvenile fish varies between 50% and 100%. *Miamiensis avidus* has also been reported as a causative agent of scuticociliatosis in turbot (Kayış et al., 2011) and common dentex (*Dentex dentex*) (Turgay et al., 2015) cultivated in Türkiye. *Uronema marinum* is another species that causes the disease called scuticociliatosis. In Türkiye, it has been reported in turbot larvae (Türe, 2021). While the early methods used to distinguish the scuticociliate species isolated from diseased fish were based on morphometric methods, for the four most common scuticociliates (*P. longisetus, P. persalinus, U. marinum* and *M. avidus*) small subunit (SSU) ribosomal (r)DNA or mitochondrial cytochrome c oxidase 1 (cox1) gene-based sequence analyzes are used (Whang et al., 2013).

Species in the genus *Ichthyobodo* (Kinetoplastea), such as the *Ichthyobodo necator* complex, cause the disease called Ichthyobodiasis (or costiasis), although pathological cases caused by flagellated protozoa have not been reported frequently. These parasites are called secondary disease agents that trigger disease outbreaks depending on the health status of the fish or stock density (Isaksen, 2013). This protozoan species usually affects fish skin. It causes thickening of the mucous layer, characteristic symptoms such as hyperplasia and dullness in colour. The parasite has a free lifestyle that consists of a motile stage with a long and short flagellum and a trophozoite form that attaches to the host as a pear-shaped non-motile parasite. Infection has been reported in the European seabass population in Portugal (Santos, 1996) and in Türkiye (Yardımcı et al., 2016). Also, Tokşen (2000) reported mortality rates up to 60% in gilthead sea bream stocks cultured in Türkiye. Gilthead sea bream larval deaths were also been reported, particularly in heavily stocked hatcheries in Spain (Alvarez-Pellitro et al., 1995).

Myxozoan parasites constitute another important parasite group of cultured marine fish. The life cycle of myxozoan species is usually completed in two successive hosts; fish and annelids. The myxozospores ingested primarily by annelids, infecting the intestinal epithelium or epidermis of the infected ringworm, and then producing actinospores. Actinospores released from the annelid float freely in water or sediments until they reach the surface of a fish (skin or gills). After entering the fish host through the epithelium, migration continues until the parasite's developmental stages reach the final site of infection and the parasite develops into myxospores. It was first reported in gilthead sea bream and myxozoans belonging to the genus *Enteromyxum* have been reported in various marine fish to date (Sitjà Bobadilla and Palenzuela, 2012). These myxozoa members of the Cnidaria phylum have a two-host life cycle in marine fish: myxospore stage as a parasite in fish and actinospore stages in aquatic invertebrates. In this group, *Ceratomyxa* spp. (Ceratomyidae, Myxozoa) are coelozoic myxosporeans and have been detected in the gallbladder of cultivated European seabass and other wild sparids. They cause a seasonal infection that is more common in the winter. Katharios et al. (2007) reported an outbreak caused by *Ceratomyxa diplodae* in cultivated sharpsnout seabream (*Diplodus puntazzo*) and *Ceratomyxa sparusaurati* was identified in sea bream cultured in Spain. Alama-Bermejo et al. (2013) examined seasonality and infection dynamics in the marine environment and reported that while no significant infection was observed in winter, this species increased the production of actinospores in benthic invertebrate hosts

with an increase in temperature. In Türkiye, it has been identified to genus level in European seabass (Özer and Öztürk, 2011) and common dentex (Tokşen and Çilli, 2010).

Enteromyxum leei (Enteromyxidae, Myxozoa) is the most important myxosporean. It was reported by Breton (1999) as the parasite species that most negatively affects aquaculture in the Mediterranean. It has been isolated from many fish species such as gilthead sea bream, common two-banded seabream, red porgy, and its transmission can occur directly from fish to fish (Diamant, 1997). The target organ is the intestine of fish, which causes an inflammatory response in the intestinal epithelium, in short, enteritis with a chronic course (Sitja-Bobadilla et al., 2007). Clinically infected fish show atrophy with loss of appetite and weight. The feed conversion rate (FCR) of fish with cachexia decreases to an alarming level. The intestines are fragile and translucent, filled with mucous fluid. There is focal obstruction and bleeding in the intestines. In addition to the decrease in perivisceral fat, the organs are pale in color. It is diagnosed by the visualization of myxozoospores in the microscopic examination of the intestinal contents by the rectal biopsy (Sitja-Bobadilla et al., 2021). Samples can be collected even by gently squeezing the moribund fish. For molecular diagnosis, samples may be taken by probing the rectum with a sterile cotton swab. Myxospores can pass into the water with faeces or be transmitted directly from dead fish. *Enteromyxum scophthalmi* is another parasitic agent belonging to the genus *Enteromyxum*. It causes a serious disease affecting turbot (*Scopthalmus maximus*). This disease, which is defined as sunken head syndrome or turbot enteromyxosis, causes similar syndrome in various other fish species besides turbot (Sitjà-Bobadilla and Palenzuela, 2012). It develops in the intestinal epithelium of turbot, causes severe catarrhal enteritis and impairs intestinal function, and the fish dies within a few weeks. Enteromyxosis is greatly suppressed below 15°C, especially in winter. In summer, uncontrollable acute deaths have been reported in sharpsnout seabream in Greece (Rigos et al., 1999).

Monogeneans, also called flatworms, are species with a high degree of host and organ selectivity. Monogenean species other than *Sparicotyle chrysophrii*, which has been reported in gilthead sea bream, are defined as opportunistic pathogens in gilthead sea bream and other farmed marine fish. *Lamellodiscus* species (Diplectanidae, Monogenea) are the primary parasites of sparids and cause limited pathology. Due to their direct life cycle, they reproduce in large numbers and rapidly. It has been reported that *Lamellodiscus elegans* has the highest prevalence in the Adriatic sparid hosts in autumn and spring and reaches the highest abundance in the summer months (Mladineo, 2004; Sanchez-Garcia et al., 2011). *Lamellodiscus* species together with *Microcotyle* sp., *Trichodina* sp., and *Vibrio* sp., were reported as a secondary pathogen in gilthead sea bream during winter in Portugal (Cruz e Silva et al., 1997)

and mixed infections with epitheliocystis in northeastern Spain (Padros and Crespo, 1995). Another species, *Lamellodiscus echeneis* (formerly *Furnestinia echeneis*), which causes high mortality rates and economic losses in gilthead sea bream among cultured fish, was reported in Spain, Italy, Greece and Türkiye, depending on seasonal changes, the lowest occurrence in spring and highest in autumn. The increase in temperature is the most obvious factor limiting the development of the parasite. Paperna et al. (1977) reported that they have encountered this parasite in cultured gilthead sea bream every season, while Revarsat et al. (1992) reported an increase in the number of this parasite in spring, while Oliver (1969) reported that it mostly attached to the 2nd and 3rd gill arches; a study at three different locations in Corsica showed that among cultured gilthead sea bream and wild fish, the highest prevalence and abundance occurred in farmed fish (average 18-25°C) and in autumn (Antonelli et al., 2010). Diagnosis of the parasite can be made easily under the light microscope with squash preparations of the gill. In the protocol defined by Wong et al. (2007), the measurements of sclerotized structures of wet preparations can be made with more precision when prepared with 2.5% sodium dodecyl sulfate. Their bodies are wide at the level of the ovary, and this species is 560-890 microns long. The squamodisc in the haptor of the parasite, which has two pairs of non-crystalline eye spots in its dorsal, has a lamellar structure with a diameter of 180-220 microns. The complex-structured hooked attachment organelles in the haptor cause damage to the gills of the fish. Harris et al. (2000) reported that resistance of fish to monogenean parasites was decreased with increased cortisol levels as a result of stress.

Another monogenean commonly known to cause gill pathology is *Diplectanum* species (Diplectanidae, Monogenea). The most known species to cause problems in marine fish farming is *Diplectanum aequens*. The haptor of this monogenean, which is 650-1700 microns long and 260-500 microns wide, is 110-300 microns in size, and its squamodisc is 180 microns in diameter. The length of the lateral bars is 53-78 microns, and the medial bar length is 155-187 microns. It has a copulatory organ 150 microns long (Oliver 1968). This species, which is found in the gills of the host in the adult stage and on the skin in the young stage, was reported in European sea bass cultured in Türkiye (Tokşen, 1999). In a study conducted in Southern Israel, it was observed that this species caused mixed infections with *Diplectanum sciaenae* in European seabass. In the identification of species, it is sufficient to connect the fastening apparatus or opisthaptors consisting of fixation disks and make measurements under the microscope with marginal hooks or hamuli. In a study investigating the life cycle of *D. aequans*, temperature was reported to be a limiting factor (Cecchini, 1998). Over a two-year study in the Mediterranean region of Spain, it was observed that *D. aequans* is at its maximum

prevalence and intensity in winter, with young individuals reaching their peak in November and also in February and May (Gonzalez-Lanza et al., 1991). It was stated that 83-89% of the eggs of the parasite hatched at 15-30°C, 75% of them hatched at 10°C, and no hatching was observed at 5°C in cultured European seabass (Cecchini et al., 2001).

In recent years, *Neobenedenia girellae*, a capsalid species from monogeneans called "skin parasite", was described in gilthead sea bream farmed in Israel (Smirnov et al., 2022). In Türkiye, *Benedenia sciaenae* was defined in the meagre (*Argyrosomus regius*) (Tokşen *et al*., 2007), and it is very difficult to detect these transparent colored parasites that attached to the skin of fish with the naked eye. They feed on blood of the host fish and cause a serious pathological disorder. The life cycle is direct and reproduction in these egg-reproducing parasites is directly related to water temperature. A single larva (oncomiracidium) emerges from an egg. Another blood-feeding monogenean is *Polylabris* sp. (Microcotylidae, Monogenea). In France, *P. tubicirrus* has been reported in the gills of *Diplodus* sp. sparids reared in Corsica (France), and it has been reported to cause epidemics in Italy and Greece in farmed gilthead sea bream (Athanassopoulou et al., 2005).

No digenean parasites were encountered in fish reared in marine cages, and crustacean class parasites, which are considered to be the most common but less important parasite groups, were reported in the gills of cultured gilthead sea bream and European seabass with well-known seasonal population growth. In Türkiye, *Caligus* and *Lernanthropus* species from copepoda, have a broad ventral surface concave and dorsal surface convex, dorsoventral flattened head. Cephalothorax acts like a sucking disk to attach itself to the host so that the parasite is not affected by the flow of water. The 2nd antennae and the strong hooks of the maxipedes also strengthen attachment to the host. Sea lice species belonging to the genus *Caligus* (Caligidae, Hexanauplia), which have two suckers and feed on tissue fluid by piercing the host skin with the mandibular they extract from these suckers, showed seasonal epizootics in European seabass. *Caligus elongatus* is the most well-known species, especially along with *Lernanthropus salmonis*, which causes restrictions and problems for the salmon industry worldwide in terms of costs associated with prevention and control. Severe mortalities have been reported as a result of fluid electrolyte loss related to hemorrhage, anemia, edema in salmon farms caused by *C. elongatus* in Scotland, Ireland and Norway. This parasite, which is a carrier of Infectious Salmon Anemia (ISA), also causes lesions progressing to the muscle. In some cases, fibroblast proliferation, neutrophil, lymphocyte and macrophage infiltration in the dermis and epidermis, and foreign body giant cell formation in chronic inflammation have been observed (Jones et al., 1990). In Türkiye, *Caligus minimus* and other *Caligus* species

were reported in cultured and wild marine fish (Altunel, 1983; Tokşen, 1999; Akmirza, 2010; Uluköy and Kubilay, 2005; Öktener, 2009; Özer and Öztürk, 2011; Er and Kayış, 2015; Alaş and Öktener, 2017). Although the life cycle of caligids has been stated as having eight stages in some studies, Kabata (1989) suggested that there are ten stages. After hatching, the two free-swimming nauplii stages are followed by the infective copepodid stage. The nauplii stage is the light-sensitive planktonic stage that does not feed. The active host passes from this stage to the chalimus stage within a few days after it attaches to the host. It usually attaches to the posterior part of the fins in the caudal region where the current is the least. Inflammation or epithelial hyperplasia may be developed where it is attached. After the chalimus stages on the host, it goes through two immature/preadult stages until it becomes an adult. In these stages, individuals on the host are motile. The adult parasite mates on the host. Infestation occurs especially during the period when the water temperature is high. Rarely, chronic infestation may occur during the winter months. In a study on the genus *Caligus*, sea bass fish were observed over a two-year period in the Bardawil Lagoon on the Mediterranean coast of Egypt (Paperna, 1980). As a result of the study, *Caligus minimus* densities were determined at higher levels in winter and early spring, and at lower levels in summer and autumn. While the maximum number of copepodites and chalimus were recorded in May, temperatures in the lagoon were reported to vary between 10-16°C in January and 28-34°C in July-August. In another study, the prevalence and density of the parasite was examined in Greece, and it was reported that the European seabass for four *Caligus* species (*C. minimus, C. pageti, C. mugilis*, and *C. apodus*) followed the same trend throughout the year, while no high mortality rate was recorded. It has been suggested that higher rates may also occur in the lagoons in winter (8-10°C). It has been mentioned that it may be related to fish migrating for winter catchments, which may promote parasite infection, albeit minimal locally.

There is another copepodid reported as parasitic in marine fish, including species belonging to the largest genus of the Lernanthropidae family, *Lernanthropus*. The most characteristic feature of these parasites is their feet. The third foot is in the shape of a shoe or horn and is extended outward from the body. Pedigric segment in the fourth foot is leaf-like and ridge-shaped dorsal bark, which is important for species identification. In addition, shell shape and dimensions are among the important morphometric characters for species identification. Male individuals are much smaller than females and are in the form of a buccal apart siphonostome. *Lerneanthropus kroyeri* (Lernanthropidae, Hexanauplia) has been reported intensively in marine fish cultured in Türkiye from these parasites that attach to gill lamellas with their secondary antennae. By infecting the gills of the European seabass, it causes mechanical damage to the gill epithelium and opens a port for secondary bacterial infections (Manera and Dezfuli,

2003). It causes degeneration of the epithelial tissues to which the host is attached, sometimes necrosis of the connective tissue and excessive mucus secretion. During attachment to the gill filaments with their third legs, they may cause capillary damage and cause occlusion in the branchial arteries due to thrombi. In our country, this parasite was reported in cultured European seabass by Korun and Tepecik (2005). Although the prevalence of this parasite in Corsica suggests that higher water temperatures are preferred in spring and summer (Antonelli and Marchand, 2012), it was reported by Tokşen (2007) in our country even at lower water temperatures (17,5°C). This parasite, which can be seen in the gills with the naked eye, is sufficient to examine under a light microscope for species identification.

2.3. Other potential parasitic pathogens and treatment

Some other parasitic pathogens reported in aquaculture fish include members of the phylum Apicomplexa such as the endoparasitic protozoans *Eimeria*, *Goussia* and *Cryptosporidium* responsible for fish coccidiosis, myxosporean parasite *Sphaerospora* species and the nematode *Hysterothylacium aduncum*. Parasites in the phylum Apicomplexa have a complex life cycle that includes sexual and asexual phases and infectious stages. *Eimeria sparis* (Eimeriidae, Conoidasida) has been widely reported in gilthead sea bream aquaculture. This endoparasite, which causes mortalities especially in gilthead sea bream in the spring in Spain, is also difficult to distinguish with a new *Goussia* species, *Goussia sparis*, which co-infects the intestines of gilthead sea bream, and there is a case report in which it was identified together (Sitja-Bobadilla et al., 1996). *Eimeria dicentrarchi* is a little-known but common coccidian parasite. The parasite infects the intestinal epithelium of European seabass. In a study conducted in Croatia, the overall prevalence of parasites was 16,7% in adult European seabass sampled in March and October (Gjurcevic et al., 2017). While there are no reports in farmed fish in Türkiye, different apicomplexan species, *E. merlangi* (Özer et al., 2015) and *E. sardinea* (Özer et al., 2014) have been identified in whiting (*Merlangius merlangus*) fish caught in the Black Sea. *Cryptosporidium molnari* (Cryptosporidiidae, Conoidasida) is another apicomplexan parasite, transmitted orally through ingestion of oocysts. The parasite infects the gastrointestinal epithelium and is released from the host in feces. It has been isolated from cultured gilthead sea bream and has been reported to a lesser extent in European seabass (Alvarez-Pellitro and Sitja-Bobadilla, 2002). Maximum prevalence and intensity were reported in the spring in Spain (Sitja-Bobadilla et al., 2005). A study of the transmission mechanism revealed that infection was initiated at 23.3-26.8°C (Sitja-Bobadilla et al., 2003).

Sphaerospora sparis (Sphaerosporidae, Myozoa, formerly *Polysporoplasma sparis*) is a myxosporean parasite species that causes relatively benign infections of fish muscle tissue.

This parasite adversely affects the market value of fish and it causes mortalities especially in farmed gilthead sea bream. It is widely distributed in gilthead sea bream farms on the Mediterranean and Southern Spanish Atlantic coast. This parasite infects the kidney and is responsible for the so-called glomerular disease of gilthead sea bream (Palenzuela et al., 1999). *Kudoa dicentrarchi* (formerly *Sphaerospora dicentrarchi*) and *Sphaerospora testicularis* are myxosporeans that affect European seabass reared in Italy (Fioravanti et al., 2004). *Kudoa dicentrarchi* is a histozoic parasite that infects connective tissues. The intestine, gallbladder and kidney tissues are the target organ (Sitja-Bobadilla et al., 1992). *Sphaerospora testicularis* species is coelozoic in the seminiferous tubules of the testicles and causes infertility (Sitja-Bobadilla et al., 1990). Infection in cultured European seabass was also reported in Spain during the warmer seasons (Sitja-Bobadilla et al., 1993).

Hysterothylacium aduncum parasite (Raphidascarididae, Nematoda), one of the helminth nematodes, has been reported to cause diseases in wild European seabass in Norway (Sterud, 2002), in the Gulf of Mexico (Deardorff and Overstreet, 1980) and in marine fish farms in Chile (Gonzalez, 1998). The relationship between the prevalence and temperature of sparidae member fish caught in the wild in the northern Aegean Sea, Kalay et al. (2009) reported that the prevalence and mean intensity were in a positive correlation and highest between March and June. Therefore, like *Anisakis* species, it poses a potential threat to cultivated species.

Chemotherapy is a common practice to control parasite infestations and disease outbreaks. In a review study on the disease problems and treatments of marine fish reared in Türkiye, it was found that bath applications with formaldehyde and hydrogen peroxide were intensively applied against ectoparasite infestations. It has been stated that copper is used for the treatment of *Cryptocaryon irritans*, which is common in earthen ponds rather than offshore facilities in the sea, responds well to fish and is more widely used. The study also reported that the free-floating dinospore form of the high virulence *Amyloodinium ocellatum* is sensitive to drugs, but it is difficult to eradicate with copper therapy because the trophont and tomont forms of the parasite are resistant, and periodic treatment is required for the risk of infection. In the same study, it was reported that effective treatment was performed against isopod infestation with emamectin benzoate and that garlic-based food additives were more effective than a bath in the treatment of parasites orally in Türkiye (Demircan et al., 2020). An easy and efficient treatment against *C. irritans* in shrimp farming is to chlorinate equipment and sea water using sodium hypochlorite at a concentration of 50 mg/l (Vaughan et al., 2018). To reduce the likelihood of re-infection, Stija-Bobadilla et al. (2006) advise net changing together with the bath treatments (formalin and hydrogen peroxide) for monogenean. *In vitro* tests have

shown that a 30-minute bath in formalin (300 ppm) from *S. chrysophrii* is 100% effective for eggs, oncomiracidia, and adults. However, the efficacy of hydrogen peroxide (200 ppm) for oncomiracidia and adults has not been determined. As all results were obtained *in vitro*, *in vivo* studies should be carried out to determine the ideal exposure dose and timing (Stija-Bobadilla et al., 2006). A modified diet including caprylic acid (CA), an efficient eight-carbon saturated fatty acid, was created by Rigos et al. (2013). Antinematoda medicaments include piperazine, mebendazole, thiabendazole, pyrantel, ivermectin, carbamazine dimethyl and others that are effective against pinworms, hookworms, whereas praziquantel, bithionol sulfoxide, oxamniquine, and metrifonate are used against to trematoda. Niclosamide, are used to treat tapeworms as anti-helminths. Also levamisole is frequently used as anti-parasitic medications for nematodes. According to the European Union 169 Commission Regulation No. 37/2010, metronidazole, the active component of nitroimidazole used to treat diseases brought on by protozoa, is not allowed to be used in animals utilized for food production in member states. One of the many key challenges in delivering safe products is the absence of hazardous drug residues in farmed fish. Intensive use of parasitic drugs causes drug residues to accumulate in the body. Drug resistance has also been reported for most of the commonly used anti-parasitic drugs such as organophosphates, synthetic pyrethroids and avermectins. Especially development of multi-resistance to chemotherapeutants, and alternative delousing strategies have been raising concerns about the environmental impacts and the UK provide another alternative herbal treatment to chemotherapeutic use. Recently medicinal plants which are widely used, either as a single drug or in combination with synthetic drugs. According to Costello (2009), salmon lice infestations cost the global salmonid farming business a total of $480 million in losses in 2006. Another alternative is stocking cleaner fish (*Ballan wrasse*, *Labrus bergylta* or lumpfish, *Cyclopterus lumpus*) to control sea lice infestations in Atlantic salmon farms is widespread and is viewed as a salmon welfare-friendly alternative to current delousing control treatments in Norway. Experimental vaccine studies have been carried out for the protection of fish against parasites, especially in recent years, various concerns about both food and environmental safety related to the use of chemicals have increased the tendency to different protection measures (Table 1).

There are 24 vaccines available in the fish farming industry, one licensed for bacterial and viral diseases, including lobster, only in Chile there is a commercially licensed parasite vaccine against sea lice (Adam, 2019). The development of parasite vaccines is limited. Most of the experimental studies are on ectoparasites for which more information is available, and very few studies are available in endoparasites. Although these studies focused on the purpose

of developing vaccines, it is necessary to develop vaccines for parasite species about which little is known, the host-parasite interaction and the life cycle of the parasite with different stages. Recent attempts to genetically produce parasite antigens with modified microorganisms have increased the possibility of some parasite vaccines. It is a widely used genome-based approach to identify potential vaccine candidates for the development of protein subunit vaccines. Protein sequences from the pathogen are screened and then potential vaccine candidates are selected using software programs. Known vaccine candidates for other pathogens can be selected on the basis of highly immunogenic proteins or other criteria. Recombinant subunit vaccines (or DNA vaccines) are then produced and in vivo efficacy tested. DNA vaccines are now approved for use in Europe, and many more will likely be developed in the future. With reduced cost of technologies such as whole genome sequencing, it enables targeted vaccine design for specific pathogens or heterogeneous species. In recent years, omics studies are the most powerful methods recommended for vaccine development by providing information about potential vaccine candidates with transcriptomic and proteomic analysis started with genomics, which aims to examine the entire gene content (genome) of an organism. Whereas, currently, there are species for which there is not much information about the pathobiology and/or diseases of marine fish parasites.

Table 1. Experimental marine parasites vaccines*

Disease	Parasite	Host	Vaccine	Target Antigen	Delivery Method	Reference
Marine White Spot	*Cryptocaryon irritans*	Grouper	Live	Theronts	Bath	Yambot and Song 2006
			Killed	Theronts	IP	
		Tiger pufferfish (*Takifugu rubripes*)	Subunit	Recombinant proteins	IP	Watanabe et al., 2021
Sealouse infestations	*Lepeophtherius salmonis*	Atlantic salmon	Crude parasite extract	Adult female	IP	Grayson et al., 1995
PKD	*Tetracapsuloides brysalmonae*	Rainbow trout	DNA	Micro-exon-gene (TB-MEG1)		Faber et al., 2019
Myxobolosis	*Myxobolus koi*	Gold fish	Subunit	Crude protein spore	IM	Kane et al., 2016
Scuticociliatosis	*Uronema marinum*	Grouper	Subunit	i-antigen	IP	Harikrishnan et al., 2012
	Philasterides dicentrarchi	Turbot	Subunit	Membran protein	IP	Fontenla et al., 2016

*This table was adapted from Shivam et al. (2021). IP: Intraperitoneal enjection, IM: Immersion.

3. Fungal Pathogens in Turkish Mariculture

3.1. Main fungal pathogens

3.1.1. Saprolegniasis

Saprolegnia, which cause saprolegniasis in fish, is a filamentous fungus that produces abundantly branching vegetative micelles without a septum. Saprolegniasis is a common name used for the disease caused by species belonging to the genera *Achlya* or *Dictyuchus*. The reproductive units of these fungal species are different from the species belonging to the genus *Saprolegnia*. However, these microscopically distinguishable species cause similar clinical findings in eggs and fish. These fungi, which can reproduce both sexually and asexually in their life cycle, usually occur in water temperatures that are not optimal for fish and cause infection with reduced immunity of the fish. Focal gray to white lesions develop on the skin of the fish, such as cotton wool. In asexual reproduction, the terminal portion of the hyphae swells slightly. The zoospores, which form masses of cytoplasm and nuclei, accumulate at the enlarged end called the sporangium. The zoospores are released from the sporangium contain two flagella and they move freely in the water for a while or form cysts and then, the hyphae begin to burst. Secondary zoospores, which are reniform, are released. In sexual reproduction, female (oogonium) and male (antheridium) sex organs develop at the ends of the hyphae. The antheridium is slender and curved, while the oogonium has a spherical body. The zygote formed by fertilization is diploid. Fertilization, germination and hyphae development take about 3 months. It begins to spread in eggs, especially in hatcheries. Diagnosis of this disease in fish is made by the presence of hyphae in scraping wet preparations from shallow lesions. PCR is required for definitive species identification, and in some studies, a strong staining is obtained with gomorimethenamine silver staining in addition to the presence of haematoxylin-eosin staining in histopathological tissue sections. *Saprolegnia parasitica* is a pathogenic water mold species (Stramenopiles; Oomycota; Saprolegniales) that causes economic losses in this group, although it usually causes disease in freshwater fish, it causes co-infection with *Aeromonas hydrophila* in European sea bass reared in Türkiye (Dinçtürk et al., 2018). It was reported that it causes infection in gilthead sea bream (Dinçtürk et al., 2019) cultured in brackish water conditions. Several freshwater and euryhaline species have been reported worldwide as primary pathogen (Stueland et al., 2005; Van West, 2006). In another study, RxLR effector protein-containing *S. parasitica* is the pathogenic oomycete was detected by Van West et al. (2010). In fish, the infection is usually localized in the epidermis, dermis and subcutaneous muscle tissue. However, there are reports of *S. parasitica* hyphae infect deeper tissues even organs and spinal cord when infected fish is small (Gieseker et al., 2006) or when it forms a cyst (Fregeneda Grandes et al., 2001). The

infectivity of *S. parasitica* is affected by the virulence of the strain and the number of zoospores present in the water. Laboratory studies have shown that zoospore production decreases when the water temperature rises above 20°C (Dieguez-Uribeondo et al., 1996), while their germination is inhibited in acidic conditions (Kitancharoen et al., 1996). In a study reporting the development of loop-mediated isothermal amplification (LAMP) for the detection of *Saprolegnia* spp.; LAMP was compared by quantitative PCR (qPCR) and the internal transcription spacer (ITS) region of ribosomal DNA and cytochrome C oxidase subunit 1 (coxI) target gene. While the LAMP method can detect *S. salmonis* DNA as low as 10 fg, it has been reported that the qPCR method has a detection limit of 2 pg *S. salmonis* DNA. When applied to detect the pathogen in water samples, it was mentioned that both methods can detect the pathogen when only one zoospore of *Saprolegnia* is present (Ghosh et al., 2021). Digital droplet PCR (ddPCR) test, which is a fast, sensitive and specific method, has been developed for the detection of *S. parasitica* and the quantification of pathogens in environmental samples (Pavić et al., 2022).

3.1.2. Ichthyophoniasis

The fungus called *Ichthyophonus hoferi*, which is the causative agent of Ichthyophoniasis, causes a systemic granulomatous disease in many species of freshwater and marine fish. Since it was first described in brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) cultured by von Hofer in Germany in 1893, it is a common fish disease with many fish species from freshwater, brackish and marine environments and a wide geographical distribution. *I. hoferi*, a member of the phylum Mesomycetozoa, a phylogenetically close microorganism group in the animal-fungal boundary (Ichthyophonidae, Ichthyosporea), usually has a round or oval form and contains many nuclei in its cytoplasm, reported in farmed gilthead seabream, European seabass and turbot (Athanassopoulou, 1992; Franco-Sierra et al., 1997). The size of the fungus is 6-20 µm in young forms and 110-120 µm in adult and mature ones and transmitted through contaminated food, spreads to fish entering marine cages and the aquatic environment, mostly through the consumption of infected fish in fish fed with garbage or discarded fish. Stress facilitates proliferation in the host, and *Ichthyophonus* taken from the intestine enters the circulatory system through the blood and reaches other organs. A granulomatous reaction consisting of epithelioid and giant cells against *I. hoferi* has been reported in body muscles in flounder. The life cycle includes at least four different stages. Thick-walled spores are usually present in the center of the granulomatous tissue of infected organs. This resting stage turns into a hyphae stage showing multiple germination tubes. At each end, the round, thin-walled spherical structure separates to form a nucleated structure and then subdivides the motile stage transitioning to the stage called endospores or infective stage. Spanggaard et al. (1995) reported that they

observed that changing the pH in the stomach from 7 to 3.5 triggered the germination of spores and transformed into hyphae. This suggests that pH plays an important role in the development of hyphae. In another study by the same authors, it was reported that temperatures between 0-25°C have no effect on its development, the growth of the pathogen outside this temperature range is affected, and beyond -20°C and +40°C are lethal temperatures (Spanggaard and Huss, 1996). *Ichthyophonus hoferi* was the only species of this genus to cause disease in marine fish until 2000. However, morphological criteria and phylogenetic DNA sequences, another species was identified by Rand (2000) in yellowtail flounder. This species, which is morphologically different from *I. hoferi* and causes disease in fish, was defined as *Ichthyophonus irregularis* according to the 18S SSU rDNA sequence result. For the diagnosis, quantitative PCR (qPCR) assays specific for genus *Ichthyophonus* were developed for parasite detection and surveillance (White et al., 2013; Wurdeman, 2019). In Türkiye, *Ichthyophonus hoferi* was first reported in seabass with pasteurellosis (Timur et al., 1999) then the fungus was reported causing mortalities in aquarium kept goldfish (*C. auratus*) (Öztürk et al., 2010). In squash preparations from the heart, liver, spleen and kidneys; *Ichthyophonus hoferi* was observed as surrounded by a thick fibrous membrane, having an average diameter of 200-250 µm, spherical shape with a multinucleated morphology. The host reaction and granuloma development stages against this pathogen were described in detail (Öztürk et al., 2010).

3.2. Less important fungal pathogens

3.2.1. Rosette agent disease

Sphaerothecum destruens was first described in the USA and has already been reported in more than 14 fish species, including important aquaculture species, and reproduces asexually in the cells (Andreou and Gozlan, 2016). To refer to the parasite's distinctive clusters that can be observed in stained tissue impression smears from infected fish, the term "rosette agent" is used to describe the parasite. The spores of the pathogen released into the environment through feces are transmitted directly between hosts via the alimentary route or indirectly through the attachment of free-floating spores to the gills (Andreou et al., 2009). When spores enter the water, they divide and each spore produces an average of 5 motile zoospores (Arkush et al., 2005). These motile zoospores stay alive in water for a maximum of 15 days (Andreou et al., 2009). After demonstrating their relationship through phylogenetic analyses of their small subunit ribosomal DNA (SSU rDNA) sequences, *Ichthyophonus hoferi*, the rosette agent, and *Psorospermium haeckeli* were placed in a new clade (DRIPs, for the first letter of each of their names) by Ragan et al. in 1996. Cavalier-Smith (1998) classified the DRIPs clade's members as belonging to the class Ichthyosporea, but when Herr et al. (1999) made

their initial suggestion, Mendoza, Taylor, and Ajello (2001) changed the name and established the class Mesomycetozoea. Infection by *S. destruens* in host leads to destruction of internal organs or even death (Arkush et al., 2005). *S. destruens* has been found to replicate in cells of the epithelial, mesenchymal, and hematopoietic types during the histozoic stage (Arkush et al., 1998). Because the parasite can be seen histologically in the lumina of renal tubules and bile ductules and has been re-isolated from ovarian fluid, the scientists hypothesized that infected fish may shed spores via bile, urine, gut epithelium, and seminal and ovarian fluids (Arkush et al., 2005). In Türkiye, this fungus was first identified in European seabass reared in Milas in 2012, by histopathological and molecular methods, and then it was reported periodically in European seabass in the same region in autumn (Ercan et al., 2015).

3.3. Other potential fungal pathogens and treatment

Other histozoic intracellular parasitic fungi belonging to the Microsporidia class pose a potential threat to the marine fish farming in Türkiye in the future. These fungi, which are reported to cause deformation in the muscle tissues of fish, induced weakening syndrome or cystic formations and high economic losses, exist as spores outside the host. Their highly resistant spores allow these fungal-like parasites to survive in the environment for long periods, even in extreme conditions, until a suitable host is found. *Glugea* spp. (Glugeidae, Microsporidia) fungi have been reported in gilthead sea bream farmed in Mediterranean coast in France (Mathieu-Daude et al., 1992). In the same study, xenomas were reported in the muscles close to the pectoral fins of fish, and the viability of fungal spores and the extrusion rate of filaments, which are considered parameters of infectivity, and the resistance of spores to heating and freezing were analyzed. Heating at 60°C for 30 minutes or freezing at -15°C for 30 minutes completely killed all spores. To the contrary, temperature treatment of 40°C for 30 minutes did not reduce spore viability, but it has been reported to reduce filament extrusion rates. *Pleistophora* sp. (Glugeidae, Microsporidia) were reported to be embedded in the gastrointestinal muscles or dorsal body muscles of gilthead sea bream at temperatures as low as 10-12°C in February and March in Greece (Athanassopoulou, 1998). Another microsporidian pathogenic fungus, *Microsporidium aurata* (Microsporidium, Microspora), has been reported to form large cysts in the peritoneal cavity, connective tissue and intestinal epithelium in gilthead sea bream in the Red Sea (Morsy et al., 2013).

While the control of fungal diseases is possible by eliminating stress factors, fresh-salt or hot water baths are applied for therapeutic purposes in marine fish in Türkiye. For aggressive and long-term infections, there are various chemotherapeutic and chemical applications. The use of hydrogen peroxide (H_2O_2) is approved by FDA in the United States for the prevention of fungal

infections in fish eggs. Formalin is also used for aggressive long-term problems in Türkiye. In a study, it was stated that fish fed with diet supplemented with 0,02% dietary pyridoxine were protected from *S. parasitica* fungal infection after 45 days of feeding. In the same study, in the challenge study with *S. parasitica*, immunological parameters such as Hb, PCV, MCV, MCH, NBT, total plasma protein, albumin, globulin contents, lysozyme and phagocytic activity were significantly higher ($p < 0.05$) in the group fed with pyridoxine vitamin B6 (Saha et al., 2016). Görmez and Diler (2014) reported that the essential oils of three Lamiaceae species (*Thymbra spicata, Origanum onites* and *Satureja thymbra*) have the potential to be used to develop alternative and natural control methods in their study where they examined the chemical composition and antifungal activities against *S. parasitica* strains. There is no commercially licensed vaccine against aquatic fungal diseases not only in Türkiye but also in the world.

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CHAPTER 5

CURRENT DISEASE ISSUES IN FRESHWATER AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.005

1. Introduction

Fish have been fed in ponds for various reasons since ancient times. In the middle ages, these fish were used for nutritional purposes. Commercial aquaculture activities have a history of a little more than 100 years around the world. Commercial aquaculture activities in Türkiye, which started with rainbow trout eggs brought from abroad in the 1970s, have shown great diversity in terms of both the number of species grown and the culture sites over time. The development of aquaculture in Türkiye, which is now one of the leading countries in Europe, especially in terms of rainbow trout farming, will be discussed in the other sections of this book. According to the 2022 data from the Turkish Statistical Institute (TUİK, 2022), the amount of aquaculture in Turkish freshwater ecosystems is 136.000 tons as the most cultivated freshwater fish species is the rainbow trout (*Oncorhynchus mykiss*). Today, more than 20 freshwater fish species, in addition to rainbow trout, various carp, sturgeon and tilapia species are cultured in Türkiye for research, recreational or commercial purposes. One of the main obstacles for aquaculture is the pathogenic diseases and related mortalities. The aim of this section is to list the diseases caused by bacterial, viral, parasitic and fungal pathogens that have been diagnosed in cultured freshwater fishes since the beginning of commercial aquaculture activities in Türkiye.

2. Bacterial Diseases

Bacteria are microorganisms that show prokaryotic cell organization characteristics and can live and reproduce in living or non-living environments. Some bacteria commensally colonize the gill, skin and digestive tract in fishes and may beneficially affect the digestion and immune system of the host. Some aquatic bacterial species that are non-pathogenic to the fishes may also be beneficial for the maintenance of the natural balance. However, some bacteria are considered pathogenic since they can affect fish health under stress conditions. Fish are in constant contact with the microorganisms in the aquatic environment in which they live with their skin and gills covering the body. The development of a disease case is a result of the combination of environmental conditions, pathogenicity of the bacteria and immune functions of the fish (Austin and Austin, 2012; Roberts, 2012).

Although Gram-negative rods are the main pathogens of fishes, some species of Gram-positive rods, cocci and acid-fast bacteria were also reported to be pathogenic to fishes. Bacterial fish diseases are among the important limiting factors in fish production triggering serious mortality rates, especially in hatcheries. In this section, detailed information will be given about common bacterial infections observed in cultured freshwater fish species in Türkiye.

2.1. Furunculosis

Aeromonas salmonicida subsp. *salmonicida* is a Gram-negative non-motile bacterium known as the causative agent of systemic furunculosis in salmonids and non-salmonids. Infected fish show few symptoms that are characterized by abscesses in the acute form of disease and furuncles on the body surface in the subacute and chronic forms. Furunculosis structures are in the form of ulcers filled with bloody fluid. Outbreaks are often induced by stress factors, inadequate environmental conditions, poor handling and high stock density (Bruno et al., 2013; Dar et al., 2022). The first case in Türkiye was reported in rainbow trout (Timur et al., 1999) and was reported later in various regions of Türkiye (Kayış et al., 2009a; Türk et al., 2013).

2.2. Motile aeromonas septicemia (MAS)

The disease caused by the motile members of the *Aeromonas* genus (mostly by *Aeromonas hydrophila* and others such as *A. sobria* and *A. caviae*), which are commonly found in water and fish flora, is known as motile aeromonad septicemia. *A. hydrophila* is the most commonly identified cause of MAS. All fish in freshwater, marine, warm and cold waters are susceptible to this disease. The typical clinical findings of MAS are the darkening of the skin, dropsy, exophthalmia, erosion and necrosis of the fins, hemorrhagic gills and edema of superficial lesions on the skin (Bruno et al., 2013).

A. hydrophila, *A. caviaea* and *A. sobria*, which are regarded as the most important aetiological agents of MAS, have been recovered and identified from moribund rainbow trout in different regions of Türkiye (Onuk et al., 2013; Balta, 2020; Yardımcı and Turgay, 2021). In a recent study, it has been reported that *A. hydrophila* causes blue sac fry syndrome in trout (Kayış et al., 2015). *Aeromonas* sp. was found to be the cause of inflation in the swim bladder with low mortality in diseased Russian sturgeon (*Acipenser gueldenstaedtii*) in Türkiye (Türe et al., 2018a). Moreover, opportunistic MAS agents, *A. hydrophila* and *A. sobria*, have been reported in sturgeons (*Acipenser gueldenstaedtii* and *Acipenser baerii*) (Kayış et al., 2017). Recently, various Aeromonads such as *A. schubertii* (Akayli et al., 2011a), *A. veroni* (Onuk et al., 2013) *A. bestiarum*, *A. dhakensis*, *A. encheleia* (Duman et al., 2018) and *A. media* (Özcan, 2023) were isolated and identified from moribund cultured rainbow trout in different regions of Türkiye.

2.3. Vibriosis

Vibriosis is the most common septicemic disease of marine and brackish water fish caused by *Vibrio* species. Infected fish have clinical findings such as darkening of the skin, anorexia with swollen abdomen, increased mucous production, periorbital edema, and hemorrhages at the fin bases as well as extensive hemorrhage in the internal organs and peritoneal surface. Vibriosis is usually observed in hot weather, especially when stocking density is high, as well as when salinity and organic load are high (Bruna et al., 2013; Dar et al., 2022).

Throughout the history of aquaculture, *Listonella anguillarum* (formerly classified as *Vibrio anguillarum*), *V. ordalii*, *V. vulnificus*, *V. harveyi*, and *V. alginolyticus* were identified as the general causative agents of vibriosis worldwide with several other cases of various *Vibrio* species (Austin and Austin, 2012). *L. anguillarum* is still the main vibriosis agent in rainbow trout in Türkiye (Tanrıkul, 2007; Akaylı et al., 2014; Alkhunni et al., 2017; Akaylı et al., 2018). Also, *V. alginolyticus* was isolated in rainbow trout cultured in the Black Sea (Rize) with similar vibriosis symptoms (Duman et al., 2023) and from asymptomatic cultured rainbow trout in the Aegean region (Duman et al., 2023a). Moreover, it has been reported that *V. parahaemolyticus*, and *V. fluvialis* were isolated from rainbow trout (Aydın, 2000) and Seven khramulya (*Capoeta capoeta*) (Akayli et al., 2019).

2.4. Yersiniosis

Yersiniosis (Enteric Red Mouth Disease, ERM) is the name used to describe infections caused by *Yersinia ruckeri* which is among the most important salmonid disease which results in grand economic losses in the aquaculture sector worldwide. It develops in an acute or chronic form. In the chronic form of the disease, external signs include ascites, exophthalmia,

cutaneous petechiae and localized hemorrhages (Austin and Austin, 2012; Bruno et al., 2013; Dar et al., 2022;). It was first reported from cultured rainbow trout in southern Anatolia (Timur and Timur, 1991a), then spread to other parts of the country (Akaylı et al., 2013; Altun et al., 2013a; Onuk et al., 2019; Dinçtürk et al., 2021; İspir et al., 2021).

2.5. Pseudomoniasis

Pseudomonas septicemia is a bacterial disease case especially in wild and farmed fish caused by pseudomonads, a group of opportunistic Gram-negative oxidative bacteria. Disease outbreaks occur under stress conditions and periods of inadequate water conditions. The most characteristic signs of pseudomonasis include fin rot, hemorrhagic skin ulcers, darkening, ascites and petechiae on internal organs (Austin and Austin, 2012; Bruno et al., 2013). Although pseudomonads such as *P. aeruginosa*, *P. anguilliseptica*, *P. baetica*, *P. chlororaphis*, *P. fluorescens*, *P. koreensis*, *P. luteola*, *P. plecoglossicida*, *P. pseudoalcaligenes* and *P. putida* (Duman et al., 2023b) have been identified from various cases, the most common causative agent is *P. fluorescens* (Austin and Austin, 2012). *P. fluorescens* has been defined as a primary or secondary disease agent in cultured rainbow trout and sturgeon in Türkiye (Akaylı and Timur, 2004; Kayış et al., 2009a; Kayış et al., 2017). *P. putida*, which causes ulcer and hemorrhagic ascites, was reported in cultured rainbow trout (Altınok et al., 2006; Bektaş et al., 2009) and sturgeon (Kayış et al., 2017) in different regions of the country. Other pathogenic pseudomands isolated and identified from moribund cultured rainbow trout are *P. luteola* (Altınok et al., 2007) and *P. plecoglossicida* (Akaylı et al., 2011b).

2.6. Flavobacteriosis

Flavobacteriosis, is the general name of the disease cases induced by various filamentous Gram-negative bacteria and cause serious mortalities in trout culture. Among them, rainbow trout fry syndrome (RTFS or bacterial cold-water disease) caused by *Flavobacterium psychrophilum*, columnaris disease caused by *F. columnare* and bacterial gill disease caused by *F. branchiophilum* are the most destructive forms for salmonid aquaculture (Austin and Austin, 2012). RTFS occurs in low water temperatures (below 10 ºC) and its most distinctive symptom is the lesions descending to the muscle on the fins of juvenile fish. In most columnaris cases, pale skin color, damages on the dorsal fins and necrotic gill lamellae lesions were reported (Bruno et al., 2013). Balta (1997) first reported *F. psychrophilum* from rainbow trout in Türkiye and following reports have been documented in cultured rainbow trout from different parts of the country (Korun and Timur, 2001; Kayış et al., 2009a; Satıcıoğlu et al., 2018).

A mixed infection case of *F. hydatis* and *A. hydrophila* was reported from cultured Russian sturgeon in Sakarya (Timur et al., 2010), *Flavobacterium johnsoniae* was identified from diseased Russian sturgeon (Karatas et al., 2010) and rainbow trout (Yıldırım and Özer, 2010) in Türkiye. Various *Flavobacterium* species such as *F. bernardetii* sp. nov (Satıcıoğlu et al., 2021a) *F. turcicum* sp. nov., *F. kayseriense* (Satıcıoğlu et al., 2021b) and *F. muglaense* sp. nov. (Duman et al., 2021) have been reported in diseased trout in Türkiye. Classification of the genera *Flavobacterium*, *Cytophaga*, *Flexibacter, Chryseobacterium* and their marine representative *Tenacibaculum* is complicated where researchers are still working on the phylogenetic analysis and taxonomic studies (Bernardet et al., 1996; Suzuki et al., 2001; Satıcıoğlu et al., 2023) and new species are proposed frequently.

2.7. Streptococcosis

Gram-positive cocci, representatives of the genera *Lactococcus*, *Streptococcus* and *Vagococcus* are occasionally identified as causative agents of cultured fishes worldwide. Freshwater streptococcosis cases are divided into two groups. The warm water streptococcosis or lactococcosis cases caused by *Lactococcus garvieae*, *Streptococcus agalactiae, Streptococcus iniae* and *Streptococcus parauberis* are generally observed when water temperatures are above 15 ºC. The other form of the disease known as cold-water streptococcosis cases are by *Vagococcus salmoninarum* below 15 ºC water temperature. Lactococcosis outbreaks caused by *L. garvieae* are associated with environmental conditions such as stress, high stock density, rapid alterations in water temperature and inadequate water quality parameters, but water temperature is the most crucial factor. The most common clinical symptoms of lactococcosis in cultured fish species include congestion, hemorrhages, skin pigmentation, lethargy, exophthalmia, corneal clouding, anemia in the liver, splenomegaly and erratic swimming (Wendrell et al., 2006; Öztürk and Altınok, 2014; Ürkü and Timur, 2014; Dar et al., 2022).

L. garvieae is frequently isolated and identified from diseased rainbow trout as a streptococcosis agent with high mortality (80%) in Türkiye (Diler et al., 2002). *L. garvieae* was first identified from a rainbow trout farm in 1995 in Türkiye (Çağırgan and Tanrıkul, 1995). Subsequently, it was reported from many other regions of Türkiye in the last decade (Timur et al., 2011a; Türe and Çimagil, 2018; Balta and Balta, 2019; Akaylı et al., 2020; Önalan et al., 2020; Altan and Korun 2021). In recent studies, it has been reported that the streptococcosis agent (*L. garvieae*) isolated in cultured rainbow trout in the Black Sea region was described as *L. petauri* according to whole genome analysis of the strain (Altınok et al., 2022). The only S. *parauberis* case in Türkiye was reported from diseased trout (Bektaş et al., 2017). The first *Vagococcus salmoninarum* infection of rainbow trout was reported in the USA in 1968 (Austin and Austin, 2012). Didinen et al. (2011) reported the first *V. salmoninarum* case

in Türkiye from broodstocks of rainbow trout cultured in the Mediterranean region. Later, other outbreaks were also reported in cultured rainbow trout in Türkiye (Tanrıkul et al., 2014; Satıcıoğlu et al., 2021c).

2.8. Staphylococcosis

Staphylococci are very common in nature and are part of the normal flora of the skin and mucous membranes. In addition, due to their zoonotic potential, their transmission mechanism via feed, environment and other wild animals is of interest of the researchers worldwide (Foster, 1996). The clinical signs of staphylococcosis in fish may be variable depending on the severity of the disease, causative agent and the fish species, but the eyes are the most affected organ in infection. The main clinical signs are exophthalmia, loss and degeneration of the eye (Austin and Austin, 2012).

The most common primary pathogens of fish staphylococcosis, *Staphylococcus epidermidis* and *S. aureus* have been frequently reported from various marine (Çanak and Timur, 2020) and freshwater fish species (Timur and Akaylı, 2003) in Türkiye. Few outbreaks of staphylococcosis caused by other representatives of the genus occurred in cultured freshwater fishes in Türkiye. *S. cohnii* subsp. *cohnii* (Akaylı et al., 2011a), *S. warneri* (Metin et al., 2014; Akaylı et al., 2019), *S. hominis* (Turgay et al., 2015) and *S. capitis* (Akaylı et al., 2019) have been reported as a staphylococcosis agent in cultured freshwater fishes.

2.9. Other potential bacterial pathogens of freshwater fishes in Türkiye

Recently, various bacterial infections of freshwater fishes that have not been previously isolated in Türkiye were reported where it is possible to name them as emerging pathogens. Bacteria in this group are not commonly detected in cultured freshwater fish species in Türkiye, but as the aquaculture sites showed diversification, also due to the interaction with various environmental factors, new bacterial species were recovered in the disease cases of freshwater fishes.

In recent studies, Gram-negative bacterial species such as *Plesiomonas shigelloides*, *Shewanella putrefaciens*, *Citrobacter* spp., *Edwardsiella ictaluri*, *Acinetobacter* spp., *Chryseobacterium* spp., *Serratia liquefeciens*, *Hafnia alvei*, *Sphingomonas echinoides* and Gram-positive bacterial species such as *Bacillus gibsonii* and *Frigoribacterium faeni* have been defined in this group (Keskin et al., 2004; Kayış et al., 2009a; Altun et al., 2013b; Altun et al., 2014; Kayış et al., 2017; Akaylı et al., 2020; Duman et al., 2020; Satıcıoğlu et al., 2020; Akaylı et al., 2021; Çanak et al., 2021; Satıcıoğlu et al., 2023).

3. Viral Fish Diseases

Viruses, the smallest and most primitive microorganisms, are obligate pathogens and are capable of replicating only within the host's body cells. It is not possible to cure most of the viral diseases in wild and cultured fish species. Hence, these diseases hit the sector a great blow, especially because they cause high mortalities and economic losses in fish farms (Evensen and Santi, 2008; Austin and Austin, 2012; Roberts, 2012). Several viral disease cases were observed and reported in cultured rainbow trout and common carp (*Cyprinus carpio*) in Türkiye more abundantly, namely IHN, IPN and VHS. In addition, some other viral diseases were also occasionally reported in Turkish freshwater aquaculture (Table 1).

Table 1. Viral infections of cultured trout reported in Türkiye (modified from Öztürk and Altınok, 2014)

Disease	Host	Reference
Infectious pancreatic necrosis (IPN)	*O. mykiss*	Candan (2002); Albayrak and Ozan (2010); Işıdan and Polat (2011); Kalaycı et al. (2012); Gürçay et al. (2013); *Öğüt et al. (2013); *Büyükekiz et al. (2017); Albayrak et al. (2018); *Işıdan et al. (2019); Tamer et al. (2020)
Viral haemorrhagic septicemia (VHS)	*O. mykiss*	*Albayrak and Ozan (2010); *Işıdan and Polat (2011); Kalaycı et al. (2012); Öğüt (2013); Öğüt et al. (2013); *Albayrak et al. (2018)
Infectious hematopoietic necrosis (IHN)	*O. mykiss*	*Albayrak and Ozan (2010); *Öğüt et al. (2013)
Erythrocytic inclusion body syndrome (EIBS)	*O. mykiss*	Timur et al. (2011b)
Carp pox	*C. carpio*	Timur (1991b)
Epidermal Papilloma	*C. carpio*	*Diler and Özen (2002)
*: reference added after Öztürk and Altınok, 2014		

3.1. Infectious pancreatic necrosis (IPN)

Infectious pancreatic necrosis (IPN) is a major infectious viral disease seen in cultured Salmonid fishes worldwide (Faheem et al., 2021). Infectious pancreatic necrosis virus (IPNV) of the Aquabirnavirus genus of the Birnaviridae family, which contains RNA in its structure is the causative agent of the disease. The most characteristic symptom is the necrosis of the pancreas in infected fish (Evensen and Santi, 2008; Austin and Austin, 2012).

The course of the disease is more acute in the broodstock stage and young (10-20 gr) fry and causes high mortality. However, while adult fish do not show any symptoms of the disease, they can carry the disease chronically. Despite the disease spreading horizontally from

fish to fish mostly through contaminated water, the eggs of carrier broodstock individuals can also carry the virus. The mortality rate caused by the disease develops depending on the fish species, age, genetic resistance of the fish, virus serotypes and strains, viral load and environmental stress factors. For example: depending on the virulence of the virus, the mortality rate may reach 100% in young trout. Abnormal behavior such as darkening of the skin color, turning around its own axis or swimming sideways, as well as swelling in the abdomen and whitish thread hanging from the anal orifice are the most prominent clinical findings of IPN. Internal examination reveals findings such as ascites and bleeding in the perivisceral adipose tissue and pallor in the liver. In histopathological examination, it has been reported that the virus causes acute multifocal necrosis in the exocrine pancreatic tissue of fish (Candan, 2002; Evensen and Santi, 2008; Austin and Austin, 2012; Roberts, 2012; Faheem et al., 2021). In Türkiye, IPN was first reported by Candan in 2002 from juvenile rainbow trout followed by other reports (Table 1).

3.2. Viral hemorrhagic septicemia (VHS)

Viral Hemorrhagic Septicemia (VHS) is the most common viral disease seen in wild and cultured fish, especially in rainbow trout. The causative agent is a RNA-virus, VHS-V of the Novirhabdovirus genus of the family Rhabdoviridae (Einer-Jensen et al., 2004; Austin and Austin, 2012; Roberts, 2012; Faheem et al., 2021).

Depending on the severity of the disease, high fish mortality can be observed in infected fish, sometimes without any clinical signs. Rotation and abnormal swimming behavior, exophthalmos, widespread hemorrhages on the body surface and edema in the abdominal cavity are among the most prominent clinical symptoms of the disease. This disease, which can be observed in all seasons of the year, is more common especially in the spring months when water temperatures start to increase (at water temperatures around 9–12°C). Disinfection of eyed and fertilized eggs is an effective and cost-effective preventive measure to stop the spread of disease (Einer-Jensen et al., 2004; Austin and Austin, 2012; Roberts, 2012; Faheem et al., 2021). After the first detection in rainbow trout cultured in Türkiye by Albayrak and Ozan (2010), it spread to the other parts of the country (Table 1).

3.3. Infectious hematopoietic necrosis (IHN)

Infectious hematopoietic necrosis (IHN) is a viral disease of rainbow trout and other salmonid fishes worldwide. The virus (IHN-V) is a bullet-shaped RNA virus of the genus Novirhabdovirus of the Rhabdoviridae family (Austin and Austin, 2012; Roberts, 2012; Dixon et al., 2016; Faheem et al., 2021). The mortality rate is very high in young fish. Water

temperatures below 15°C are suitable for IHN to occur. Clinical symptoms of this infection are hemorrhages in the mouth, on the back of the head, pectoral fins, muscles and around the anus, swelling in the abdomen, skin darkening, abnormal behavior, anemia, and pale gills (Austin and Austin, 2012; Roberts, 2012; Dixon et al., 2016; Faheem et al., 2021). This viral disease was reported by Albayrak and Ozan (2010) and Öğüt et al. (2013) in cultured trout in Türkiye (Table 1).

3.4. Erythrocytic inclusion body syndrome (EIBS)

Erythrocytic inclusion body syndrome (EIBS) is a highly contagious viral disease seen in various trout species (Winton and Purchell, 2016). The pathogen (EIBS-V) is an RNA virus belonging to the Piscine othoreovirus (PRV) family (Takano et al., 2016).

The disease is more common during cold water periods in autumn, winter and spring, possibly due to reduced host immune response. The direct cause of mortalities in fish affected by EIBS varies depending on the fish, the intensity of the infection, the presence of secondary pathogens and some other environmental factors. Diseased fish are usually anemic and lethargic, with pale gills, pigmentation abnormalities and a severe decrease in blood hematocrit levels (Takano et al., 2016; Winton and Purchell, 2016). This viral disease was reported by Timur et al. (2011b) in marine cultured rainbow trout in Türkiye (Table 1).

3.5. Carp pox/ Koi herpesvirus disease (KHVD)

Cyprinid herpesvirus 1 (CyHV-1) is a viral disease that causes carp pox, with characteristic epidermal papillomas occurring in carp species. The virus (CyHV-1) is a DNA virus from the Herpesvirus genus of the Alloherpesviridae family (Rahmati-Holasoo et al., 2020; Faheem et al., 2021. The mortality of the disease is relatively low. Lesions, epidermal hyperplasia and light-colored spots (papillomas) are seen on the body surface. This viral disease was reported by Timur (1991b) in common carp (*Cyprinus carpio*) in Türkiye (Table 1).

3.6. Epidermal papilloma

Many different papillomas in fish can be the result of different infectious and non-infectious reasons. Epidermal papillomas on the fish body can be seen as brownish-white, smooth, sometimes loosely adherent tumors on the skin and fins. The viral etiology of epidermal papillomatosis in fish has been supported, but virus particles have not always been found in papillomas (Korkea-aho et al., 2006). This disease is caused by viruses belonging to the Retroviridae family (Coffee et al., 2013). Diler and Özen (2002) made the histopathological diagnosis of viral epidermal papilloma in cultured common carp in Türkiye.

Note: Infectious Hematopoietic Necrosis (IHN), Spring Viremia of Carp (SVC) and Viral Hemorrhagic Septicemia (VHS) infections in fish are notifiable viral fish diseases (Kaplan and Karaoğlu, 2018). There is no cure for these viral diseases in cultured fish. However, implementing preventive measures such as obtaining high water quality, keeping low stocking density, providing certified eggs instead of possibly infected fish eggs, producing in spring waters and closing infected hatcheries for disinfection is very important for the prevention and control of the disease (Austin and Austin, 2012; Faheem et al., 2021; Roberts, 2012).

4. Parasitic Infestations

Parasites are members of the animal kingdom, which can be microscopic or macroscopic in size and can cause diseases in fish. Although the harmful effects of parasites on fish in the natural environment are less, they cause serious disease outbreaks in aquaculture populations. It is known that the parasite population reaches a high level, especially under negative environmental conditions and where a high fish stock density is preferred. Parasitic infestations; vary considerably with the number of parasites, the species, size and health status of the host. Due to the wide variety of parasites that infest fishes, a complete and precise classification can not be made by scientists. With this, parasites that cause disease in fish are examined under 2 main groups: protozoans and metazoans (Bruno et al., 2006; Woo, 2006; Özer, 2019).

4.1. Protozoan parasites

Protozoan parasites are the most primitive animals that are unicellular, mostly motile and that can only be seen under a microscope. Parasites in this group live in fresh water and marine environment, soil and in the intestinal flora of various animals. Protozoans are the most commonly observed parasitic group observed in hatchery units. However, the intensity of the infestation depends on the parasite's morphology, size, number and attachment pattern (Lom and Dyková, 1992; Bruno et al., 2006; Ozer, 2019). Protozoan species can show a great variety in morphology and size. They are mostly found attached to the gills, fins and skins of fish. They can be classified according to their motility patterns (flagella, pseudopod or cilia) or the presence of immobile spores (Sporozoans). Parasitic protozoans belonging to *Ichtyophthirius*, *Trypanoplasma* and *Myxosoma* genera cause serious infestations in fish. Parasites of the genera such as *Costia*, *Chilodonella* and *Octomitus* become parasitic when there is another disease in the fish with decreased immune resistance. Parasites of the genera *Hexamita*, *Cryptobia* and *Myxozoa* are generally found attached on and in the internal organs of fishes. Among these groups, *Ambiphyra* spp., *Apiosoma* spp., *Hexamita*

sp., Ichthyobodo necator, *Ichthyophthirius multifiliis*, *Trichodina* spp. and *Trichophyra* species were reported to be the principal pathogenic protozoan parasites in aquaculture (Lom and Dyková, 1992; Bruno et al., 2006; Kayış et al., 2009b; Özer, 2019). Parasitic infestations caused by protozoan parasites observed in rainbow trout and other freshwater fishes cultured in Türkiye were given in Table 2.

Table 2. Protozoan parasites observed in cultured freshwater fish in Türkiye (Modified from İnnal and Stavrescu-Bedivan, 2022)

Pathogen	Host fish species	Reference
Ichthyobodo sp.	*O. mykiss*	Soylu (1985); Altunay (2006); Altunay and Yıldız (2008); Balta et al. (2019)
Ichthyobodo necator	*O. mykiss*	Burgu et al. (1988); Soylu (1996); Kayış (2006); Öğüt and Akyol (2007), Balta et al. (2008)
	S. fontinalis	Kayış (2006), Balta et al. (2008)
Hexamita salmonis	*O. mykiss*	Öğüt and Akyol (2005; 2007); *Timur et al. (2009), *Kayış et al. (2009b); Balta et al. (2018)
Spironucleus salmonicida	*O. mykiss*	Balta et al. (2019)
Ambiphrya sp.	Hybrid bass	Tokşen (2006)
	M. saxatilis	Tokşen (2006)
	O. mykiss	Özcelep (2009)
Apiosoma piscicolum	*O. mykiss*	Öğüt and Akyol (2007)
Apiosoma sp.	*O. mykiss*	Burgu et al. (1988), Altunay (2006); Altunay and Yıldız, (2008); Özçelep (2009); *Şimşek and Aldemir (2020)
Epistylis sp.	*M. saxatilis*	Tokşen (2006)
	O. mykiss	Öğüt and Akyol (2003); Altunay (2006); Altunay and Yıldız (2008), Özçelep (2009)
	Hybrid bass	Tokşen (2006)
Ichthyophthirius multifiliis	*C. idella*	Uzbilek and Yıldız (2002)
	S. fontinalis	Balta et al. (2008)
	Hybrid bass	Tokşen (2006)
	M. saxatilis	Tokşen (2006)
	O. mykiss	Burgu et al. (1988); Tokşen (2002); Uzbilek and Yıldız, (2002); Tabakoğlu (2004); Öğüt et al. (2005); Mefut et al. (2007); Öğüt and Akyol (2007); Balta et al. (2008); Özçelep (2009), *Kayış et al. (2009b); Özer et al. (2010); Sözeren Çevrimel and Soylu (2017); Balta et al. (2019); *Şimşek and Aldemir (2020)
Ichthyophthirius sp.	*O. mykiss*	Soylu (1996)
Trichodina acuta	*O. niloticus*	Cengizler and Can (1999)
Trichodina claviformis	*O. mykiss*	Öğüt and Akyol (2007)
Trichodina domerquei	*O. mykiss*	Burgu et al. (1988); İnce and Uslu (2017)

Table 2. Continue

Pathogen	Host fish species	Reference
Trichodina sp.	*C. idella*	Uzbilek and Yıldız (2002)
	M. saxatilis	Tokşen (2006)
	O. mykiss	Burgu et al. (1988); Soylu (1996); Ogut and Akyol (2003); Altunay (2006) Altunay and Yildiz (2008); Balta et al. (2008); Özçelep (2009); Sözeren Çevrimel and Soylu (2017); Balta et al. (2019)
	S. fontinalis	Balta et al. (2008)
	A.gueldenstaedtii	*Kayış et al. (2017)
	Acipenser baerii	*Kayış et al. (2017)
Trichodina nigra	*O. mykiss*	Burgu et al. (1988), Dal (2006), Özer et al. (2010),
Trichodina fultoni	*O. mykiss*	*Şimşek and Aldemir (2020)
Trichodinella sp.	*O. mykiss*	Burgu et al. (1988)
Tripartiella sp.	*O. mykiss*	Altunay (2006); Altunay and Yıldız (2008)
Chilodonella cyprinid	*O. aureus*	Cengizler and Can (1999)
	O. niloticus	Cengizler and Sarıhan (1992); Cengizler and Can (1999)
	S. galileus	Cengizler and Sarıhan (1992)
	T. rendalli	Cengizler and Sarıhan (1992)
	T. zilli	Cengizler and Sarıhan (1992)
	O. mykiss	Burgu et al. (1988); Soylu (1996); Öğüt and Akyol (2007)
Chilodonella sp.	*C. idella*	Uzbilek and Yıldız (2002)
	O. mykiss	Altunay (2006); AltunayandYıldız (2008); Özer et al. (2010); Balta et al. (2019)
Eimeria truttae	*O. mykiss*	Sağlam and Pala (2008)

*: reference added after Innal and Stavrescu-Bedivan, 2022

3.2. Metazoan parasites

Metazoan parasites are primitive multicellular parasitic animals with tissues and organs. In general, parasitic infestations in this group are associated with low water quality and poor fish health management practices. Various metazoan groups such as acanthocephalans, cestodes, helminths, leeches, monogeneans (trematodes), nematodes and small parasitic crustaceans were reported in parasitic infestation cases of farmed fish. These pathogens generally cause gill infestations and damages on the eye and internal organs with secondary effects such as starvation due to attachment in the buccal cavity and inflammation in infested fishes. In addition, the parasites in this group provide suitable portals for the entry of bacteria through the holes they make in the fish skin (Bruno et al., 2006; Özer, 2019).

Monogeneans generally live attached to the fish gills and skin of the fish they infest. Diseases caused by the members of this group, especially by *Dactylogyrus* sp. and *Gyrodactylus* sp. results in high mortality in farmed fish. Parasites such as helminth, cestode and nematode groups can cause high mortality in infected fish due to tissue damage and irritation, obstruction of the digestive tract and damage to intestinal functions (Bruno et al., 2006; Dezfuli et al., 2021). Disease reports caused by metazoan parasites in cultured freshwater fish in Türkiye were given in Table 3.

Table 3. Metazoan parasites observed in cultured freshwater fish in Türkiye (Modified from İnnal and Stavrescu-Bedivan, 2022)

Pathogen	Host fish species	Reference
Dactylogyrus sphyrna	*O. mykiss*	İnce and Uslu (2017)
	O. niloticus	Cengizler and Can (1999)
Dactylogyrus sp.	*C. idella*	Uzbilek and Yıldız (2002)
	O. mykiss	Burgu et al (1988); Ogut and Akyol (2007)
Ligula intestinalis	*C. idella*	Uzbilek and Yıldız (2002)
Schyzocotyle acheilognathi	*C. idella*	Uzbilek and Yıldız (2002)
Clinostomum complanatum	*O. mykiss*	Burgu et al (1988)
Crepidostomum farionis	*O. mykiss*	Dörücü (2000)
Diplostomum sp.	*O. mykiss*	Avsever et al. (2016), *Sözeren Çevrimel and Soylu (2017)
Hysterothylacium aduncum	*O. mykiss*	Pekmezci and Umur (2015)
Schulmanela petruschewskii	*O. mykiss*	Pekmezci and Umur (2015)
Argulus foliaceus	*O. mykiss*	Öktener and Ünal (2020)
Lernaea cyprinacea	*O. mykiss*	Tokşen et al. (2014); *Ürku and Önalan (2018); *Şimşek and Aldemir (2020)
Lernaea sp.	*O. mykiss*	*Şimşek and Aldemir (2020)
Pomphorhynchus laevis (Acanthocephalan)	*O. mykiss*	Sözeren Çevrimel and Soylu (2017)

*: reference added after Innal and Stavrescu-Bedivan, 2022

4. Fungal Diseases

Fungi are simple microorganisms that are commonly found in nature and live parasitically on humans, animals and plants. The majority of them are saprophytic, as they can obtain the nutrients they need from the inanimate environment (Deacon, 2005). Only a few fungal species cause pathogenicity in fish (Roberts, 1989; Roy and Yanong, 2003).

4.1. Saprolegniasis

Saprolegniasis is a term commonly used to describe a fungal disease of the skin, gills and fish eggs. *Saprolegnia* species are fungi in the Oomycota class, the most important of these

fungi in freshwater with their septaless and densely branching mycelium structures. Outbreaks of waterborne fungal infestation (Saprolegniasis) in fish cause significant losses mostly in fish eggs and in breeding brood fish (Çelikkale, 1988). Saprolegniasis may be a secondary infection among other infections. Physical injuries on the fish body are the main target for *Saprolegnia* infection to settle. It forms cotton-like piles on dead eggs. Factors such as poor water quality and high stocking density make fish susceptible to fungal infections in bacterial, viral and parasitic diseases, and in this case, *Saprolegnia* also attacks the muscle tissue (Roberts, 1989; Roy and Yanong, 2003).

Diler and Timur (1995), reported the presence of *Saprolegnia diclina, S. ferax*, *S. glomerata*, *S. litoralis, S. terrestris* and *Saprolegnia* sp. in fungal fish disease cases in Türkiye. While Kayış et al. (2017) reported the presence of *Saprolegnia* sp. in a sturgeon hatchery, Özçelik et al. (2020) studied on the *S. parasitica* infestations in rainbow trout hatcheries.

4.2. *Candida* infections

Candida is a yeast genus that causes fungal disease in humans and animals and the most commonly observed species of this genus are *Candida albicans* and C. *dubliniensis* (Deacon, 2005). Candida representatives very rarely cause diseases in fishes. Metin et al. (2019) reported that the white-colored structures in the gill tissue of diseased cultured trout were caused by *Candida* sp.

4.3. *Aspergillus* infections

The genus *Aspergillus* consists of common molds suspended in soil and water, vegetation, feces, decaying matter, and air (Deacon, 2005). Özcan and Barış (2020) isolated *Aspergillus flavus* and *A. niger* from cultured trout in Türkiye.

4.4. Other fungal infections of freshwater fishes

In nature, various fungi are present in living or non-living plants, in different environments such as forests, grasslands and polluted waters (Deacon, 2005). Various fungal species such as *Paecilomyces marquandi, P. lilacinus, Phoma sorghina, Phoma glomerata, Mucor circinelloides* and *Rhizopus microsporus*, as well as *Cladosporium* sp., were isolated from cultured trout in Türkiye (Özcan and Barış, 2020).

5. Conclusions

While a great number of bacterial species are harmless and many of them are even beneficial to their hosts, some bacteria that are called (opportunistic or obligate) pathogenic bacteria have the potential to cause diseases by taking advantage of occasional weakness in the host (e.g., injury and/or insufficient immune capacity). Sniezko Circle explains this as the pathogenic diseases can occur in fish with a lowered immunity when the pathogen is present in the environment with unwanted conditions (Plumb and Hanson, 2006). As listed above, during the development of aquaculture in Türkiye during the last 50 years, the production amount and the number of species cultured and the aquaculture sites showed a diversity. Simultaneously, with the rise of the population, agriculture and industrial activities also showed a great increase, which can also affect water resources. With the effect of all these factors, the pathogens affecting cultured freshwater fishes also showed great diversity over time. During the early stages of fish health studies in Türkiye, the main aim was to diagnose the pathogens with routine laboratory methods. Later, newly introduced methods, which will be discussed in another section of this book, were used for diagnosis at the academic level. Also, academic and commercial vaccination and treatment studies were initiated. Today the focus of academia and commercial fish health services is the diagnosis and identification of pathogens with novel molecular techniques, characterization of the pathogens, reducing the use of antimicrobial chemicals and prevention studies via probiotics.

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PART II

TRENDS IN FISH NUTRITION

CHAPTER 6

CURRENT RESEARCH IN FISH NUTRITION

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DOI: 10.26650/B/LS32LS24.2024.005.006

1. Introduction

Fish nutrition is an important area of research that has gained significant attention in recent years due to the increasing demand for fish products and the need for sustainable aquaculture practices. With the growing population and limited resources, it has become crucial to optimize fish nutrition and maximize their growth, health, and quality while minimizing environmental impact.

The proper nutrition of fish is essential for their growth, survival, and overall health, as well as the economic success of the aquaculture industry. The study of fish nutrition has evolved from the early identification of nutrient requirements to the current focus on optimizing feeding strategies to improve growth rates, reduce feed costs, and minimize the environmental impact of aquaculture (Hardy et al., 2022). Fish nutrition research has encompassed a range of disciplines,

including animal nutrition, biochemistry, physiology, and genetics. These disciplines have been used to investigate the nutritional requirements of various fish species, develop and evaluate diets, and understand the mechanisms underlying nutrient metabolism and utilization in fish.

Research in fish nutrition in Europe began in the 19th century when researchers focused on determining the nutrient requirements of different fish species, which involved conducting feeding trials to assess the effects of different levels of nutrients on fish growth and survival (Jobling, 2016). These studies have led to the development of commercial aquaculture feed (aquafeed) that provide the necessary nutrients for optimal growth and health. In the early 20th century, research focused on understanding the nutritional requirements of different fish species (Hardy et al., 2022), leading to the development of specialized feeds for different fish species. The discovery of vitamins and other micronutrients in the mid-20th century led to the development of more sophisticated and nutritionally balanced feeds.

Traditionally, fish meal (FM) and fish oil (FO) have been the main protein and lipid sources respectively, in aquafeeds. FM contains a well-balanced protein and amino acid (AA), which are highly digestible and palatable to cultured species, without any antinutritional factors. Undefattened FM has some oil fraction rich in highly unsaturated fatty acids (HUFAs, C ≥ 20 and $n \geq 3$). FO on the other hand has a unique fatty acid (FA) composition, with roughly equal amounts of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and HUFAs. There are few if any ingredients commercially available today that can match the unique composition and attributes of dietary FM and FO in aquafeeds. Increasing demand for FM and FO, as well as a relatively static supply and continuous growth in intensive fish farming and competition from other animal producing sectors and the pet feed industry, have resulted in increasing prices. Hence, there is a concerted research effort from scientists and other stakeholders in the aquaculture and aquafeed industry to find alternative dietary ingredients.

Recent research in fish nutrition is focused on developing sustainable aquaculture practices that are economically viable, environmentally friendly, and socially acceptable (Rombenso et al., 2022). With the growing demand for fish as a source of food, researchers have been developing feeds and feeding strategies that optimize fish growth and health while improving the efficiency of nutrient utilization and reduce the environmental footprint of aquaculture. These studies have also focused on understanding the effects of different feed ingredients on fish growth, health, and flesh. This includes research into alternative and/or novel sources, mainly plant-based proteins and oils, to reduce the reliance on fishmeal and FO. Additionally, researchers are investigating the use of novel feed additives, such as probiotics and prebiotics, to enhance fish health and disease resistance.

This book chapter aims to provide an overview of present research in juvenile fish nutrition, including the latest developments in fish feeding, nutrient requirements, feed ingredients, and feed additives. This chapter also includes studies carried out by authors from Türkiye.

2. Nutrient Requirements

Fish are heterotrophic animals that require a diverse range of nutrients to maintain their physiological processes, such as growth, reproduction, and immunity. Fish nutrition research has encompassed animal nutrition, biochemistry, physiology, and genetics. These have been used to investigate the nutritional requirements of various fish species, develop and evaluate diets, and understand the mechanisms underlying nutrient metabolism and utilization in fish. Nutrient requirements vary widely among different fish species, life stages, and environmental conditions. Hence, variations in nutrients requirements among classes should be expected between warmwater and coldwater species, freshwater and marine species, and finfish and crustaceans (Nates, 2016). The most common nutrients required by fish include protein, lipids, carbohydrates, minerals, and vitamins.

The proper balance of these nutrients in the diet is crucial for the growth and development of fish, as well as for their resistance to diseases and stress. Mostly, these requirements are rough approximations of the optimum levels of nutrients needed in practical diets to grow aquaculture species to harvestable size and are fundamental in formulating diets that are productive and cheap for commercial aquaculture (Nates, 2016). Nutrient requirements of a known species can be approximated in formulating diets for closely related species of unknown dietary requirements. It is crucial to know the nutritional requirements for optimal growth of the species as well as formulating a balanced diet. Improper dietary protein and energy levels increases the cost of production and often leads to deterioration in water quality. Insufficient dietary energy level causes protein waste, which can reduce the water quality. Understanding the nutrient requirements of fish is essential for developing balanced and effective fish diets.

2.1. Dietary protein and AA requirements

Proteins and AAs constitute the most critical nutrient for fish, as they are required for growth and repair of tissues. Fish require high-quality protein in their diet, which contains all the essential AAs. Protein deficiency can result in slow growth, weak immune system, and reduced resistance to stress and disease. Proteins constitute the major building block and the most expensive component of feeds.

Like terrestrial animals, aquatic organisms do not have a dietary requirement for proteins per se. Instead, they require a well-balanced blend of essential and nonessential AAs obtained

from digestion of dietary proteins (Wilson and Halver, 1986). Fin and shellfish have a relatively higher dietary protein content compared to land animal feeds (NRC, 2011). When dietary protein level is too low, fish or shrimp cannot sustain their normal physiological functions, with poor growth and health. Excess dietary protein intake does not necessarily lead to more growth but leads to increased nitrogen losses, even decreased growth, as well as increasing the environmental footprint. It is important to supply adequate levels of proteins in the diets of finfish and shellfish with the right mix of essential and nonessential AA in order to promote good growth, development and good health as well as reduce nitrogenous losses.

Dietary protein content is dependent on the species and their life stages. Available data on dietary protein requirement are mostly for juveniles and on-growing fin and shellfishes. The requirements published by various authors range from 24% to 62% and even as high as 70% (Mai et al., 2022). Protein requirements range for larval fishes ranged from 25% to 60% while 20% to 60% for on-growing ones.

Aquafeed containing optimal balance of essential and nonessential AAs and sufficient protein content is a prerequisite for improving the utilization of AAs for growth as well as reducing nitrogen excretion. This is particularly important for carnivorous species that prefer using proteins as an energy source.

Practical and commercial aquafeeds produced in Türkiye meet the protein and AA requirements of most salmonids and carnivorous fish species.

2.2. Dietary lipid and fatty acid requirements

Lipids are utilized in fish diets as a main energy source to spare proteins, which have led to the formulation, production and use of high-lipid diets in the aquaculture industry. As a rich energy source, lipids supply 8–9 kcal/g, almost double that supplied by either carbohydrate or protein (Chuang, 1990), and are required for maintaining cell membranes and nerve function. Dietary lipids are important for fish growth and development, supply EFAs, and are vectors for fat-soluble vitamins. Contrawise, insufficient or excess dietary lipids may be deleterious to fish growth and immunity (Li et al., 2012; Lu et al., 2013; Sáez-Royuela et al., 2015). Lipids also influence the flavor and texture of prepared diets and fillet quality (Stickney and Hardy, 1989). The exact lipid requirement in aquafeeds is not practical as it is defined by the metabolic interactions among protein, lipid, and carbohydrate (Sargent et al., 2002). In general, dietary lipid content of about 10–20% (dry weight) is sufficient for fish growth without excess lipid deposition in tissues (Cowey and Sargent, 1979; Watanabe, 1982; Sargent et al., 1989). However, marine carnivores as well as salmonids have lipid requirements of 15-22%

dry weight of the diet (Vergara and Jauncey, 1993; Vergara et al., 1996, 1999; Santinha et al., 1999) for optimum growth and development.

Fish naturally have a high HUFA content and consequently require these fatty acids (Sargent et al., 1997). The HUFAs, ARA (arachidonic acid, 20:4*n*−6), EPA (eicosapentaenoic acid, 20:5*n*−3) and DHA (docosahexaenoic acid, 22:6*n*−3) provide a range of benefits including the maintenance of normal cell functions in fish when there is the decline in water temperature (Sargent et al., 1997, 1999). All animals including fish have absolute dietary requirements for essential fatty acid (EFA). Different fish species have different physiology and metabolism and require different EFA levels.

Freshwater fish are capable of converting both *n*−3 and *n*−6 PUFA to their more highly unsaturated FA (Glencross, 2009; Köse Reis et al., 2023). Studies so far have revealed that the EFA requirements for freshwater species can generally be met by α-linolenic acid (18:3*n*−3, ALA) and linoleic acid (18:2*n*-6, LA) (Sargent et al., 2002). The dietary FA requirements for these species are 0.3–2.0% ALA and 0.5–1.0% LA of dry diet (Bell and Koppe, 2011), although some freshwater fish can have elevated levels of LA in their diets (Sargent et al., 1997; Smith et al., 2004). However, a supply of HUFA in the range of 0.2–1.0% of dry diet is enough to overcome requirement for ALA and LA (Bell and Koppe, 2011).

Marine fish have very limited capability for converting short-chain PUFA to HUFA (Ofori-Mensah et al., 2020a,b). Marine fish have HUFA requirement in the range of 0.4–3.7% of dry diet (Bell and Koppe, 2011), and particularly require 1-2% each of EPA and DHA in diet (NRC, 2011). For marine fish larvae, the ratio of DHA to EPA of ≈ 2 has been shown to be sufficient (Sargent et al., 1999). Larvae and early juveniles of sea bream and sea bass require dietary HUFA in the range of 1.5-5.5% (ratio of DHA to EPA ranging from 0.3-2%) (Takeuchi and Watanabe, 1977). Older juvenile require 0.9 to 1.9% n-3 HUFA of their diet with DHA:EPA ranging from 0.5 to 1 (Kalogeropulos et al., 1992; Ibeas et al., 1994, 1997).

Aquaculture scientists and fish nutritionists in Türkiye have been following these recommendations in formulating practical diets for experimental purposes and for commercial diets for fish farms.

2.3. Dietary carbohydrate requirements

Carbohydrates are a cheap energy source and their dietary inclusion allow amino acids and lipids to be spared from being used in energy production, which enables proteins and lipids to be used for growth and the biosynthesis of important compounds (Wilson, 1994; Stone,

2003). This is due to a lack of ability to digest and regulate plasma glucose concentrations (Zainuddin and Aslamyah, 2014). Fish can obtain their energy from other sources, such as proteins and lipids. However, carbohydrates can be used to reduce the cost of aquafeeds, and they can improve the texture and palatability of the feed.

Unlike terrestrial animals, aquatic organisms including fish and shrimp have limited ability to make use of them (Shiau, 1997). Hence, dietary carbohydrate levels in aquafeed should be kept low, as high levels can lead to digestive problems and reduced growth (Hemre et al., 2002; Zhou et al., 2015).

The ability of aquatic organisms to use dietary carbohydrates is closely associated with their natural feeding habits, position on the trophic level, inclusion level, composition of carbohydrates, and technological treatment, which alters the gelatinization degree of starch (Kaushik et al., 2022). Accordingly, herbivores and omnivores are able to tolerate diets with relatively high carbohydrates levels (40-60%) (Wilson, 1994; Hemre et al., 2002; Enes et al., 2009; Polakof et al., 2012). Contrawise, fish of high trophic levels, such as salmonids and most marine carnivores, have limited tolerance for inclusion levels greater than 20% (Gümüş and İkiz, 2009; Zhou et al., 2015; Castro et al., 2016).

Aquaculture scientists, fish nutritionists and feed formulators from Türkiye prepare and manufacture practical and/or commercial diets with dietary content of carbohydrate less than 20%. This is because most of the species cultured in Türkiye are carnivorous.

2.4. Dietary vitamins requirement

Vitamins are essential micronutrients that are required in small quantities by fish. Fish require different water and lipid soluble vitamins for various physiological functions. Vitamins included in aquafeeds are usually in the form of a premix. Unlike carbohydrates, proteins, and fats, they do not yield energy but are important cofactors for many cellular processes and pathways (Mai et al., 2022), and are key for fish growth, immunity, cell membrane integrity, endocrine system operation, and reproduction. Aquafeed should contain adequate levels of vitamins, and their deficiency may present several health challenges, such as poor growth, reduced immunity, and eye and skin disorders. Vitamin premixes are formulated to meet or exceed the dietary requirements of aquatic organisms at recommended levels of inclusion in aquafeeds without considering any contributions to vitamin requirements by feed, and are usually included in feeds at 0.5 - 2% (Hardy and Brezas, 2022).

Fish nutritionists and feed formulators in Türkiye use a premix of vitamins that satisfy the needs of the species grown locally.

2.5. Dietary minerals requirement

Minerals are essential micronutrients required by fish for various physiological functions, such as skeletal development, osmoregulation, and enzyme activation (Lall, 2022). Fish require different minerals, such as calcium, phosphorus, magnesium, sodium, and potassium, in their diet. Fish feed should contain sufficient levels of minerals, as inadequate dietary levels may result in several health issues, such as skeletal deformities, reduced growth, and poor reproductive performance. Dietary mineral supplementation varies with diet formulation and mineral content of fish rearing water, and are usually included as premixes. Mineral premixes contain concentrates of essential compounds that provide both macro and trace minerals in diets to make up for low levels in the formulation (Hardy and Brezas, 2022).

Practical and commercial diets from Türkiye are prepared using mineral premix, which meet the nutritional needs of the cultured species.

2.6. Feed ingredients

Dietary ingredients that are nutritious, locally available, cheap and have consistent supply that can be included in fish diets are important to the nutrition of fish. Availability and utilization of such feed ingredients have the added advantage of contributing to the sustainable growth in aquaculture production with less environmental impact. The evaluation of the nutritional content and levels is key to the development of aquafeeds.

Aquafeed ingredients can be broadly categorized under proteins (amino acid), energy sources (lipids and carbohydrates), vitamins, minerals as well as additives to boost physical properties, palatability, or preservation of the feed or to promote growth, pigmentation, or sexual development and maturation of the organism (Hardy, 2000).

In Türkiye, aquafeeds are manufactured using dietary ingredients produced locally, with increased substitution of FM and FO with alternatives from terrestrial origin.

3. Feed Formulation and Production Technology

3.1. Feed formulation

Feed is the largest single cost item in commercial fish farming and may account for up to 60% of the total cost of production. Any reduction in the cost of feed that can be made, however small it may be, can drastically cut down the total cost and increase returns. The aquafeed industry is responsible for converting raw feed ingredients into feeds. These feeds must be relatively affordable to farmers as well as supply the needed nutrition for good growth, normal development, and health, and have the least negative impact on the environment. Adopting

alternative ingredients that are locally available for the formulation of aquafeeds is a logical step for the aquaculture farms to remain profitable (Nates, 2016).

The nutrient content and balance of the ingredients used in producing the feeds influence feed utilization and, hereby, the growth and normal development of aquaculture species. Major producers of the ingredients commonly used in producing aquafeeds have databases that contain the nutritional content of the various ingredients. Major aquafeed producers have an inventory of the ingredients (and the nutrient content of each of these ingredients) included in the feed formulations. Formulating aquaculture feeds requires the inclusion of several feed ingredients since these ingredients have significant nutrient and functional restrictions, which may inhibit their inclusion at high levels in the diets of aquatic organisms.

Prior to the middle of the 20th century, feed formulation was by trial and error, which aimed at producing feeds that supported fish growth, maintained rearing water quality, and prevented clinical signs of nutritional deficiencies (Hardy et al., 2022). This made use of simultaneous equations to solve simple feed formulations once ingredients have been chosen. Currently, there are improved models and computer softwares used in formulating aquafeeds, essentially reducing the time needed for calculation of ration formulation. The immense effort from academia and commercial sectors, coupled with pressure from regulators have led to the formulation and production of modern diets with the ability of maximizing production performance, facilitating immune resilience, and achieving a tailored edible product, while also meeting acceptable social licenses and minimizing the environmental footprint (Rombenso et al., 2022).

Modern compounded aquafeeds are sophisticated, specifically engineered blend of dietary ingredients that adequately meets the nutritional needs of the organism for proper growth, development and well-being. These include feed meals, oils, and concentrates that supply the required macronutrients and premixes and specialty products that are included as sources of minerals, vitamins, pigments, binding agents, and other ingredients based on the rationale for preparing the feed. Due to this, feed formulators, aquaculture scientists and/or fish nutritionists are expected to have an in-depth knowledge of the nutritional status of the ingredients available to them and to recognise a blend of ingredients that satisfy the dietary needs of target species and can be processed to the desired pellet specifications at the lowest cost.

3.2. Feed production technology

This has the objective to convert a homogenous blend of ingredients into feeds with longer shelf life that have characteristics that make them suited to feeding fish or shrimp in water (Hardy and Brezas, 2022).

3.2.1. Compressed pellets

These diets are produced by forcing a homogeneous feed mixture through holes in a rotating metal die by means of a static roller located inside the die. The force involved generates heat that gelatinizes starch and compresses the mixture into pellets. As the feed emerge from the rotating die, a static adjustable knife cuts it into desired pellet length, mostly 1–2 times pellet diameter. The combination of heat, moisture, and pressure compresses the feed mixture as it exits the die.

The lipid content of the ingredient mixture should range between 3 and 11% to lubricate the openings in the die and decrease dustiness. The moisture level is critical as inadequate or excess level in the mixture reduces pellet hardness, affecting pellet quality. Pellets made with inadequate moisture are dry and easily break, while excess moisture restricts compression of the feed mixture, which leads to the production of soft pellets. Changes in atmospheric humidity and/or the rate at which the mixture is presented into the steam chamber can alter the quality of pellet. Hence, aquafeeds produced in this way are influenced by factors such as lipid level, moisture, and humidity in the feed mixture.

In Türkiye, this technology is mostly used by scientists and research laboratories in universities to produce practical diets for research purposes. Small and private aquaculture farms that prefer to manufacture their own aquafeeds also use this technology.

3.2.2. Extruded diets

This is prevalent technology used to produce aquafeeds currently. The process involves three main steps: conditioning, extrusion, and cooling/drying. During conditioning, steam is added to a chamber in which the feed mixture is being agitated using counter rotating augers. The feed mixture is heated by the steam and friction associated with mechanical force, reaching 125–150˚C with a moisture content of 20–24% (Hardy and Brezas, 2022), which initiate starch gelatinization. The mixture is worked to a dough-like consistency and then forced into a long, tapered barrel with steam ports containing an auger with increasingly tight flights. The feed is extruded through the die as long noodles that are cut to desired length by a rotating knife on the exterior of the die. As the feed mixture exits the die, high pressure is released, that instantly converts moisture to water vapor, creating small air pockets to form within the pellets. Pellets immediately pass through a dryer on a moving belt where they are exposed to forced, heated air. After cooling and drying, the air pockets in the pellet enable it to float or slowly sink in water, depending on conditions under which the extruder is operated. Proper combinations of ingredients to include starch and to limit fat in the mixture produce water-stable, hard pellets.

This technology is used by commercial aquafeed factories in Türkiye to produce diets for fry, juvenile, on-growing, adult and broodfish.

3.2.3. Cold extrusion

Modified pasta-type extruders are used to manufacture semi-moist pellets. By varying the moisture content and through careful selection of ingredients with binding activity, stable moist pellet can be produced. Moist pellet extrusion does not involve heating the mixture with steam or other means prior to extrusion, instead the mixture is forced through a plate containing tapered holes to compress the feed mixture. This means that sanitation standards throughout the process must be very high to prevent microbial contamination. The resulting products are cut into desired lengths by an external rotating knife as they exit the die. Subsequently, the pellets are screened, "quick-frozen," bagged, and stored. Moist pellets can be kept at room temperature if feeds contain ingredients that lower water activity and prevent mold growth. Such pellets are typically packed in foil bags that are flushed with N_2 to eliminate O_2 and then sealed. This reduces the chance of lipid oxidation in the pellets.

With this technology, the ingredients and their limits are chosen based on the water content and water activity. Pellet binders, such as pre-gelatinized potato starch, must be added at quantities that provide adequate binding capacity to produce a high-quality pellet. Additional binders must be included in formulations containing moisture levels over 30%, such as those containing liquefied or ground fish products.

This technology is used in producing diets for broodstock in government research institutes and large aquaculture farms that prefer to produce their own diets in Türkiye.

3.4. Fish feeding

Fish feeding is a critical aspect of fish nutrition as it directly affects their growth, survival, and health. Researchers have been investigating different feeding strategies and technologies to improve fish feed utilization, reduce waste and environmental impact, and enhance fish performance. For example, studies have shown that feeding fish with multiple small meals throughout the day can improve feed efficiency, reduce feed wastage, and enhance fish growth. Additionally, researchers are exploring the use of automated feeding systems that can adjust feeding rates and timing based on fish behavior and environmental conditions, thus optimizing feeding efficiency and reducing labor costs.

4. Recent Advances in Fish Nutrition Research Since 2010

Fish nutritionists have for several decades endeavored to formulate fish diets that support or boost growth of cultured fish as well as control costs, mostly focusing on minimizing the dependence on scarce marine resources (Turchini et al., 2019). Variations in the feed ingredients and aquafeed formulations used in preparing aquaculture diets globally are very high. These variations are due to the target species, age and/or developmental stage, size, producer, farming methods as well as the target markets. However, when it comes to dietary protein and lipid sources, modern aquafeeds generally contain relatively small quantities of FM and FO, typically from 0% to 30% of the dietary ingredients. Such research efforts have already started paying off as the dependence on marine ingredients in aquafeed formulations has reduced, especially in the culture of salmonids (Turchini et al., 2019).

4.1. Protein

Proteins are polymers of amino acids arranged into the structure of many polypeptides, with critical physiological functions. Proteins are large complex organic nitrogenous molecules. They can be found in the cells of all living organisms, and are essential for growth and maintenance of life. Over tens of thousands of different proteins are produced by organisms with each one having a specific structure, function, and a unique amino acid sequence (Mai et al., 2022). The protein used in the formula must be balanced in amino acid composition with respect to the needs of the species. The optimal dietary protein level in fish is generally dependent on the fish species, its physiological state (age and size), protein quality, environmental parameters and feeding level.

Traditionally, FM has been the main protein in aquafeed due to its palatability, high digestiblity, unique amino acid composition and ability to supply the required protein and/or amino acid requirements of most species under culture. However, increasing demand, limited supply and continuous growth in intensive farming of aquatic organisms, have led to variable but generally increasing prices. This issue has been worsened by the surge in competition from other animal producing sectors. There is a concerted effort to minimize the over dependence on FM in aquafeed formulations due to the considerable economic incentive. Hence, researchers and fish nutritionists, for the past decades, have worked on replacing FM either totally or partially with complementing and/or novel ingredients.

Earlier studies into replacing FM in aquafeeds produced good results in salmonids when plant proteins were used (Ytrestøyl et al., 2015). On the contrary, similar formulations in the diets of marine carnivorous species produced poor growth (Takagi et al., 2006; Lunger et al.,

2007; Takagi et al., 2008; Sarker et al., 2012). The discovery of the importance of taurine and its subsequent inclusion in the diets of these marine fish species yielded positive results (Lunger et al., 2007; Chatzifotis et al., 2008). Hence, soy concentrates, casein, gluten and other plant proteins can be included in the diets of aquatic organisms, especially freshwater fish, at high levels without compromising growth once these diets are fortified with minerals and vitamins (Nates, 2016).

Over the last decade, research on novel ingredients, such as blood meal (Hussain et al., 2011; Aladetohum and Sogbesan, 2013), hydrolysed feather meal (Yu et al., 2020), poultry by-product meal (Hekmatpour et al., 2019; Karapanagiotidis et al., 2019), microalgae (Madeira et al., 2017; Shah et al., 2018; Yarnold et al., 2019; Valente et al., 2021), single cell proteins from fungi and bacteria (Blomqvist et al., 2018; Glencross et al., 2020; Jones et al., 2020; Agboola et al., 2021), macroalgae (review by Aragão et al., 2022), and insect meals (Nogales-Mérida et al., 2019; Liland et al., 2021; Alfiko et al., 2022; Tran et al., 2022; Wethasinghe et al., 2022), has proliferated and will continue to expand. These studies reported on the effects of the alternative and/or novel ingredients on growth performance, feed utilization, and whole-body amino acid profile, and protein pattern in the fillet, digestive enzyme activities and histology of the digestive system, mostly in juvenile and on-growing fishes.

Researchers in Türkiye have also been working on reducing dietary reliance on FM in aquafeeds by developing more sustainable and cost-effective feed formulations by incorporating locally available feed ingredients, such as red lentil meal (Yürüten Özdemir and Yıldız, 2019), feather meal (Damir, 2023), poultry by-products (Damir, 2023) and black soldier fly prepupae (Ozturk et al., 2023). Studies have shown that these feed ingredients can replace a significant portion of fishmeal and reduce the cost of feed without compromising the growth and health of fish.

4.2. Lipid

Lipids are a group of natural organic compounds comprising fats, oils, phospholipids, and sterols. Lipids and fatty acids are major organic constituents of living organisms, providing metabolic energy required for growth, movement, reproduction and migration (Turchini et al., 2022).

It is an established fact that fish just like all animals require the essential fatty acids (EFA), especially the HUFAs. These include EPA, DHA, and ARA, which are abundant in FO. These HUFA are needed by fish for optimal growth and good flesh quality and as such sufficient amounts of these must be provided in their nutrition in captivity. FO, produced from wild

fisheries, has traditionally been the principal fat and energy source in aquafeed formulation (Turchini et al., 2019) with an estimated consumption of 88.5% of the global supply of FO in 2006 (Tacon and Metian, 2008). However, the production of FO has been decreasing and therefore the price of this feed ingredient has been increasing (Shepherd and Jackson, 2013; Boyd, 2015). The reliance on a scarce feed ingredient like FO will limit production of fed aquaculture species in the future. Consequently, the continuous growth in global aquaculture production together with dearth of raw materials from capture fisheries, environmental and economic concerns over the use of FO, have led to a soaring need to replace FO.

In the recent decade, extensive research has been devoted to alternative and reliable lipid sources to replace FO in aquafeeds. These include vegetable oils (VO) (Tocher et al., 2011; Eroldoğan et al., 2013; Hixson et al., 2014a,b,c; Castro et al., 2016; Yıldız et al., 2018; Ofori-Mensah et al., 2020a,b, 2022a,b), animal fats (Trushenski et al., 2011; Nogales-Mérida et al., 2011; Friesen et al., 2013; Gause and Trushenski, 2013; Emery et al., 2016), alternative oils from the marine environment (krill, copepod and amphipod oils) (Miller et al., 2011; Olsen et al., 2011), single celled organisms (Miller et al., 2011; Tibbetts et al., 2017), microalgae (Tibbetts et al., 2020), by-products of aquaculture and wild capture fisheries (Tocher et al., 2019; Hamilton et al., 2020), and insects (Dumas et al., 2018). Among these, VOs have received the further attention due to their increased output, especially of seed oils, price stability and sustainability (Turchini et al., 2009). Such investigations have examined the effects of VOs and their blends on growth and feed efficiency, fillet fatty acid profile, welfare, reproduction and physiology of cultured organisms. Recent results have shown that FO can be totally replaced with VOs in diets of freshwater fish and salmonids (Arslan et al., 2012; Hixson et al., 2014a,b; Yıldız et al., 2018). However, marine carnivores require marine resources to constitute 15%–20% of dry matter for similar levels of dietary replacement (Eroldoğan et al., 2013; Hixson et al., 2014c; Betancor et al., 2016; Ofori-Mensah et al., 2020a,b).

Another area of research interest in the inclusion of alternative dietary oils is the ability of fish to use them to supply their energy needs as well as bioconvert these precursor 18–C PUFA into HUFA *via* the *de novo* pathway. It is known that dietary fatty acid composition as well as trophic level modulate the activity of enzymes, which affects the biological conversion of fatty acids in cultured fish species. These enzymes are in-charge of the biosynthesis of HUFAs in the *n*–3 and *n*–6 series from precursor 18–C PUFA (Torstensen and Tocher, 2011; Thanuthong et al., 2011). A variety of genes such as fatty acid elongases, desaturases and oxidases are involved in the bioconversion of dietary precursors into their respective HUFAs. Majority of the genes encrypting the enzymes for the *de novo* synthesis of dietary FAs

have been cloned from diverse freshwater and marine teleosts (Hastings et al., 2001; Agaba et al., 2004; Zheng et al., 2004; Tocher et al., 2006). Unlike the herbivores, carnivorous fish have minimal extent of fatty acid bioconversion although they may express active elongases, Δ-6 and Δ-5 desaturases (Eroldoğan et al., 2013; Hixson et al., 2014c; Betancor et al., 2016; Ofori-Mensah et al., 2020 a,b).

The effect of alternative lipid on the molecular and metabolic responses in fish is another research area in fish nutrition. These studies have reported that dietary lipids can modulate molecular response by regulating the mRNA response and the activity of key enzymes involved in lipid metabolism (Morais et al., 2011, 2012a,b; Thanuthong et al., 2011; Benedito-Palos et al., 2014, 2016; Betancor et al., 2016; Castro et al., 2016; Houston et al., 2017; Jin et al., 2017; Torno et al., 2018). For example, dietary inclusion of *n*–3 and *n*–6 18–C PUFA were reported to upregulate lipases, enzymatic activities of desaturase, elongase, fatty acid synthetase, in aquatic organisms.

Authors from Türkiye have been active in the area of lipid nutrition by publishing manuscripts in high reputable journals. Some of the studies regarding effect of alternative oils focused on on growth performance and fatty acid profile (Arslan et al., 2009; Arslan et al., 2012; Eroldoğan et al., 2013, 2018; Yildiz et al., 2018; Ofori-Mensah et al., 2020a,b, 2022b), histology of the digestive system (Ofori-Mensah et al., 2022b), fate of ingested fatty acids (Eroldoğan et al., 2013; Ofori-Mensah et al., 2020a), molecular and metabolic responses (Ofori-Mensah et al., 2020a,b, 2022b; Köse Reis et al., 2023), and organoleptic property and quality of the final product (Ofori-Mensah et al., 2022a). On the other hand, a study (Arslan et al., 2013) focused on the dietary lipid level and its effect on growth, FA composition, digestive enzymes activities, and mineral content.

4.3. Carbohydrates

Carbohydrates cover a large number of neutral chemical compounds, formed of carbon, oxygen, and hydrogen atoms in proportions, and almost always correspond to the empirical formula $(CH_20)n$, where $n \geq 3$. As a group, carbohydrates are made up of the sugars, polyhydroxy aldehydes, ketones, alcohols and acids, their simple derivatives, and any compound that may be hydrolyzed to these (Kaushik et al., 2022). Carbohydrates are not essential macronutrients as the form which they can be utilized by most fish, glucose, can be synthetized *in vivo* from non-glucose precursors (lactate, AAs), and are underutilized as energy source by carnivorous fish.

Plant materials have high carbohydrate content, composed of digestible (such as starch) and non-digestible polysaccharides (such as fibres) carbohydrates (NRC, 2011).

The right proportion of carbohydrate in aquafeeds can have protein and lipid sparing effects as well as providing substrates for biological syntheses (Arnesen and Krogdahl, 1993; Wilson, 1994). It is known that carbohydrates in the form of glucose could be converted to fat and vice versa in tissues *via* gluconeogenesis in fish. Recent research has shown that overabundance of dietary carbohydrates may lead to surplus fat retentions, impaired immune response, and declining health (Gao et al., 2010; Li et al., 2012; Polakof et al., 2012). Consequently, establishing the optimum relative levels is quintessential to aquaculture as dietary lipid and carbohydrate can impact assimilation and use of one another, inadvertently affecting growth and other metabolic processes (Li et al., 2014; Miao et al., 2016). Recent studies now focus on the effects of varying dietary carbohydrate and lipid contents on the molecular and metabolic responses in fish (Kamalam et al., 2012, 2013; Castro et al., 2016; Li et al., 2019).

In 2009, authors from Akdeniz University investigated the impacts of dietary lipid and carbohydrate contents on growth, proximate composition and digestibility in rainbow trout (Gümüş and İkiz, 2009). By feeding high lipid and carbohydrate diets, Türkoğlu et al. (2022) evaluated the relationship between anxiety and obesity, histopathological and immunohistochemical analysis in the brain tissues, the molecular responses related to lipid and carbohydrate metabolisms in zebrafish.

4.4. Functional feed additives

Aquafeeds are formulated with a wide variety of ingredients intended to meet the nutritional needs of target species so that the species can grow, survive and perform their normal physiological functions. Modern diets for aquaculture species are formulated to contain feed additives that are non-nutritive but perform functions such as improving the quality of the feed and feed efficiency, as well as promoting growth, immune response, and health performance in species fed such diets. These functional ingredients include the following.

4.4.1. Phytogenics

Phytogenics, also known as plant-based feed additives, represent a relatively young class of additives that have gained significant attention in animal nutrition. Derived from various parts of plants, such as herbs, spices, and essential oils, phytogenics are incorporated into animal feed to enhance animal performance and overall health.

The use of phytogenics as feed additives stems from their natural bioactive compounds, which possess a range of beneficial properties. These compounds include essential oils, tan-

nins, flavonoids, saponins, and many others, each with unique physiological effects on animals. Phytogenics have the potential to elicit various effects on animals, including increased appetite stimulation, antimicrobial activity, direct modulation of intestinal bacteria, stimulation of gastric juices, boosting the immune system, and possession of anti-inflammatory and antioxidant properties (Chakraborty and Hancz, 2011; Chakraborty et al., 2014; Reverter et al., 2014). Phytogenics offer several advantages over traditional feed additives, such as antibiotics and synthetic growth promoters, which have faced increasing scrutiny due to concerns about antibiotic resistance and environmental impacts.

Researchers from Türkiye have conducted studies in aquatic animals fed diets supplemented with extracts from plants. Yilmaz et al. (2016) reported that dietary inclusion of thyme, rosemary or fenugreek in commercial diets improved some hematological, immunological and biochemical status in sea bass. Dietary inclusion of blackberry syrup (Yilmaz, 2019) and walnut leaf extracts (Yilmaz et al., 2023) enhanced haemato-immune responses, fish immune parameters, antioxidant status, as well as survival rate in Nile tilapia. Diler et al. (2021) reported that administration of sumac powder through diet had a positive impact on the growth performance, intestinal tissue morphology, and antioxidant enzyme stimulation in rainbow trout.

4.4.2. Organic acids

Organic acids, including acetic, butyric, citric, formic, lactic, propionic, malic, and sorbic acids, along with their salts, have been employed as acidifying agents in animal feed formulations (NRC, 2011). These compounds represent a promising feed additive for enhancing gut health and optimizing animal performance. They are commercially available in various forms, primarily as adsorbates or salts (FEFANA, 2014).

The beneficial effects of inclusion of organic acids in the diets of aquatic organisms can vary based on factors such as the specific fish species, size or age of the fish, type and dosage of organic acids or salts used, composition and nutrient profile of the experimental diets, buffering capacity of dietary ingredients, culture and feeding practices, and water quality conditions (FEFANA, 2014). Notably, positive outcomes regarding the digestibility of proteins, amino acids, and lipids have been observed in rainbow trout (Morken et al., 2011) and Nile tilapia (Liebert et al., 2010) when organic acids were incorporated in their diets.

4.4.3. Yeast and its derivatives

The inclusion of yeast has become more prominent in the animal feed industry due to its exceptional nutritional composition and widespread availability. At present, there is a notable

surge of interest in incorporating yeast and its derivatives into aquafeeds as a functional feed additive. This includes the use of probiotic live yeast, yeast fractions such as yeast cell walls and yeast extracts, as well as the utilization of yeast as a source for the production of purified products (prebiotics) such as mannan-oligosaccharides (MOS) and β-glucans.

The most extensively commercialized species, commonly recognized as baker's yeast, is *Saccharomyces cerevisiae*. The inclusion of yeast and its products in diets has been shown to boost growth, enhance survival rates, facilitate gut maturation, and improve the immune and antioxidant systems in fish and shrimp. The effect of yeast and its products on animals is dependent on the form (whole yeast, autolyzed yeast) in which they are administered, processing (mechanical or chemical disruption) and method of delivery (Encarnação, 2016).

Yeast components at the cellular level can offer valuable non-nutritive compounds that have the potential to promote fish health. These include mannan oligosaccharides (MOS), glucose polymers (β-glucans), chitin, and nucleic acids (Rumsey et al., 1992). The application of yeast β-glucans in aquaculture has been explored as immune-stimulants to modulate the innate immune system of fish and shrimp, ultimately enhancing their survival (Welker et al., 2012; Meena et al., 2013; Bai et al., 2014).

4.4.4. Probiotics

Probiotics refer to live microbial feed supplements that exert beneficial effects on the host animal by enhancing the balance of intestinal microorganisms. They achieve this by producing inhibitory compounds, such as lactoferrin, lysozyme, and bacteriocins, which combat pathogens. Probiotics also compete for essential nutrients and adhesion sites, inhibit the expression of virulence genes or disrupt quorum sensing, provide essential nutrients and enzymes to enhance nutrition, modulate interactions with the environment, and contribute to the development of favorable immune responses (Merrifield et al., 2010).

Among the probiotics, lactic acid bacteria (e.g., *Lactobacillus spp*., *Pediococcus spp*., *Enterococcus spp*.) and *Bacillus spp*. have been extensively utilized. These probiotics have been documented to improve the growth and/or nutrient utilization of aquatic organisms (Merrifield et al., 2010; Avella et al., 2011), while also bolstering their resistance against pathogenic bacteria. (Merrifield et al., 2010; Ringø et al., 2010; Ai et al., 2011; Merrifield and Carnevali, 2014).

The addition of enzyme-producing probiotics resulted in enhanced activity of digestive enzymes (such as lipase, proteases, and amylase) and improved feed utilization in gilthead sea bream larvae (Suzer et al., 2008). Çelik et al. (2018) reported increased serum glucose whereas LDH and ALP were decreased in rainbow trout fed diets supplemented with Bacillus subtilis.

4.4.5. Prebiotics

Prebiotics are a substance resistant to the acidic environment in the stomach, can be fermented by gut microbiota and can promote the growth of gut microbiota to improve host health (Davani-Davari et al., 2019). They can also be any compound, substrate, long chain sugar, nutrient, or fibre that serves as food or energy to the beneficial microorganisms in a host digestive system (Mountzouris, 2022; Tran and Li, 2022). Prebiotics are mostly dietary carbohydrates that are non-digestible in the upper gastrointestinal tract but provide energy to probiotics in the gut to enhance an organism's health. Dietary inclusion of prebiotics have been reported to stimulate the growth of gut probiotics (Ringø et al., 2010; Sanders et al., 2019; Rohani et al., 2021).

Prebiotics commonly used in aquaculture include β-glucan, inulin, arabinoxylan oligosaccharide, MOS, galactooligosaccharide, fructooligosaccharides and oligosaccharides, which were reported to increase feed utilization efficiency in rainbow trout (Shoaei et al., 2015), promote growth performance in juvenile chu's croaker (Li et al., 2019), and enhance immune system in Pacific white shrimp (Li et al., 2021). Additionally, prebiotics have been reported to stimulate disease resistance in aquatic organisms (Abdel-Latif et al., 2022; Li et al., 2018; Baumgärtner et al., 2022; Yılmaz et al., 2022).

The incorporation of β-glucans into the diet as feed additives has been found to stimulate the immune response in aquatic organisms (Welker et al., 2012). Multiple studies have demonstrated the immune effects of β-glucans, particularly in terms of antibody production, expression of immune system genes, survival rates, resistance to infectious diseases, and improved stress resistance (Meena et al., 2013).

MOS are non-digestible carbohydrates that are derived through the partial hydrolysis of mannans. MOS can expel harmful bacteria by binding to them (Dimitroglou et al., 2010). Consequently, MOS promotes the growth of bacterial populations associated with a healthy and well-functioning gut. Inclusion of MOS in the diets of aquatic organism has resulted in enhanced growth performance, feed efficiency, immune response, increased gut absorption surface area, and enhanced activities of specific digestive enzymes (Dimitroglou et al., 2010; Torrecillas et al., 2011; Gelibolu et al., 2018a,b).

In Türkiye, Şahan and Duman (2010) reported non-specific immune response in Nile tilapia fed diets supplemented with β-glucan. Gültepe et al. (2011, 2012) reported improved growth, feed utilization and digestibility in gilthead seabream fed diets containing MOS. Gelibolu et al. (2018a, b) reported higher survival and significant increase in live weight and protein efficiency rates with the dietary inclusion of MOS in gilthead seabream. It was reported in rainbow trout that nucleotide (Özlüer-Hunt et al., 2016) and prebiotic inulin (Özlüer-Hunt et al., 2019) supplemented diets led to significantly better activities of digestive enzymes and antioxidants and immune responses.

4.4.6. Enzymes

Increased inclusion of feed ingredients of plant origin has increased the dietary content of anti-nutritional factors, which negatively affect the absorption and bio-availability of nutrients in monogastric animals such as fish. Plants contain a significant proportion (up to 80%) of phosphorus in the form of phytate, which has the ability to form chelates with various mineral cations (such as K, Mg, Ca, Zn, Fe, and Cu) and complexes with proteins and amino acids. As a result, the presence of phytate reduces the bioavailability of other minerals and the digestibility of proteins (Morales et al., 2016).

Dietary inclusion of exogenous enzymes has been shown to inhibit antinutritional factors. This inturn improves nutrient bioavailability, which then enhances growth performance in fish (Kumar et al., 2012; Maas et al., 2018). Although a number of microbial/fungal enzyme products are available on the market, phytase has received the widest application in aquaculture (Encarnação, 2016). Dietary phytase supplementation in Atlantic salmon (Carter and Sajjadi, 2011), crucian carp (Nie et al., 2017) and Nile tilapia (Nwanna and Olusola, 2014; Abo-Norag et al., 2018) significantly improved whole-body dry matter composition, crude protein content, ether extracts, ash content, carbohydrate content, calcium and P content.

Researchers from Suleyman Demirel University (Isparta, Türkiye) reported inclusion of cellulase enzyme to canola meal-based diets showed no significant differences in growth performance in Nile tilapia after 90 days of feeding (Yiğit and Ölmez, 2011). Yiğit et al. (2018) documented that incorporation of phytase and protease to a soybean meal-based diet did not increase growth or nutrient digestibility in rainbow trout after 12 weeks of feeding. However, in a masters thesis submitted to Istanbul University, Kaiza (2020) reported improved growth performance in juvenile rainbow trout fed diets high in plant-based proteins supplemented with phytase, xylanase and monocalcium phosphate for 90 days.

4.4.7. Mycotoxin binders

Mycotoxins are harmful secondary metabolites produced by diverse fungi, capable of contaminating crops and animal feeds, including aquafeeds. These toxins can have a range of deleterious effects on aquatic animal health, including reduced growth rates, impaired immune function, increased susceptibility to infectious diseases, and even mortality. In addition, the

presence of mycotoxins can adversely affect the quality and safety of aquaculture products as well, potentially posing a risk to human health. To mitigate the negative effects of mycotoxins in aquaculture, various strategies have been developed, including the use of mycotoxin binders in aquafeeds.

Mycotoxin binders are substances that can be added to aquafeed to mitigate or alleviate the detrimental effects of mycotoxins, which are toxic substances produced by certain types of fungi. They bind to mycotoxins in the gastrointestinal tract of aquatic animals, preventing their absorption into the bloodstream and reducing their negative effects on animal health. These binders can be of natural or synthetic origin, and they are commonly added to aquafeeds to protect fish and shrimp from mycotoxin exposure.

Several types of mycotoxin binders have been studied in aquaculture, including clays, activated carbon, zeolites, and chitin and chitosan derivatives. These binders work by different mechanisms, such as physical adsorption, chemical reaction, and ion exchange. Extensive evidence has demonstrated the efficacy of mycotoxin binders in mitigating the toxicity associated with diverse mycotoxins such as aflatoxins, ochratoxins, zearalenone, and deoxynivalenol, in aquafeeds (Hassan et al., 2010; García-Pérez et al., 2013; Selim et al., 2014; Jin et al., 2018 Gonçalves et al., 2018; Yang et al., 2020; Zhang et al., 2020). These studies have shown that mycotoxin binders can effectively reduce the negative effects of mycotoxin exposure in various aquaculture species.

5. Conclusion

Research in fish nutrition has come a long way since its inception in the 19th century. From understanding the essential nutrients required for fish growth to developing specialized feeds for different fish species, research has played a critical role in the development of sustainable aquaculture practices. Fish nutrition is a dynamic and rapidly evolving field of research that plays a crucial role in the sustainability and profitability of aquaculture. The proper nutrition of fish is essential for their growth, survival, and overall health. The evolution of research in fish nutrition has led to the development of commercial fish feeds and the optimization of feeding strategies to improve growth rates, and reduce feed costs. Researchers are continuously exploring new strategies, technologies, and ingredients to optimize fish nutrition, reduce environmental impact, and enhance fish health and quality. The integration of multidisciplinary approaches such as genetics, physiology, and ecology is essential for developing innovative and sustainable fish nutrition solutions that can meet the increasing demand for fish products while preserving the environment.

Researchers from Türkiye have contributed to the advancement in fish nutrition and have kept up with their colleagues in Europe and North America. Most of the studies published from these authors are on rainbow trout, gilthead seabream and European seabass. However, research in the area of aquaculture nutrigenomics in Türkiye is quite a new field, hence, few studies have been published by researchers from Türkiye.

The future of research in fish nutrition will be focused on developing more sustainable and environmentally friendly aquaculture practices that can meet the growing demand for fish as a source of food. Additionally, advances in genetics and biotechnology will allow researchers to develop fish strains that are more resistant to diseases and have improved growth rates.

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CHAPTER 7

ALTERNATIVE PROTEIN SOURCES IN AQUAFEEDS

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DOI: 10.26650/B/LS32LS24.2024.005.007

1. Introduction

Global aquaculture production has reached almost 122 million tons that 57.5 tons of which is classified as total farmed finfish production level in 2021 according to FAO Fishery statistics (FAO, 2022). Farmed fish and crustacean species totally rely on the properly manufactured nutritionally balanced protein rich aqua feeds in order to reach market size rapidly and feed cost comprises of almost 60 to 70% of all the farm expenditures globally. Although fishmeal produced by wild catch is considered as the best possible protein source in aqua feeds because of its excellent amino acid composition and high amount of vitamins and minerals, its availability is not expected to increase in the future due to the status of finiteness of wild fish stocks and their strict management to prevent overfishing (Tacon and Metian, 2008; FAO, 2022). Therefore, sustainable farming of aquatic animal species necessitates the production

of aqua feeds using economically viable sustainable feed ingredients specifically the protein sources alternative to fishmeal.

Myriads of alternatives that are in animal and plant origin and other potential sources like single cell protein and insect meals have been considered to replace fishmeal in aqua feeds (Gatlin et al., 2007). However, several inherited characteristics regarding to their imbalanced amino acid profiles, poor digestibility and palatability and the presence of Anti-Nutritional Factors (ANFs) such as trypsin inhibitors, phytate and non-starch polysaccharides restricts the use of these protein sources in aqua feeds (NRC, 2011). Nevertheless, animal-by product meals produced from the wastes of meat and fish processing factories are traditionally considered as best possible sustainable fish meal replacer in aqua feeds specifically for carnivorous species since they have high protein and lipid content and presents no issues with palatability for fish (Robinson and Li, 1999; Psofakis et al., 2021;Sharma et al., 2021; Lee et al., 2023). Plant protein meals however appears to be more problematic in terms of not only the presence of low amounts of essential nutrient but also the comparatively numerous ANFs per unit weight to animal by-product meals (Prabhu et al., 2019; Fountoulaki et al., 2022). Furthermore, lysine and methionine are the two essential amino acids that are categorically imbalanced in almost all of the plant protein meals compared to that of those in animal protein sources. Amino acids are the building block of living organisms and need to be supplied in a balanced fashion with sufficient amounts for a healthy growth and physiological functions to occur. Phytate phosphorous is a predominant type of phosphorous source in plant proteins and has no bioavailability in monogastric animals including fish. Moreover, indigestible fraction of the total carbohydrates namely non-starch polysaccharides represent a considerable part of the chemical composition of plant meals and lower the digestible energy content of raw seed meals. Previously the lignin was demonstrated to significantly lower the nitrogen digestibility in rainbow trout fed experimental diets containing 300 g/kg diet of 76 cultivar of lupin meal (*Lupinus angustifolius*) (Glencross et al., 2008). Single cell protein meals and insect meals are considered as two best possible sustainable protein sources for aqua feeds. Single cell ingredients are relatively broad class of products that encompasses mostly bacterial, fungal and micro algal derived protein meals or PUFA rich oil source specifically produced from microalgae (Glencross et al., 2020). Insects as potential protein source in aqua feeds has gained momentum in recent years for not only having a good source of essential amino acids and fatty acids and vitamins and minerals but also for their ability of growing and reproducing quickly and easily on low-quality organic waste and manure labeling them the most environmentally sustainable protein source for farm animals (van Huis et al., 2013; Bruni et al., 2018).

Several strategies have been used to improve the nutrient composition of alternative protein sources specifically the plant proteins. Dietary enzyme and probiotic supplementation and microbial fermentation of plant meals before incorporating into the diets have all been demonstrated to be useful methods in increasing the bioavailability of NSP's (Non-Starch Polysaccharides) as an energy source and phytate-P to monogastric animals including fish without compromising growth and intestinal integrity (Yamamoto et al., 2010; Collins et al., 2018; Hulefeld et al., 2018; Kumar et al., 2020; Lee et al., 2020; Picoli et al., 2022). Traditionally oil seeds are extracted to produce oil, the quality, and the chemical composition of the remaining press cake and meals are mainly affected by the genotype and the growing conditions of oil seeds as well as the extraction method used during the process (Li et al., 2023). Generally, it is considered that mechanical force during oil extraction decrease some of the ANF's and make NSP's more susceptible to hydrolysis by enzymes (Stone et al., 2003). In addition, the protein and energy digestibility values of spray-dried blood meals in fish were demonstrated to be significantly increased to the same meals produced by rotoplate, steam tube and ring drying techniques (Bureau et al., 1999; Xu et al., 2022).

This chapter intend to outline the most up to date information about the fishmeal alternatives in aqua feeds specifically in reference to their nutrient composition and usability in diets for farmed fish and crustaceans. Furthermore, the research targeting the improvements in nutrient composition of alternative animal and plant meals and press cakes using novel technology and equipment is highlighted in the chapter. The contribution by Turkish university labs and institutions in above research subjects is also acknowledged throughout the text.

2. Animal By-Products

Animal by-products contain all types of slaughterhouse and meat packaging wastes generated during the processing of terrestrial farm animals for human consumption. These wastes mainly include cattle, sheep and porcine blood meals, scraps of meat and meat and bone meals, poultry by-product meals containing skin, internal organs, crest and feet and feather meals. These raw materials are refined as dry powder using several techniques for drying, compression and lipid removal before available for aqua feed and other mono-gastric animal feed production (Sharma et al., 2021). Animal by-product meals are considered as best FM replacer in aqua feeds because of their high crude protein, fat and vitamin and mineral content for fish. No reported ANFs in animal by-product meals makes these meals even more valuable candidates for dietary fishmeal replacement in aqua feeds. However, these protein sources should be critically evaluated for an estimate of protein digestibility

using *in vitro* enzyme assays since they contain high levels of bone, feather and connective tissues. BSE (Bovine Spongiform Encephalopathy) has also been a major concern of meat packaging by-product meals produced specifically from cattle being used in feeds of monogastric animals including fish for almost three decades. The latest lifting of the ban imposed on all cattle and porcine meat packaging by-product meals by the EU is also expected to increase their use as sustainable protein sources in feed formulations for non-ruminant farm animals and fish. Some of the latest information regarding to the fishmeal replacement levels by various animal by-products in farmed finfish and crustaceans in Türkiye and around the world is summarized in Table 1.

2.1. Meat and meat and bone meals

Meat meal and meat and bone meals are the rendered products arising from mammalian (mainly beef, pork or lamb) meat packaging and should not contain added blood, hair, hoof, horn, hide trimmings, manure and stomach and rumen contents. The tissue and bone fractions with tissue are subject to the dry rendering process in which preground raw material is cooked by dry heat (at appx. 135-140°C) without no steam and hot water added in a steam jacketed cooker until the moisture is evaporated (Li et al., 2008). The sterilization of the meal is achieved during the cooking process according to the requirement of hygiene standards in meat by-product meals outlined in EU-Directive 90/667/EEC in Europe (Hertrampf and Piedad-Pascual, 2000). Following cooking and sterilization and sieving of pulp, de-oiling is carried out by draining off and the screw press of the remaining meal. Compared to meat meal, meat and bone meal is characterized with much higher crude ash content since it has the added variable bone material depending on the product quality assessment criteria of individual meat rendering company. The nutrient composition of meat by-product meals is varied substantially between rendering companies due to the variable ratio of bones and/or fat in the raw material used to produce meals. Therefore high ash and/or fat content in the meals reduces the protein content in the products. In this respect, crude protein content of meat meal is around 55% whereas crude protein varies between 45 to 50% in meat and bone meal (Li et al., 2008). Crude fat content of both meals appear to be around 7 to 9% of their chemical composition. The crude ash content of meat and bone meals are generally 10 to 15% higher than meat meals averaging 33% of the chemical composition. Meat industry by-product meals are commonly characterized for their inferior protein quality to fish meal and whole egg protein since they contain less lysine, isoleucine and methionine+cystine.

2.2. Blood meal

Large quantities of fresh blood is generated in slaughterhouses across the world and small portion of it is used for human consumption and pharmaceutical purposes and the rest is generally discarded due to lack of processing facilities that turn fresh blood into animal feed protein ingredient. However the portion that is discarded has been reported to be as low as 15 to 25% in some countries like Norway that has the infrastructure and the processing technology used for producing blood meal for animal feed (Hertrampf and Piedad-Pascual, 2000). Fresh animal blood is basically dried using three different type of drying process to produce blood meal. Basically fresh blood is spray-dried, ring-dried and cooker-dried in order to produce blood meal (Li et al., 2008). In spray-drying the water from fresh and uncoagulated blood is removed using low temperature evaporator (approximately at 50°C) under the vacuum until the 30 to 50% of solids remaining. Following water removal, solids are spray-dried at higher temperatures reaching 250 to 300°C. The large proportion of water in fresh blood in ring drying process is, however, removed by centrifugation and then transferred to rotating ring where very high temperatures at around 400 to 550°C force off the remaining moisture leaving a dry product. Conventional cooker is also utilized to produce blood meals. The duration and the temperature used in the blood meal production process largely defines the quality of the products (Hertrampf and Piedad-Pascual, 2000; Li et al., 2008). Spray-drying is reported to be a better processing technique to produce blood meals because lower temperatures and retention time is used. The crude protein content of blood meals is over 90% but it has very low fat and carbohydrate averaging around 1.2 and 3.3% respectively (NRC, 2011). Even though the essential amino acid variation in blood meal is not as wide as it is seen in other feed protein ingredients, methionine and isoleucine are two limiting amino acids compared to whole chicken egg protein.

2.3. Poultry by-product meal

Poultry by-product meal is the rendered parts of slaughtered poultry that generally includes heads, feet, undeveloped eggs and viscera excluding the feathers. It is also called as poultry offal meal. Rendering of fresh parts is generally achieved using either wet or dry processing techniques. Dry rendering is the most widely used method of producing poultry by-product meals in which raw material is finely chopped to increase the heat penetration and release of fat. During the cooking of raw material the initial temperature is set to 100°C then increased to 125°C gradually until the water evaporates completely. Fat from the remaining slurry is then removed by screw pressing or the solvent extraction methods. Following fat removal, the product is finely ground and packaged for marketing. The biggest concern in

poultry based products is the existence of dangerous pathogens such as salmonella but the higher than 100°C processing temperature and its duration and stringent evaluation of the final product for this pathogen ensures the safety of poultry by-product meals (Hertrampf and Piedad-Pascual, 2000). Proximate composition of poultry by-product meals varies substantially because of much variability in raw poultry cuts used to produce poultry by-product meals. The average crude protein content of poultry by-product meals is around 60% and it is predominantly from connective tissues. Poultry by-product meal is considered an animal protein meal that has one of the best balanced essential amino acid composition. However, it generally contains 0.5% urea and higher than that amount indicates adulteration by indigestible material such as feathers that could substantially lower the protein quality in poultry by-product meals. The fat content varies depending on the type of raw poultry cuts to produce the meal and whole poultry meal contains 2.5 times more crude fat averaging 44% compared to conventionally manufactured meals. Total unsaturated fatty acid content of poultry by-product meal fat almost doubles its total saturated fatty acid contents in which linoleic acid is the highest reaching nearly 17% of all the fatty acids (Hertrampf and Piedad-Pascual, 2000). The crude ash content of poultry by-product meals is generally about 14% and proportional to the amount of bones that goes into offal.

2.4. Feather meal

Feather meal is a by-product of poultry processing plants and very large quantities are produced globally giving the fact that a chicken weighing 2 kg produces 180 g of feathers (Hertrampf and Piedad-Pascual, 2000). Raw feathers contain 90% protein in the form of keratin which is a tough insoluble protein with the strong disulphide bonding lowering the protein digestibility around 5% in untreated feather meals. However, several processing techniques such as hydrolysing or autoclaving or treatment with sodium hydroxide (0.25% NaOH) and fermenting of feathers with a bacterial culture of *Bacillus licheniformis* or treating with bacterial kinase and even the gamma ray irradiation are all suggested in literature for the improvement of protein digestibility of feathers (Grazziotin et al., 2006; Campos et al., 2017; Psofakis et al., 2021; Ren et al., 2020; Herath and Yakupitiyage, 2022). Hydrolysing is actually a process whereby feathers are pressure cooked to partially denature some compounds keeping the properties of protein intact. Except hydrolyzation, all other methods to improve the nutrient quality of feathers are largely based on the data obtained *in vivo* animal and *in vitro* laboratory experimentations and therefore considered as uneconomical. The quality of feather meal is closely related to the duration of hydrolyzation because too much autoclaving generally will produce overcooked meal with a lower protein quality. Following cooking,

feather meal is dried at 60°C and finely ground and packaged. Histidine, lysine, methionine and tryptophan are limiting essential amino acids in processed feather meals and their amount is, on a certain extent, related to the level of pressure being used during the cooking process. High pressure, bigger than 207 kPa, has been reported to cysteine being partly destroyed and converted to lanthionine (Robbins and Bauer, 1980). Fat content of feather meal averages around 3.5% and the variation is generally between 1.5 and 5%. If the fat content exceeds 5% in the feather meal, it indicates contamination with skin tissue in the product.

2.5. Milk by-product meals

Several milk industry by-products including dried whey, casein and dried skim milk are considered as useful ingredients for fish feeds (Hardy and Barrows, 2002). Of all three, casein is the most widely used milk by-product in fish diets because of its very high protein content compared to the other two. Casein is the protein of milk and basically the skimmed milk derived from full fat milk after separation of butterfat constitutes its composition (Hertrampf and Piedad-Pascual, 2000). Pasteurized skimmed milk is then coagulated with an acid or rennet enzyme to precipitate the casein as casein curd. The curd is washed, dried and ground to produce powdered casein. If the lactose is not removed before drying, brown colored dehydrated casein occurs as a result. Two other type of casein salts are also produced during its manufacture and they are basically named as sodium caseinate and calcium caseinate. Average crude protein content of casein is around 88% of its proximate composition because not less than 78% of the milk total nitrogen content is casein-nitrogen (Hertrampf and Piedad-Pascual, 2000). Casein contains adequate amount of essential amino acids for farmed animals including fish but much variability is expected because of the large geographical differences of dairy farms and wide range of species and breeds used to produce milk. Since the basis of casein is skimmed milk, casein is almost fat free ingredient comprising just 0.7% of its nutrient composition. In this respect, fat soluble vitamins are almost nonexistent whereas water soluble vitamins are available in nutritionally significant amounts in casein. It is rather considered a protein ingredient and generally used in semi purified experimental diet formulations for fish because of its incomplete nutritional profile compared to other protein meals.

3. Fish Processing Waste and By-Product Meals

This category includes all the waste generated from fish and crustacean processing plants that are converted to many fish protein meals including fish meal, fish protein concentrates and solubles and silages and crustacean meal supplements rich in pigment. Conventional fish meals are generally produced from whole small oily fish species such as the herring and

anchovy and sardines caught mainly off the coast of Peru, Chile East Coast of the U.S.A. and Gulf of Mexico and the Nordic countries, Japan and Russia (Hardy and Barrows, 2002; Hardy et al., 2005; Forster, 2008). However, small fish species not destined for human consumption such as sand eels, Norway pout (*Trisopterus esmarki*) and sprat are also used in fish meal production in Denmark (Hertrampf and Piedad-Pascual, 2000). This section will mainly concentrate on the production techniques and nutritional quality of the meals and by-products manufactured using remnants of fish processing plants. Fish meal produced using raw materials remaining after filleting of whole fish is classified as lower quality product due to its lower crude protein and high ash content that basically comes from the muscle tissue attached vertebra and head bones, viscera and fin rays. The manufacturing is similar to conventional fish meal production using whole small fishes and involves the cooking of the raw materials and continuous oil and water separation at the same time during the process before drying and grinding the material into meal form. The fish meal quality is closely related to the temperature being used during cooking as air drying process of the raw material reaching up to 500°C reduces the crude protein and fat content almost 10 and 2% respectively compared to wet reduction process using steam-jacketed cylinder where steam is injected into the cooking material (Hertrampf and Piedad-Pascual, 2000). Protein content of fish meal produced from whole fish varies with the species, season and the latitude where they are caught and is generally reported to be between 63 to 83% of its proximate composition (Tacon, 1993; Hertrampf and Piedad-Pascual, 2000; NRC, 2011). However, fish meal produced from fish waste contains much less crude protein averaging around 50% compared to conventional fish meal. It also contains similar fat but almost twice the amount of ash present in conventional meals. Regardless of its source, fish meal is an excellent source of essential amino acids, fatty acids phospholipids, vitamins and minerals for farmed animals including fish.

The same raw material remaining after fish processing could also be turned into hydrolyzed fish protein concentrate that is specifically characterized as a feed ingredient by its extraordinarily high protein content and the conversion of insoluble fish protein into polypeptides and amino acids through enzymatic or chemical hydrolysis (Forster, 2008). During the manufacturing process, raw materials need to be thoroughly minced in order to increase the surface area for the hydrolysation at which temperature, pH and time are strictly controlled for the maximum production of short-chained polypeptides (Forster, 2008). Following hydrolysation, bones and scales and other indigestible matter are removed first before the application of de-fattening, being concentrated, pasteurizing and spray-drying to the product (Hertrampf and Piedad-Pascual, 2000). The crude protein content of fish hydrolysates are generally very high averaging around 80% with negligible amount of crude fat and fiber. Hydrolysates are also rich source of Sulphur containing amino acids and taurine that is reported to be almost 10 times higher than that of the conventional fish meal produced from anchovies (Picone, 1987).

Fish silage and many other silages are liquid feeds and produced to acid preserve the fish and crustacean waste against putrefaction specifically in tropical regions of the world. The freshest raw material with low fat content is more suitable for silage making than materials with high fat content. Silages are made using organic and inorganic acids as formic and propionic acids are the most commonly used acids in the process. In addition, fermentation of raw materials using molasses (generally between 10 to 12% of the total volume) and lactic acid bacteria (200 million of lactic acid bacteria per m^3 of silage) is another way of making silages and is reported to autolyze and liquefies the raw material in the same way as acid silages as a result of the conversion of sugar into lactic acid (Raa, 1994). The principle behind acid silage making is to lower the pH of the raw material as quickly as to 3.8 to 4 by adding around 2.5% (by weight) of a single or a combination of acids in order the internal proteolytic enzymes to autolyze and break down the tissue structures (Hertrampf and Piedad-Pascual, 2000). When autolysis is complete, the raw fish material liquefies and fat rises to the top whereas bones and undissolved portions remain on the bottom as a sludge. The degree of autolysis depends upon the digestive enzyme activity in the raw material, pH and the temperature and the process is more quick and effective with the material containing the highest amount of gut. Fish processing waste containing no viscera hardly autolyze. The manufacturing process of fish silages is simple and no need to have substantial and specialized investment requirements. Silages could be concentrated by lowering the moisture level from 80 to 50% making the dry matter of the final product averaging between 20 to 48%. Crude protein seem to be highly varied in dry matter (28 to 74%) averaging 58% in most of the silages (Wood et al., 1985; Espe et al., 1992). The nitrogen fraction is composed of short-chained polypeptides, free amino acids, ammonia, non-protein nitrogen compounds and urea that could reach 20 to 30% of the total N content (Hertrampf and Piedad-Pascual, 2000). Fat content of silages is averaging around 16% but hugely varies depending on the fat content of the raw material used. Ash is much higher in silages produced from fish waste compared to the product prepared from whole fish and generally varies between 10 to 19% in dry matter. Essential amino acid content of silages is similar to fish meal but only difference in free amino acids being significantly higher in silages due to the degradation of proteins. The common characteristics of fish hydrolysates, silages and soluble is to have large amount of free amino acids that are considered as the chemo-attractant properties in these protein sources.

Fish soluble is another by-product of fish meal manufacturing and it is basically the liquor part of cooked fish. The solids are removed and the remaining is centrifuged to separate the oil from the press-liquor. Further vacuum evaporation concentrates the product down to 50% solid. This is then de-hydrated by spray-drying and marketed as fish soluble. Crude protein content of fish soluble varies between 60 to 80% in commercial products and much of it is in non-protein nitrogen category. If the product is produced using fish wastes, the ash content is much higher compared to that of fish soluble produced from whole fish. Fish soluble is a rich source of selenium and particularly the solubility of its macro and micro elements is also very high. Furthermore, it is an excellent source of water soluble vitamins.

Table 1. Recent investigations targeting dietary fish meal replacement levels by animal by-products in several farmed fish and crustacean species in Türkiye and around the world based on the growth, growth and some of the associated nutritional physiology parameters

Species	Animal By-product	Opt. Repl. Level (%)	Effect	Reference
Tilapia zillii	Poultry By-product	50	Significantly better growth and nutrient retention efficiencies compared to 100% replacement	Yıldırım et al. (2009)
Cherax quadricarinatus	Poultry and/ or plant protein mixture replacing total fish meal (48%)	100	No significant difference in growth and feed utilization efficiencies	Eroldoğan et al. (2022)
Sparus aurata	Poultry By-Product replacing dietary total fish meal (36%) at 50 and 100% in diets formulated according to balanced AA profile of *Sparus aurata*	100	No significant difference in growth other measured parameters such as haematological, digestive enzymes and liver histology	Sabbagh et al. (2019)
Micropterus salmoides	Spray dried Chicken Blood meal replacing dietary fish meal at 14.3, 28.6, 42.9 and 57.1% levels.	57	No difference in growth but SDCP significantly increased protein and lipid retention along with reduced Malondialdehyde and significantly increased catalase activity	Xu et al. (2022)

Table 1. Continue

Species	Animal By-product	Opt. Repl. Level (%)	Effect	Reference
Sparus aurata	Hydrolyzed Feather meal replacing dietary fish meal at 50 and 100% without AA suppl. and 25 and 50% with Lysine and Methionine suppl.	25	Replacement levels higher than 25% without AA supl. significantly reduced growth and nutrient metabolism but digestive Protease and heamatological parameters were not affected	Psofakis et al. (2021)
Sparus aurata	Meat and Bone Meal replaced dietary fish meal at 50 and 75%	50	Significantly lower growth and increased feed intake observed at 75% replacement along with lower energy retention efficiency	Moutinho et al. (2017)
Anguilla australis australis	Meat Meal replacing dietary fish meal at 42% (23% of fish meal crude protein)	42	Compared to other plant proteins replacing fish meal at same level growth parameters were significantly higher for fish fed MM diet	Engin and Carter, (2005)

4. Plant Proteins

Plant protein sources, specifically the oil extracted cakes and by-products of food and beer and liquor industries, are considered as sustainable and economically viable protein ingredients for aqua feeds globally. However, as it was mentioned before they contain myriads of Anti Nutritional Factors (ANFs) that might seriously hinder the bioavailability of nutrients in monogastric animals including fish. These ANFs are classified according to their effect on certain nutritional physiology or just being toxic itself or non-bioavailable to aquatic animals. Protease inhibitors such as trypsin inhibitors in soybean and most of the pulses is an example to this classification. Phosphorous in plants is another ANF that falls in this classification and exists in the form of myo-inositol-hexakis dihydrogen phosphate known as phytic acid and it is simply non-bioavailable because fish and many other aquatic animals cannot utilize from it due to the absence of internal phytase in their digestive system to hydrolize it (Hua and Bureau, 2010; Greiling, 2019; Engin and Koyuncu, 2023). Phytic acid has also a very high chelating capability with many micro elements such as zinc and iron preventing their absorbance and bioavailability to the fish. Lectins or hemagglutinins are proteins that binds to carbohydrates and more importantly to the cells lining in the gut interfering the absorption of many minerals including calcium, ıron,

phosphorous and zinc and considered another category of ANFs that distrupt the nutrient utilization by the fish. Plant protein sources also contain large amount of oligosaccharides in the form of NSPs (Non-Starch Polysaccharides) that fish cannot effectively utilize as an energy source. However, mechanical treatment and de-hulling of the raw seeds before processing them into feed ingredient lowers the crude fiber content and improves their utilization as an energy source in fish. Herbivorous and omnivorous fish tend to utilize from digestible carbohydrate fraction of plant proteins as energy source better than carnivorous fish species (Wilson, 1994). Plant protein concentrates produced from grains are also available for aqua feeds after much of the non-protein constitute is removed from the seeds during starch production and named as glutens. Soybean protein concentrates are also advocated as fish feed protein ingredient since the most ANFs particularly the heat sensitive ones are removed or inactivated during the manufacturing process (Olli et al., 1994). Plant proteins generally contain crude protein between 20 to 50% on a dry matter basis with low levels of sulphur-containing amino acids. In some of them, tryptophan may also be a limiting essential amino acids. Glutens, on the other hand, contain much higher crude protein compared to the raw or oil extracted version of the seed itself averaging around 60 to 65% on dry matter basis. In pulses, the crude fat content generally varies between 1.5 to 8% on a dry matter basis and is a rich source of mono and polyunsaturated fatty acids (Hertrampf and Piedad-Pascual, 2000). Plant proteins are devoid of HUFAs (Highly Unsaturated Fatty Acids) specifically EPA and DHA which are classified as essential fatty acids for an optimal growth, reproduction and other physiological mechanism. Plant proteins are a rich source of vitamin B_1 and B_2 and nicotinic acid and macro and micro elements. However, palatability of diets containing large amount of plant proteins in aquatic farm animals has always been an important issue to be addressed to replace the conventional fish meal in aqua feed formulations and production. Plant proteins that are considered as fish meal replacer in aqua feeds will be discussed in this section of the review under three sub-headings as pulses, oil-seed meals and grains. Recent information regarding to the dietary replacement levels of fish meal by several below mentioned plant protein sources in some of the important farmed species specifically in Türkiye and around the world is summarized in Table 2.

4.1. Pulses

Pulses are the edible seeds of leguminous crop that belongs to botanical family of *Leguminosae* (Sipas et al., 1997; Hertrampf and Piedad-Pascual, 2000; Venero et al., 2008). Pulses are generally produced in Asian and African continents India being the biggest producer in the world accounting almost 30% of 95 million metric tons of annual global production (Bhat et al., 2022; Mishra et al., 2023). Canada, Australia, Türkiye and Brazil are also important

pulses producing countries. Pulses include beans such as black gram, field bean, green gram, faba and mung beans, peas, lupins, chickpeas and lentils excluding soybean and ground nuts that are exclusively used for oil extraction and therefore will be discussed in details under the oil-seed meals subheading of this review chapter (see 4.2). Before using in animal and aquatic animal feed formulations pulses need to be de-hulled and ground. Like all plant sources, they contain several ANFs and mechanical and other treatments of the raw seeds including soaking in water, toasting and autoclaving before making them into meal alleviate the amount of many ANFs substantially in the final product (Refstie et al., 1998).

Pulses contain crude protein between 20 to 35% generally peas have the lowest but lupins the highest depending between on the species and cultivars (Hertrampf and Piedad-Pascual, 2000). Australian narrow-leafed sweet lupin (*Lupinus angustifolius*) contains crude protein averaging around 32% and a very good source of digestible energy for ruminants with very low level of alkaloids (Sipas et al., 1997). However the high amount of crude fiber and oligosaccharides (a-galactosyl homologues of sucrose) have been considered as the main hurdles of lupin being utilized as an energy source in fish (Glencross et al., 2003; 2008). Peas (*Pisum sativa*) on the other hand contain the lowest amount of crude protein among the pulses averaging around 20% on dry matter basis. Cow peas has the lowest amount of crude fiber among the pulses projected as fish meal replacer in aqua feeds. The crude fat content of pulses vary between 1.5 and 8% of which faba beans has the highest (Hertrampf and Piedad-Pascual, 2000). Pulses are a fair source of phosphorous and contains low levels of calcium.

Feeding value of pulses for carnivorous, omnivorous and herbivorous farmed fish and crustacean species has been reported worldwide agreeing on the treated meals specifically for their ANFs such as ethanolic extraction and supplemented with external enzymes to break their oligosaccharides down to absorbable monosaccharides could be a beneficial way to increase the level of pulses in aqua feed formulations (Viola et al., 1988; Eusebio, 1991; Glencross et al., 2003). Some of the pulses such as Jack beans (*Canavalia ensiformis*) has been demonstrated by Martinez-Palacios et al. (1988) that there is a direct relationship between fish mortality and the increase of jack bean meal in diets in tilapia (*Oreochromis mosambicus*). Besides making their oligosaccharides more digestible to fish, eliminating non-thermal toxic substances by boiling and soaking the beans in 1:1 ethanol-sulfuric acid solution has been reported to increase dietary levels of treated Jack bean to almost 25% without compromising the growth levels (Martinez-Palacios et al., 1988). Some of the research results for dietary fish meal replacement by lupins and peas for some farmed species are summarized in Table 2.

4.2. Oil-seed meals

Oil-seed meals are solvent extracted or mechanically pressed (expeller) cakes of oil bearing seeds produced by annual plants and perennial trees. The cultivation of these plants were basically for oil consumption need for humans and on a lesser extent for industrial applications (Hertrampf and Piedad-Pascual, 2000). The most important group of these oil bearing seeds belong to plant families of legumes and crucifers. Palm and coconut oil extraction industries around the globe also produces large amount of solvent extracted and expeller cakes. Soybean oil extraction by-products are the most utilized feedstuffs in aqua feeds to replace fish meal and named as full fat flakes, defatted soy flakes, soy flour, soybean meal (de-hulled or hulls added), expelled soybean meal, soy protein concentrates and soy protein isolates following several treatments such as solvent extraction, grinding, expelling, toasting and protein and soluble carbohydrate extractions (Brown, 2008). The others are categorized as coconut, cotton seed, ground-nut, linseed, palm kernel, rape seed and canola, safflower, sunflower, sesame and mustard seed meals along with recently developed camelina oil crop for bioenergy production and will be discussed separately for their unique chemical properties and feeding values specifically for fish throughout the text under this subheading;

Soybean (*Glycine max*) is the most important oil bearing seed produced around the world and its by-products are widely used in farmed animal feed formulations including fish. It is a good source of protein averaging around 42 to 53% in the raw heated seed and oil extracted meals respectively (Brown, 2008). Soy protein concentrate and isolates contain much higher crude protein than other soy products averaging around 72 to 92% on a dry matter basis respectively. Regardless of soybean meal types, three amino acids lysine, methionine and threonine are the limiting essential amino acids compared to that of fish meal produced from herring (Brown, 2008). However the purification of soy protein into soy concentrate tend to increase the amino acid concentrations in the final product approximating the lysine and total sulfur containing amino acids 85 and 95% of the concentration found in herring fish meal (Brown, 2008). Full fat soybean meal is a good source of 18:2n-6 constituting 10% of the total fatty acid classes and only 1.4% is made up with 18:3n-3. These are the essential fatty acids required for fish and providing the need for fish is reported (NRC, 2011) to be between 0.5 to 1.7% of the diet, 50% dietary inclusion of full-fat soybean meal could provide the essential fatty acids requirements of fish requiring n-6 fatty acids but almost the significant portion of fatty acids of fish requiring n-3s. The carbohydrate fraction of soy products are often referred as soluble carbohydrates generally comprised of oligosaccharides such as sucrose, raffinose and stachyose (Brown, 2008; Glencross et al., 2012). Raw seed and oil extracted meals of

soybean contains around 27 to 35% NFE (Nitrogen-Free Extract) respectively and compared to other heat sensitive compounds, alcohol-soluble fraction of the total carbohydrates is considered more of an anti-nutrient factor that is decreasing the nutritional value of soybean meals in salmonids (Olli et al., 1994). Like in all plant sources soybean products contains several ANFs including Trypsin inhibitors, phytic acid, saponin, antigenic proteins, goitrogen and soy isoflavones some of which could be inactivated by heat during manufacturing process. Furthermore soy products are deficient of calcium, phosphorous, sodium, chlorine, sulfur, zinc and selenium in terms of concentration in per unit of dry weight compared to that of herring meal (NRC, 2011). The use of fish meal in commercial salmonid diets has been reduced from 65 to 15% over the past three decades and the biggest part of it came from soy products in combination with canola, sunflower and corn and wheat gluten meals (Gatlin et al., 2007; Kumar et al., 2020; Davies et al., 2021). This has been possible because of extensive research undertaken worldwide concentrating on the improvement of nutritional quality of plant proteins including soy products through the application of various novel techniques such as enzyme and fermentation biotechnologies details of which will be discussed under the heading of 'Technology Available to Improve Nutrient Quality of Plant and Animal By-Product Meals' of this review chapter.

Coconut meal is the by-product of oil extraction from the kernel of mature coconut (*Cocus nucifera*) by solvent or mechanically pressing. The crude protein content is around 20% with lysine and methionine are the limiting essential amino acids. Compared to whole chicken egg protein, coconut meal contains only one third of lysine and methionine+cystine exist in chicken egg protein (Hertrampf and Piedad-Pascual, 2000). Expeller cake has more fat than solvent extracted meal averaging around 20%. High crude fiber content is a major issue for feeding fish compared to ruminants and research investigating its use in farmed aquatic species is limited.

Oil bearing seeds of cotton plant (*Gossypum hirsitum*) is generally used for oil extraction after fibrous part is harvested for textile manufacturing industries. Decorticated seeds contain 50% meal and 16% fat along with a high proportion of hulls approximating around 22%. Cotton seed meal and expellers are high in crude protein averaging between 22 to 44% of its proximate composition decorticated meals in both category having the highest protein around 40% compared to that of corticated meals. Lysine and methionine are the two limiting essential amino acids in cotton seed meals. The fact that solvent extraction reduces and the gossypol binds lysine in conventional cotton seed meals, lysine availability is better in the glandless cotton seed meal (Lim and Dominy, 1991). Corticated seed meals contain high amounts of crude fiber almost

twice the amount exist in decorticated meals averaging around 23% (Hertrampf and Piedad-Pascual, 2000). It appears that only decorticated cotton seed meal and expellers are suitable for fish meal and other plant protein replacement in aquatic organisms and glandless varieties must be considered. Most of the research targeting dietary fish meal replacement in aquatic organisms has been conducted with tropical and subtropical omnivorous and herbivorous species of fish and crustaceans and is rather limited (Hassaan et al., 2019).

Ground nut meal is the solvent extracted or mechanically pressed ground nut (*Arachis hypogaea*) for oil production. It contains crude protein between 32 to 46% decorticated meals and expeller having over 40% on a dry matter basis. Lysine, methionine, threonine and tryptophan are even lower compared to that of the soy products let alone to the herring meal. It is a rich source of unsaturated fatty acids and has a very high crude fiber and NFE content. Although it is a protein rich meal, its use in aquaculture diets for fish meal replacement is very limited and investigations conducted in carp and tilapia species suggest up to 25% replacement (Jackson et al., 1982).

Linseed meal is the residual remaining of oil extraction from flax (Linum usitatissimum). Approximately 70% meal and 30% oil constitutes the linseed from flax. It has around 35% crude protein showing similar handicap to ground nut cakes having low level of certain essential amino acids such as lysine existing only 55% of that is available in soybean meal (Hertrampf and Piedad-Pascual, 2000). Fat in the linseed meal is highly unsaturated with low melting point. Crude fiber is not particularly high in linseed meal and expellers averaging around 10%. However NFE is around 35% in both meals and up to 10% of the carbohydrate fractions is made up with mucilage, a sticky water dispersible carbohydrate that is a major concern in fish due its ability to absorb large amount of water and prevent nutrient absorption in the gut. Therefore its use in fish diets is very limited and is suggested around 3 to 7% in omnivorous and herbivorous warm water species such as common carp. Selenium is particularly high in linseed meals and vitamin levels are similar to those of other vegetable oil meals.

Palm kernel meal is a by-product of oil extraction from fruit bunches of palm tree (*Elaesis guineensis*). Oil extraction from palm kernels is a very important business in some parts of Africa and throughout South East Asia particularly in Malaysia and Indonesia (Azizi et al., 2021). Kernels made up only 1/3 of the fruit bunch of palm trees and 36 kg of kernels produce almost equal amount of meal and oil when extracted averaging around 18.4 and 17.6 kg respectively (Boer and Bickel, 1981). The meal itself contains 12 to 14% crude protein, around 6% residual fat and quite high crude fiber between 17 to 20% depending on the farming conditions such as soil conditions, harvest time (Azizi et al., 2021). Palm kernel meal and

expellers are also a good source of manganese. Because of its low crude protein content and very high crude fiber, nutritional value for farmed aquatic species appears to be low but future technological improvements on the nutrient composition may increase its chance of becoming a feed protein ingredient for aqua feeds.

Rape seed meal is a by-product of oil production from rape seed (*Brassica sp*.) and is also named as colza (Hertrampf and Piedad-Pascual, 2000). It is a high protein meal and contains high amounts of deleterious erucic acid and glucosinolates. Crude protein is generally between 35 to 39% in rape seed meal and expellers. Its cultivar created by plant crossbreeding technologies is called canola and contains much lower erucic acid and glucosinolates and other ANFs and considered a better option for fish and soybean meal replacer in aqua feeds (Ngo et al., 2016). However both rape seed and canola meals are quite high in phytic acid preventing their use as a phosphourous source in diets for farmed fish. Like in all plant protein sources, rape/canola meals are also deficient for their lysine and sulfur containing essential amino acids. Rape seed contains an enzyme myrosinase which breaks the glucosinolates into several toxic compounds and these compounds are associated with the impairment of thyroid function in fish (Burel et al., 2001). Crude fiber and the NSPs are another main concern for fish meal replacement particularly farmed carnivorous fish. Nutritional value of canola meals and expellers have been actively testing by aquaculture nutrition scientist around the world after being treated with available technologies for the dietary fish meal replacement in many farmed fish and some of their findings will be outlined in Table 2. (Sajjadi and Carter, 2004; Drew et al., 2007; Ngo et al., 2016; Mohammadi et al., 2020).

Safflower (*Carthamus tinctorius*) is an oil seed plant grown for its oil around the world. The seeds are characterized with having large amount of hulls hampering the use of safflower meal and expellers as feed ingredient in diets of aquatic animals. After hulls are removed, seeds could only yield 20% of meal and 35% of oil. Crude protein content of safflower meals is around 20 to 25% in corticated seed meals but could be as high as 60 to 70% in totally decorticated meals. However total decortication is very costly and very difficult to come by in the commercial market (Hertrampf and Piedad-Pascual, 2000). Lysine and sulfur containing essential amino acids are the limiting amino acids in safflower meals. Very high amount of crude fiber, much of which is composed of cellulose and on a lesser extent indigestible lignin, make only around 3% of total carbohydrate available for energy in monogastric animals. Safflower seed meals also contain quite high levels of phenolic glucosides (0.39 to 1.62% on a dry matter basis) that are responsible for imparting bitter flavor to the meals. The use of safflower meal and expellers as dietary fish meal replacement is very limited in aquatic animals.

Sesame meal is a by-product of sesame (*Sesasum indicum*) grown in tropical and sub-tropical areas of the world for its oil. Compared to other oil bearing seeds, oil content of the seeds are quite high averaging around 52% and rest is meal containing hulls. After decortication, 30% of the seed is a meal that could be used as a protein feed stuff in animal feed formulations including fish (Hertrampf and Piedad-Pascual, 2000). Crude protein content of sesame meals is around between 42 to 45% with good amount of methionine, cysteine and tryptophan. Lysine is a limiting essential amino acids in sesame meals and expellers. Although decortication of the seeds substantially reduces the crude fiber content, it is still high in decorticated meals preventing the effective use as feed ingredient in aquaculture diets. Even though sesame meals are a rich source of calcium, magnesium, phosphorus and trace minerals, bioavailability particularly phosphorus and calcium is low due to high levels of phytin and oxalic acid in the content of the meal. The ash content of sesame meals are quite high compared to that of other oil seed meals. Its use as animal feed ingredient including fish is very limited.

Sunflower meal is the by-product of oil extraction from seeds of sunflower (*Helianthus annuus*) belonging to the botanical family of *Asteraceae*. It is the most widely cultivated oil bearing seed around the temperate regions of the world. Sunflower seeds yield almost 70% of meal after oil extraction and considering the yearly global production of the seed itself, it is the 4th highest produced meal cake around the world after soybean, rape/canola and cotton seed meal averaging around 22 million tons in 2019 (Heuze et al., 2019). Sunflower meal and expeller contain crude protein between 30 to 43% depending on the decortication of the seed (Hertrampf and Piedad-Pascual, 2000). Decorticated seed meals tend to have higher amount of crude protein than that of corticated seeds. Lysine is the limiting essential amino acid in sunflower seed meals but their methionine and arginine levels are higher than soybean meal. Corticated seed meals contain quite high crude fiber averaging around 30% in the dry matter and could be substantially reduced to almost 11 to 12% with decortication of the seeds (Hertrampf and Piedad-Pascual, 2000). Sunflower seed meals are a rich source of phosphorus and potassium as well as nicotinic acid and choline. Vitamin levels are also higher than that of soybean meal. Sunflower seed meal and expellers are generally considered as a dietary soybean oil replacer for warm water omnivorous farmed fish (Shi et al., 2023) but several investigations have also been reported for carnivores fish species such as gilthead and sharpsnout sea bream (Lozano et al., 2007; Mérida et al., 2010)

The oilseed camelina (*Camelina sativa*) is a member of the Cruciferae (Brassicaceae) family, which includes mustards, rapes, broccoli, cabbage, collards, cauliflower and many weeds (Hixson et al., 2015). Camelina plantation was initially projected for its oil being used for biofuels particularly for jet fuel (Mudalkar et al., 2014). Because its oil contains 35 and 15% of total fatty acid classes as alfa-linolenic and linoleic acid respectively and meals after oil extraction is a good source of protein (20 to 30% on a dry matter basis), cold pressed camelina meal containing 10% residual oil has recently been evaluated by several authors for dietary fish meal replacement in farmed fish including salmonids (Hixson et al., 2015; Bulurwell et al., 2016). Residual oil could be further reduced down to 4 to 5% by solvent extraction of press cake meals. Like in all oil seed meals, crude fiber (neutral detergent) content of both meal and expellers is high averaging around 34% along with 3.8 and 0.6% glucosinolates and phytate-P levels respectively (Hixson et al., 2015).

4.3. Grains

Wheat and corn are the most important grains used as a various by-product meals in farm animal feed formulations including fish and crustaceans. Corn and wheat glutens are the by-products of starch production from these grains and contains very high amount of crude protein between 60 and 75% on a dry matter basis (Engin and Carter, 2005). Germ meal and fiber fractions are also produced during the process (Wu et al., 1999). Wheat germ is a by-product meal of wheat milling and contains 25 to 30% crude protein depending on the milling process (Hardy and Barrows, 2002). The high apparent digestibility of dietary protein in corn gluten has made corn gluten being used as dietary fish meal replacer in across all farmed fish diets depending on the size and the nutritional requirements of species. Corn gluten meal contains almost similar crude protein to fishmeal but lysine is the first limiting amino acid in corn gluten meal preventing its dietary substitution for fish in generous amounts (Güroy et al., 2013). Gluten meals are generally considered as best plant protein ingredient for monogastric animals including fish since it is a by-product of starch production through which many ANFs exist in the raw seed are reduced including crude fiber and heat labile compounds (Güroy et al., 2013).

Distiller's dried grains (DDG), distiller's dried grains with soluble (DDGS) and distiller's yeast are the three main by-products of distiller and commercial ethanol production industries (Glencross et al., 2020). The most important grains that are used in these industries are corn, sorghum, wheat and barley and as a by-product distillers dried grain products contain undigested grain components resulting from grain ethanol fermentation (Buenavista et al., 2021). These products are often used in diet formulations of dairy cattle and swine farming. But after the application of enzyme and fermentation technologies and de-hulling and degerming of grains to decrease the indigestible carbohydrate fractions, these products are increasingly being considered as protein ingredients in diets for farmed fish around the world (Øverland et al., 2013; Buenavista et al., 2021; de Macêdo et al., 2023). The crude protein and fiber

content of DDGs are around 30 and 14% on a dry matter basis (Hardy and Barrows, 2022). However crude protein content in DDGSs could be increased up to 40% via further separation of indigestible fiber, coupled with refinement of the dry grinding process of the remaining product (Nazeer et al., 2023). Lysine and methionine are the two limiting amino acids in DDGSs preventing their use without dietary supplementation of these in aqua feeds. DDGS has been reported to be included in aqua feeds between 15% to a maximum 40% depending on the ingredient being replaced, species, size, age of fish, ingredient quality, use of limiting nutrients, culture system, feeding conditions, and protein level of remaining ingredients in the diet (Lim and Yıldırım-Aksoy, 2008; Buenavista et al., 2023).

Table 2. Recent investigations targeting dietary fish meal replacement levels by plant protein sources in several farmed fish and crustacean species in Türkiye and around the world based on the growth, growth parameters and some of the associated nutritional physiology indicators

Species	Plant Protein Source	Opt. Repl. Level (% DM)	Effect	Reference
Oncorhynchus mykiss	30% of Lupin Meal ethanol extracted and supplemented with enzyme	30	Improved growth nitrogen, organic matter and NFE digestibility with processing of raw lupin seed meal	Glencross et al. (2003)
Psetta maeotica	Defatted soybean replacing dietary fish meal at 10 and 20% levels	20	No significant difference was observed in growth nutrient utilization nitrogen loss or retention	Yigit et al. (2010)
Oreochromis niloticus	50% of dietary fish meal crude protein replaced by defatted soy bean meal with with 60, 80 and 100% of fish oil replacement by soy canola and linseed oils	28,4	No significant difference in growth parameters and whole body AA and FA compositions	Uysal and Engin, (2018)
Acipenser gueldenstaedtii	Dietary Soy bean meal and cotton seed meal inclusion at 20 and 40% levels	40 CSM 20 SBM	CSM replacement at both levels significantly improved growth but ALP (Alkaline Phosphatase) levels were significantly lower at 20 and 40% dietary inclusion of SBM and CSM respectively	Emre et al. (2018)

Table 2. Continue

Species	Plant Protein Source	Opt. Repl. Level (% DM)	Effect	Reference
Ctenopharyngodon idellus	Conventional and enzymatically treated Sunflower meal replaced dietary soybean meal at 25, 50, 75, 100 and 75 and 100% levels	Up to 50 SFM 75 ETSFM	Reduced growth, lipid deposition, intestinal health and disorder in microbial community at higher than 50% replacement by SFM but 75% replacement by ETSFM had no negative effect on growth and improved lipid metabolism and intestinal health of carp	Shi et al. (2023)
Pagrus major	De-hulled soybean meal replaced fish meal protein in diets supplemented with Fish soluble, Krill Meal and squid meal at 70, 80, 90 and 100% levels	100 with FS KM and SM supplementation only	Significantly higher growth at 70 and 80% replacement but feed and protein efficiency were not influenced by the treatments	Kader et al. (2012)
Oncorhynchus mykiss	Solvent extracted camelina meal (SECM) and camelina protein concentrate (CPC) replacing dietary fish meal at 6, 12and 18% and 6 and 12% respectively	18 SECM 12 CPC	No significant differences were observed in growth performance, nutrient utilization, carcass proximate composition and hindgut morphology among treatments but 18% supplementation level much higher acclimation period is require for juvenile fish around 1g	Lu et al. (2020)
Pangasianodon hypophthalmus	Corn Gluten Meal replacing dietary fish meal at 20, 40, 60, 80 and 100% levels	25,1	Significantly lower growth and protein growth and protein at 80 and 100% replacement	Güroy et al. (2012)
Scophthalmus maximus	Corn Gluten-Soybean Meal replacing dietary fish meal at a ratio of 58:42 at 15, 30, 45 and 60% replacement level with lysine, taurine and monocalcium phosphate fortification	30	Significant reduction in growth and feed and nutrient utilization parameters at 45 and 60% replacement levels	Sevgili et al. (2015)

Table 2. Continue

Species	Plant Protein Source	Opt. Repl. Level (% DM)	Effect	Reference
Oreochromis niloticus	Canola Meal Dephytinized and treated with methanolic ammonia solution replaced dietary fish meal at 12.5, 25, 37,5 and 50% levels	25	Higher than 25% replacement levels significantly reduced growth and negatively influenced the intestine and liver tissue histology but relative gene expression of antioxidant enzymes increased with increasing supplementation with canola meal	Mohammadi et al. (2020)

5. Single Cell and Other Protein Sources

Single-cell ingredients (SCI) are a relatively wide range of materials that are generally made up with bacterial, fungal (yeast), microalgal-derived products or the combination of all three microbial groups into microbial bioflocs and aggregates (Glencross et al., 2020). By far the microalgal based protein ingredients are the most investigated for fish meal replacement in aqua feeds (Sevgili et al., 2019). However bacterial meals produced from a wide variety of bacterial genera cultured on different substrates are increasingly becoming as feed protein ingredients for replacing fish meal in aquaculture diets (Zheng et al., 2023). These ingredient are broadly classified as single cell oil products (SCO) or single cell protein (SCP). The crude protein content in algal and bacterial meals could reach up to 80% dry basis and generally bacterial meals have the highest amount among SCPs. Crude fat content is also highly varied among SCP reaching up to 20% (Glencross et al., 2020). In terms of essential amino acid composition, bacterial meals show more balanced composition with high levels of methionine compared to micro algal and fungal based protein ingredients (Glencross et al., 2020; Zheng et al., 2023). Carbohydrate levels are substantially higher in fungal and biofloc meals compared to that of microalgal and bacterial meals (Glencross et al., 2020). Bioflocs meals on the other hand are considered as heterogenous mix of each of the other single-cell resources mainly combined with by-products from each other. Recent investigation by Zheng et al., 2023 reported that Methanotroph bacteria meal (MBM) produced by the fermentation of Methylococcus capsulatus could replace up to 30% dietary FM without compromising the growth performance and health of turbot juveniles, but excessive substitution levels at 80 and 100% would hamper antioxidant capacity, liver health and protein metabolism. A study from Türkiye also

investigated the effects of dietary fish meal replacement by yeast based commercial protein feed ingredient and reported that up to 40% fish meal protein could be replaced by Nu-Pro® without compromising on growth rates, feed efficiency or the fillet biochemical composition in the rainbow trout (Hunt et al., 2014). Another study conducted in Türkiye also demonstrated that diets containing biofloc meal supplemented with symbiotic was an effective strategy in mitigating the potential adverse effects of salinity on growth of Nile tilapia improving gut microbiota, body composition and tissue histomorphology (Hersi et al., 2023).

Insect meal is the novel protein source that has recently gained attention to replace fish and other protein meals in aqua feeds. The main reason of high interest existing in insect meal is that insects can be reared quickly and easily on low-cost by-products or organic waste from agriculture and the food industry with almost zero carbon emission compared to that of other protein meals (Moon and Lee, 2015; Bruni et al., 2018). Furthermore they are an excellent source of protein with all the necessary essential amino and fatty acids and trace minerals and particularly two species of insects belonging to order coleoptera (yellow mealworm, *Tenebrio mollitor*) and diptera (Black soldier fly, *Hermetia illucens*) have been investigated as fish meal replacer in diets for some of the important farmed species around the world (Bruni et al., 2018). According to literature, the average protein content of insects varies between 50 and 82% (dry matter, DM) depending on the insect species or on the method of processing the insect (Henry et al., 2015). Amino acid contents are also varied with the insect order that species from Diptera has been reported to have AA composition almost similar to fish meal whereas insects from Coleoptera and Orthoptera had an AA composition similar to that of soybean products with lysine and methionine being limiting EAA (Barroso et al., 2014). Fat content of insect could reach up to 30% on a dry matter basis with the absence of HUFAs such as EPA and DHA that are considered as essential amino acids specifically for marine carnivores farmed fish species. Since the fatty acid contents of organisms are highly dependent upon the FA profile of feed source being offered, insects FA could be tailored with using some of marine waste in combination with other biological waste as substrate for insect feeding (Ogunji et al., 2008). Insects contain high amounts of chitin a polysaccharide which is composed of an unbranched polymer of N-acetylglucosamine (Henry et al., 2015). Chitin comprises of the large amount of natural diets in some of the marine fish such as cobia and does not hamper the nutrient utilization and even increased protein digestibility was observed when fed crab and shrimp meals containing 3 and 10% chitin respectively (Fines and Holt, 2010). However, chitin severely reduced the growth and nutrient digestibility in trout and Nile tilapia fed diets containing 25 and 10% chitin respectively (Lindsay et al., 1984; Shiau and Yu, 1999). A previous investigation by (Sanchez-Muros et al., 2016) postulated that up to

50% of dietary inclusion of *Tenebrio mollitor* meal replacing largely the soybean meal in fish/soybean meal control diet significantly lowered the growth but did not affect feed intake, in vitro protein digestibility, muscle amino acid composition or biometric indexes. The muscle fatty acid profile was also significantly altered (Sanchez-Muros et al., 2016).

Krill, *Euphausia superba* and *E. pacifica*, is a major marine biomass increasingly being harvested and processed for dietary supplements to increase palatability and balance AA profile of aqua diets (Hardy and Barrows, 2002). Krill meal contains crude protein between 25 and 55% whereas lipid and ash contents could reach up to 20 and 28% respectively. It is also a rich source of carotenoids. Sustainability of krill production is in question and exploitation of these sources seriously disrupts balance in marine ecosystems. Therefore its use as a main protein ingredient for diets of farmed fish appears limited.

6. Technology Available to Improve Nutrient Quality of Plant and Animal By-Product Meals

Technologies in connection with the improvement of nutrient quality of protein sources alternative to fishmeal has always been the major research topic in aquaculture nutrition. Generally these technologies vary from being only simple mechanical to much advanced and highly know-how driven biotechnological applications involving many other related research disciplines. The main underlying concept behind the application of these technologies to various alternative protein sources is to increase their nutrient bioavailability to farmed aquatic animals. The objective is basically to increase the digestibility of nutrients such as proteins, complex carbohydrates and phosphorous in these animal and plant protein feed ingredients as well as decreasing the levels of other compounds preventing effective nutrient utilization by fish and crustaceans. Spray drying technology appears to be a better option for drying of many animal-by products since protein in general and several essential amino acid digestibility of these sources have been reported (Bureau et al., 1999). In addition several pre-processing techniques such as particle size reduction and hydrolization and even gamma-ray irradiation of feather meals have all been suggested for dietary fish meal replacement in higher levels compared to untreated feather meal in some important farmed fish (Ren et al., 2020; Psofakis et al., 2021; Herath and Yakupitiyage, 2022). The mechanical treatment such as dry roasting jet-sploding, micronisation and extrusion of raw seed or oil extracted meals or the dietary inclusion of enzymes or the fermentation of these meals by microorganisms such as bacteria and yeast to effectively hydrolize certain nutrients and phytate phosphorous in fish is an advancing research area in aquaculture nutrition. The most important applicable technology appears to be the biotechnology producing the enzymes that fish and other aquatic farmed animals do not have in their digestive system intrinsically using microorganisms such as bacteria and yeast. This section of the review will focus mainly on the application of enzyme and fermentation technologies to dietary plant proteins and their use as fish meal replacer in farmed aquatic animals.

6.1. Enzymatic treatments

Dietary inclusion of enzymes in the form of proteases, carbohydrate enzymes and microbial phytases is an active research area in fish nutrition in order to increase the nutrient availability from the feed ingredients specifically in plant origin to the fish as an energy and phosphorus source. Bioavailability of dietary nitrogen and phosphorus is crucially important for the sustainability and environmentally friendly status of aqua farms. Proteases are suggested to counteract the effects of TI (Trypsin inhibitors) and hydrolyzing the macromolecular proteins into smaller peptides more efficiently whereas carbohydrases such as xylanase, β-glucanase, cellulase and amylase are projected to attack fibrous components of plant meals in diets (Denstadli et al., 2011; Kumar et al., 2020). Phytases of different origins are included into diets for the effective hydrolysis of myo-inositol bound P to release and absorbed by guts in aquatic organisms depending on the level of available phosphorus in the diets (Greiner et al., 2019). Recently bioprocessed soybean and other plant protein meals reduced for their ANFs such as TI lectins, oligosaccharides and phytic acid are also becoming more available as commercial feed ingredients.

Ogunkoya et al. (2006) demonstrated that top coating of diets with enzyme mixture containing xylanase, amylase, cellulose, protease and β-glucanase at 1 and 2.5 g/kg activity in trout fed diets in which 0, 10 and 20% of the fishmeal protein is replaced by soybean meal had marginal effects on growth and nutrient retention but significantly reduced solid N and P waste at 2.5 g/kg coating level. Commercial enzyme called RONOZYME® containing enzyme cocktail (β-glucanase, hemicellulose and pectinase) has been tested for its effectiveness to hydrolize NSPs in soybean meal, sunflower cake and rapeseed meal *in vitro* in terms of incubation conditions or pretreatment of ingredients (set at 45°C for 45 minutes*)* and *in vivo* dietary fish meal replacement in trout (Denstadli et al., 2011). The degradation of NSPs was found to be correlated neither with the time nor the temperature. In addition trout growth was not affected most probably due to small carbohydrate polymers during enzyme pretreatment of diets either being excreted or bound to other nutrients thus lowering the absorption specifically at water temperature of 12°C (Denstadli et al., 2011). Furthermore, Lee S A et al. (2020) reported that trout fed completely plant protein based diets not supplemented with inorganic P source but containing advanced Escherichia coli phytase at 500 and 2500 FTU/

kg at two different ambient temperature (11 vs 15°C) retained P around 19 to 29% and 23 to 25% higher compared to that of fish fed diets supplemented with inorganic P source (positive control) and negative control without phytase supplementation respectively.

There are couple of studies conducted by Turkish University labs also investigated the effects of dietary cellulase and phytase inclusion on the growth and N and P discharge of tilapia and trout fed dietary fish meal replaced by canola meal and hazelnut and soybean meal respectively (Yiğit and Ölmez, 2011; Demir and Yılayaz, 2018). Nile tilapia growth performance, nutrient digestibility and body composition was not affected by dietary enzyme supplementation level but fish fed the diets containing high amount of canola meal had significantly lower growth parameters and FCR compared to that of fish fed fish and soybean meal control and low level of canola meal diet (41.7 g/kg diet) (Yiğit and Ölmez, 2011). Demir and Yılayaz (2020) was also able to demonstrate that dietary phytase supplementation at 1000 FTU/kg diet effectively reduced the particulate P discharge whilst increasing the dissolved P and N discharge in trout fed diets containing hazel nut and soybean meal as main plant protein sources in diets.

6.2. Microbiological treatments

Fermentation of fibrous plant ingredients with one or the combination of microorganisms such as bacteria and yeast before incorporating to diet formulations is considered as a viable option to increase their nutrient quality for monogastric animals including fish (Dawood and Koshio, 2020; Engin and Koyuncu, 2023). Furthermore dietary probiotic supplementation has also been suggested as possible alternative for increasing the nutrient retention of diets containing plant protein ingredients as main protein sources (Özil et al., 2023). Fermentation is sighted as a way of increasing the nutritional value of unconventional plant feed ingredients by increasing protein and lipid contents mainly because of reduction in the fiber and ANF content (Dawood and Koshio, 2020). The underlining process during fermentation is described as useful bioconversion or metabolic decomposition of complex substrates into simple and digestible small compounds by the microorganisms (Balakrishnan and Pandey, 1996). In addition, increment in the beneficial probiotic microorganism population in the gut and the consequent increase in the prebiotic activity resulting in secondary metabolites that could be easily absorbed is another positive effect of fermentation (Picoli et al., 2022).

Several investigations in Türkiye and around the world on increasing the nutrient quality of plant protein meals using different fermentation techniques as well as the incorporation of probiotic feed ingredients in diets for farmed fish and crustaceans (Yamamoto et al., 2010; Tanemura et al., 2016; Özil et al., 2023). Pre-fermentation of defatted and heat treated soybean meal with *Bacillus* spp. at 80°C using two different water levels and time duration used as fish meal replacer in diets for rainbow trout (Yamamoto et al., 2010). Results demonstrated that increased water and time duration during the fermentation of soybean meals improved growth with no visible distal abnormalities and regardless of fermentation method both lipid and carbohydrate digestibility increased in rainbow trout when replaced dietary fish meal at 48% level (Yamamoto et al., 2010). In another investigation Tanemura et al. (2016) fermented soybean, rape seed and algal meals with white-rot fungi (*Trametes coccinea* and *Lentinula edodes*) at 28 and 38°C for about 6 weeks and measured in vitro and in vivo ADCs in rainbow trout (Tanemura et al., 2016). These fungi species were found to be effective in lowering NDFs (Neutral Detergent Fiber) but increased ash content as a result of reduction in yield rates of the meals following fermentation decreased dry matter and protein digestibility most probably due to the mycelium generated during fermentation was not utilized as a protein source by rainbow trout. P digestibility however found to be significantly increased (Tanemura et al., 2016). Another study from Türkiye reported that probiotic mixture inclusion in diets containing almost 40% of feed protein ingredient from soybean (30%) and wheat gluten (10%) meals improved growth and significantly increased villi length and width and goblet cell numbers and total bacteria count (Özil et al., 2023).

7. Conclusion

Finding of alternative protein sources to fish meal in aqua feeds is an ongoing research topic in aquaculture nutrition since the traditional protein source of fish meal used in commercial diets has long been considered as an unsustainable and a finite source. Specifically animal by-product meals and oil seed expellers and solvent extracted meals have been the main alternatives for many farmed fish and crustacean species over the last three to four decades. The sustainability of aquaculture heavily depends on the finding the sustainable feed protein ingredients since almost 70% of the total expenditures in aqua farms is for the good quality and nutritionally balanced diets that should be produced with the emphasis on the sustainability and the environmentally friendliness status of the aquaculture premises. As it was explained throughout the text conventional alternative animal and plant by-product meals have myriads of disadvantages and effective use of them in aqua feeds necessitate the application of several technologies to improve their nutrient quality for aquatic animals. Although it appears that the achievement has been overseen in replacing some of the fish meal in commercial diets for salmonids and other omnivorus/herbivores fish species (Davies et al., 2021), it is far from over to make same comments neither for complete replacement of fish meal in the diets of

these species nor for the achievement of that sort for other alternative ingredients. Insect meal is the novel aqua feed ingredient and is vigorously looked at as a possible fish meal replacer for farmed fish and aquatic animals since they are considered as highly sustainable and a meal that is produced with almost zero carbon emission. It appears that complete replacement of marine products in aqua diets could only be possible with using available novel technologies to improve nutrient quality of raw ingredients along with the research conducted on all related nutritional physiologies apart from growth in aquatic organisms. As highlighted in the text and the Table 1 and 2, Turkish perspectives in aquaculture research are in line with the rest of the world but specifically research initiatives for finding the optimal replacement levels for local alternative meals and improvements on their nutrient quality are lacking. In this respect, research conducted by qualified University labs and institutions on the evaluation of local ingredients taking care of all the aspects of fish meal replacement in diets for fish commercially farmed in Türkiye would be highly beneficial not only for Turkish aquaculture but for the sustainability and environmentally friendly production efforts of global aquaculture.

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CHAPTER 8

LIPIDS IN AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.008

1. Introduction

Putting the water which is the life by itself on the side, lipids are probably the most important part of the body as they form the cell, separating it from the outer world by the membrane which is mainly composed of phospholipids. The definition of “lipid” has been traditionally established based on its solubility. Thus the lipids can be described as the compounds that cannot be dissolved in water, but can be dissolved by organic solvents such as ether, benzene, acetone, hexane, chloroform and methanol. Lipid is synonymous with fat/oil, but also includes phospholipids, sterols, and wax esters, etc.

Lipids are fuels with 9.4 kcal/g or 39.5 kJ/g energy, which is approximately 2.3-fold more in comparison to the energy content of carbohydrates. Fishes naturally consume different foods

and get different amounts of macronutrients. For example, carnivorous species get more lipids and proteins, while herbivores consume carbohydrates more. As a result, different nutrients are utilized to provide energy to different fish species which show differences in digestive system and utilizations of these nutrients. Lipids are primarily used as energy source by many fishes such as carnivores and diatom eaters, which consume prey rich in lipids, sparing protein to be mainly used for growth, tissue maintenance and other physiological functions (German, 2011).

Traditionally, fish oil is the major lipid resource for aquafeeds. The conventional commercial fish oil is mainly produced from the marine pelagic fishes such as anchovies, herrings and sardines. However, the demand of fast growing aquaculture industry for fish oil is impossible to be met by the wild forage fish stocks which have been stagnant for the last 3 decades. Thus, sustainable alternative lipid resources for aquafeed are now vital and inevitable for the industry.

2. Fatty Acids

Fatty acids can simply be described as the building blocks of most lipids. Fatty acids are carbon and hydrogen chains with a carboxyl group at one end (the alpha end) and a methyl group at the other end (the omega end).

The following factors make fatty acids different from each other:

a. The number of carbon atoms (the length of the chain): Short chain fatty acids have 2-4, medium-chain fatty acids have 6-10, and long-chain fatty acids have 12 or more carbons.

b. The degree of unsaturation: Fatty acids with no double-bonds are termed saturated fatty acids (SFA), meaning all carbon atoms are bounded with hydrogen atoms. They have higher melting points, and are usually solid at room temperature. Instead of binding to hydrogen, some carbons make double bonds with each other in unsaturated fatty acids. Unsaturated fatty acids with 1 double-bond are called monounsaturated fatty acids (MUFA), while they are called polyunsaturated fatty acids (PUFA) if they have 2 or more double bonds. PUFAs have lower melting points, and are liquid at room temperature. Whether a PUFA is omega-3 (n-3) or omega-6 (n-6) can be determined based on the location of the first double-bond on the chain. PUFAs with the first double bond between the third and fourth carbons from omega end are called n-3, while those having the first double-bond in between the sixth and seventh carbons are known as n-6 PUFAs.

c. The shape: SFAs are solid at the room temperature as they tightly pack because of their regular uniform shapes; however, unsaturated fatty acids have irregular shapes, and it makes them liquid at room temperature because they cannot be well/tightly packed.

3. Lipid Classes

Lipids can be divided into 4 major groups based on their chemical structures and biological functions:

3.1. Triglycerides

Triglycerides or triacylglycerols are the most existing lipids in biological systems. They are comprised of three fatty acids bound to hydroxyl groups of glycerol at the *sn*-1, *sn*-2, and *sn*-3 positions (Turchini et al., 2022). Triglycerides/triacylglycerols are so-called the storage lipids or energy storage, being the most abundant lipids in fish tissues. They are also the predominant lipids of the food or feeds.

3.2. Phospholipids

Phospholipids are the main components of bio-membranes. Instead of 3 fatty acids in triglycerides, phospholipids contain 2 fatty acids which are mostly dominated by long-chain polyunsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) that secure membrane fluidity. The third carbon of the glycerol backbone is bound with an alcohol-modified phosphate group. Two important phospholipids existing in plasma membranes are phosphatidylcholine and phosphatidylserine. Phospholipids are amphipathic molecules meaning both a hydrophobic and a hydrophilic component are existing in the same molecule. A phosphate group on one end is called the "head," and the part made of two fatty acids on the other end is called "tail." The head is polar and hydrophilic as the phosphate group is negatively charged. On the other hand, the tail is uncharged, non-polar and hydrophobic. The aforementioned structure of the phospholipids makes the bi-layer structure of cell membrane possible. The cell membrane consists of two adjacent layers of phospholipids, which form a bi-layer. The tail faces inside, away from water, whereas the polar head faces the outward aqueous environment.

3.3. Sterols

Sterols are polycyclic, long-chain alcohols. Characteristically they have a polar head group, a tetracyclic fused ring skeleton, and an alkyl side chain. Sterols are essential structural components to eukaryotic cell membranes. As they are amphipathic compounds they are able to incorporate into phospholipid bilayers. The sterol content is a key factor for eukaryotic cells for modulating and refining membrane permeability, fluidity, and membrane proteins functions (Haines, 2001; Ohvo-Rekilä et al., 2002). Sterols also function as precursors for vitamin D (Jäpelt and Jakobsen, 2013). Cholesterol is the most important sterol in all animals, including fishes.

3.4. Wax esters

Naturally occurring molecules wax esters characteristically have 1 fatty acid molecule bound to a long-chain fatty alcohol (Christie and Han, 2010). Wax esters are generally common in animal, plant, and microorganisms inhabiting in marine ecosystems. They play different functions such as energy storage and waterproofing agents. Through the natural food web in marine environment, wax esters become important nutrients for fish as their natural food such as calanoid copepods contain high amount of them (Sargent et al., 2002). Thus, fish inhabiting north hemisphere have high amount of 20:1n-9 and 22:1n-11 as they get them form wax esters provided by copepods.

4. Physiological Functions of the Lipids

Lipids have a number of physiological functions in the body.

4.1. Energy source

As they have higher ratio of carbon and hydrogen to oxygen than in carbohydrates and protein, the amount of energy they provide (9.4 kcal/g) is higher in comparison to carbohydrates (4.1 kcal/g) and proteins (5.6 kcal/g). Lipids are deposited in adipose tissue and provide energy to the organisms. Lipids provides metabolic energy in ATP form produced by β-oxidation of fatty acids. Lipids, fatty acids in another word, are the source of energy which is manly preferred for growth, reproduction, swimming and early development in fish (Tocher, 2003).

4.2. Source of essential fatty acids

Essential fatty acids can only be provided to the fish and other organisms by the dietary lipids. Fatty acid requirement of fish species might change depending on the species; however, in general, it appears that cold-water fish require n-3 LC-PUFAs, while warm-water fish need LC-PUFAs from either the n-3 or n-6 classes, or a mixture of both. In general, freshwater fish, can chain-elongate and desaturate 18C fatty acids, specifically linolenic acid (LNA; 18:3n-3), and linoleic acid (LA; 18:2n-6) to n-3 (EPA and DHA), and n-6 (arachidonic acid; ARA; 20:4n-6) LC-PUFAs, respectively (Tocher, 2003). This bioconversion ability to synthesize 20-22C LC-PUFAs from LNA and LA enables aquaculturists to formulate/produce diets containing less expensive plant oils that is rich in LNA and LA instead of using more expensive fish oils. Marine fish, on the other hand, does not have this ability and they need the aforementioned LC-PUFAs in their diets (Webster and Lim, 2002).

4.3. Bio-membrane structure and function maintenance

Organisms in aquatic ecosystems are subject to fluctuating and often extreme conditions that can influence their physiology. Temperature is one of the most important environmental

parameters that exhibits marked fluctuations even in short periods. Behavioral and physiological adaptations are effective responses to such stressors. Behavioral adaptation can be performed by moving to warmer or colder water to secure a reasonable thermal safety margin against extreme detrimental temperatures. However, biochemical and physiological adaptations, especially at the cell membrane level, provide the most specific and permanent response. Fluctuations in temperature force cells to arrange a new balance between their membranes and ambient environment. Sinensky (1974) described this as “homeoviscous adaptation”. Fatty acids are the key structural components of cell membranes which play important functional roles. Especially at cold temperatures, membrane fluidity must be maintained at an adequate level. LC-PUFAs such as EPA, DHA and ARA have a very low melting point (almost −50°C); thus, they have a much greater tendency to remain fluid *in situ* in comparison to SFAs. As a result, the relative proportions of fatty acids in cell membrane is very important because cell membranes need some solidity but must also maintain the membrane fluidity (Arts and Kohler, 2009).

4.4. Important for brain and sensorial organs

After adipose tissue, brain is the second to have the highest lipid level, with 50% of the dry weight. This probably well explains the essentiality of the lipids to the structure and functions of the brain. The fatty acid profile of the brain is dominated by the essential LC-PUFAs such as EPA, DHA and ARA, which must be provided to the brain. The fundamental role of lipids for the brain include neuronal differentiation, apoptosis, cell proliferation, synaptogenesis, neurotransmitter dynamics, insulation of neurons signaling of electrical impulses, and tissue repair (Premarathna et al., 2022).

4.5. Eicosanoids precursors

Eicosanoids are general term for the metabolites such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes which are biosynthesized by the enzymes so-called cyclooxygenase and lipoxygenase through different pathways. Eicosanoids are oxidized and biologically active molecules. They are mainly derived from 20C PUFAs and have various physiological functions as they act like signaling molecules to control inflammation and immunity, and regulate the vascular, renal, gastrointestinal and female reproductive systems (Calder, 2020).

4.6. Fat-soluble vitamins transport

Fat-soluble vitamins A, D, E and K, and some biologically active compounds like carotenoids can be transported by only lipids.

4.7. Hormones

Lipids are involved in the synthesis of hormones. On the other hand, some of the steroid lipids such as testosterone, and estrogens act as male and female sex hormone, respectively. As cell membranes are permeable to more hydrophobic lipid materials, steroid lipids can easily go through the membranes.

4.8. Gene expression regulation

Gene expression as well as duplication and transcription of DNA are directly regulated by lipids where they bind to nuclear transcriptional proteins and receptors. Lipids also indirectly regulate gene expression as they modulate the intra-nuclear microenvironment, membrane biophysics or other intracellular mechanisms.

5. Dietary Lipid Sources for Aquafeeds

Fish oil has been traditionally used as the main source of dietary lipids for aquafeeds; however, the fish oil production has not been able to cope with the tremendous growth in aquaculture over the last 4 decades. Fish oil is very rich in n-3 LC-PUFAs such as EPA and DHA that play very important physiological functions in fish and human health (Tacer-Tanas and Arslan, 2023). Now, aquaculture provides half of the fish eaten, and dietary raw material becomes more crucial. Thus, fish oil replacement has widely been subject to intensive research. Studies showed that dietary fish oil replacement did not reduce growth especially in freshwater fish (Turchini et al., 2003; Arslan et al., 2012; Yildiz et al., 2015); however, there was considerable amount of reduction in LC-PUFAs (Turchini et al., 2003; Arslan et al., 2008; Arslan et al., 2012; Eroldogan et al., 2013; Yildiz et al., 2015). Potentially important lipid resources as alternatives to fish oil might be categorized as follows:

5.1. Vegetable oils

The global amount of plant oils is approximately 200-fold of fish oil production. The price and availability of vegetable oils are independent of the amount used by aquaculture industry. In comparison to fish oil, vegetable oils are also more resistant to oxidation as they naturally contain antioxidant compounds (Tacer-Tanas and Arslan, 2023; Jaberi et al., 2023). On the other hand, they lack LC-PUFAs that have physiologically important functions in fish and human health. Vegetable oils can have very different and distinct fatty acid profile from each other and this allow them to be used solely or in mixture in aquafeeds. Table 1 presents the fatty acid profile of vegetable oils which are potentially important to aquafeeds.

5.2. Fats from land animals

The land animals generally store the lipids as fats. Animal fats are mainly produced from pigs (lard), cattle and sheep (beef and mutton tallows, respectively), and poultry (poultry fat). Lard is not used in aquaculture or any other feed industry in Türkiye. The fatty acid profile of animals is generally dominated by SFA and MUFA. However, poultry oil has considerable amount of PUFA mainly composed of LA (Table 2).

Table 1. Fatty acid composition (% of total detected) of some vegetable oils. Data are from Dubois et al. (2007)

	14:0	16:0	18:0	18:1	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Soybean	0.1	10.8	3.9	23.9	52.1	7.8	0.0	0.0	0.0
Cottonseed	0.8	24.2	2.3	17.4	53.2	0.2	0.0	0.0	0.0
Linseed	0.0	6.1	3.4	18.5	16.8	55.0	0.0	0.0	0.0
Camelina	0.0	5.3	3.0	18.7	16.0	38.1	0.0	0.0	0.0
Palm	1.1	43.8	4.4	39.1	10.2	0.3	0.0	0.0	0.0
Rapeseed	0.1	5.1	1.7	60.1	21.5	9.9	0.0	0.0	0.0
Sunflower	0.1	6.4	4.5	22.1	65.6	0.5	0.0	0.0	0.0
Olive	0.0	12.1	2.6	72.5	8.9	0.4	0.0	0.0	0.0
Corn	0.0	12.3	1.9	27.7	56.1	1.0	0.0	0.0	0.0

Table 2. Fatty acid composition (% of total detected) of land animal fat. Data are from Bureau and Meeker (2010)

	Tallow	Poultry oil	Lard
14:0	3.8	0.8	1.5
16:0	28.4	23.2	27.0
18:0	17.7	5.2	13.5
18:1	37.4	43.0	43.5
18:2n-6	4.6	17.0	10.5
18:3n-3	0.0	1.0	0.5
20:4n-6	0.3	0.0	0.0
20:5n-3	0.0	0.0	0.0
22:6n-3	0.0	0.0	0.0

5.3. Other marine oils

Mesopelagic fish, seal products, marine invertebrates such as copepods, euphausiids, mussels, etc., and other marine organisms can be considered the important candidate resources for marine oils other than traditional fish oil. Moreover, it is obvious that that aquaculture industry

will use higher volume of fishery by-products and discards in the future. Seasonal fluctuations are observed in the lipid and fatty acid profile of marine organisms mainly depending on the temperature and food availability. However, together with single-cell oils, fatty acid composition of aforementioned resources is more comparable with traditional fish oil in comparison to the all other lipid resources (Table 3). The annual potential was estimated to be more than 1 million tons for these resources (Olsen et al., 2010).

5.4. Single cell oils

They have a great potential as they are considered a unique and sustainable source of n-3 LC-PUFAs, especially EPA and DHA. In marine ecosystems, single-cell organisms such as diatoms, thraustochytrids, other microalgae, and some marine bacteria work as "biofactories" producing n-3 LC-PUFAs. These valuable fatty acids are then transferred to the fish through the food web (Miller et al., 2010). Fatty acid compositions of single-cell oils are very unique and mostly dominated by a single fatty acid (Table 3).

Table 3. Fatty acid profile (% of total detected) of some single-cell, krill and copepod oils in comparison to fish oil

	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3
Fish oil[a]	6.2	16.4	8.2	3.5	24.7	0.0	0.6	1.0	12.7	7.3
Schizochytrium[a]	8.9	26.1	0.6	0.8	1.7	0.6	0.1	2.4	2.2	36.7
Pavlova lutheri[a]	8.8	17.3	14.4	0.3	4.0	0.6	2.1	0.4	27.6	7.9
Chlorella vulgaris[a]	8.4	28.4	5.8	6.6	22.1	1.1	0.5	0.0	1.9	18.8
Isochyrysis galbana[b]	12.0	10.0	11.0	0.1	1.0	3.0	0.0	0.0	25.0	11.0
Crypthecodinium cohnii[b]	17.0	17.0	1.0	2.0	10.0	0.0	0.0	0.0	0.0	44.0
Motriella alpina FU30797[c]	0.0	12.3	0.0	15.9	17.2	7.9	4.9	34.6	0.0	0.0
Euphausia superba[a]	5.9	21.1	0.8	4.5	14.7	3.2	0.0	0.0	20.5	14.2
Parvocalanus crassirostris[a]	8.4	40.4	1.5	14.4	3.3	1.4	0.0	0.3	2.8	6.1

[a]Miller et al. (2010); [b]Sijtsma and de Swaaf (2004); [c]Chen et al. (2022).

6. Lipid Researches in Turkish Aquaculture

In Türkiye, researches on dietary lipids in aquaculture nutrition started in late 1990s; however, intensified in mid 2000s. Studies mostly focused on the impact of dietary lipid resources, alternatives to fish oil, on growth and fatty acid profile in rainbow trout, *Oncorhynchus mykiss* (Karayucel and Dernekbasi, 2010; Yildiz et al., 2013), European seabass, *Dicentrarchus labrax* (Eroldogan et al., 2013; Ozsahinoglu et al., 2013), gilthead seabream, *Sparus aurata* (Ofori-Mensah et al., 2020; Yildiz et al., 2008), Russian sturgeon, *Acipenser gueldenstaedtii* (Sener et al., 2005; Ustaoglu Tiril et al., 2016) and brown trout, *Salmo trutta* (Arslan et al., 2012). However, researchers have carried out experiments focusing on lipid/fatty acid metabolism in fish fed on different dietary lipid resources only for the last 10 years (Eroldogan et al., 2013; Ofori-Mensah et al., 2020; Tasbozan et al., 2023; Kose Reis, 2023). Also, some studies targeted how the final eating quality (Yildiz et al., 2015; Ofori-Mensah et al., 2022; Jaberi et al., 2023), and gamete quality (Yildiz et al., 2020; Yildiz et al., 2021) are affected by the dietary lipid resources. On the other hand, through international collaborations, some Turkish researchers carried out dietary lipid studies on some fish species such as Amazonian catfishes, *Pseudoplatystoma fasciatum* (Arslan et al., 2008; Arslan et al., 2009) and *Pseudoplatystoma* sp. (Arslan et al., 2013), which are not cultured in Türkiye.

Studies showed that total replacement of fish oil with vegetable oils such as peanut oil in common carp *Cyprinus carpio* (Yildirim et al., 2013), sesame, sunflower, linseed (Yildiz et al., 2015), canola (Karayucel and Dernekbasi, 2010; Yildiz et al., 2013), cotton seed (Yildiz et al., 2013), poppy seed (Ornek et al., 2021) and laurel seed (Dernekbasi et al., 2017) oils in rainbow trout, sunflower (Sener and Yıldız, 2003), canola (Eroldogan et al., 2012; Eroldogan et al., 2013) and cotton seed oils (Eroldogan et al., 2012) in European seabass, and chia oil in seabream (Ofori-Mensah et al., 2020) did not influence growth performance. However, total substitution of fish oil with hazelnut oil in brown trout (Arslan et al., 2012), hazelnut (Tasbozan et al., 2021) and soybean oils (Emre et al., 2016) in meagre *Argyrosomus regius*, soybean oil in Russian sturgeon (Sener et al., 2005), and camelina seed oil in seabream (Ofori-Mensah et al., 2020) caused growth depletion. On the other, linseed and soybean oil mixture (1:1) improved growth in brown trout compared to dietary fish oil (Arslan et al., 2012).

As expected, cultured fish always exhibited similar fatty acid profile with the respective dietary fatty acid composition, with a dramatic decrease in LC-PUFAs when dietary fish oil is replaced. However, studies emphasized the bioconversion capacity of Amazonian catfish (Arslan et al., 2009) and brown trout (Arslan et al., 2012) for converting 18C dietary PUFAs such as LNA and LA to 20-22C PUFAs such as EPA, DHA and ARA when they were fed diets with vegetable oils. Recent molecular studies showed that this bioconversion capacity is quite limited in marine fish species such as seabream (Ofori-Mensah et al., 2020) and European seabass (Eroldogan et al., 2013).

Regarding sensorial features, fish oil substitution with vegetable oils generally did not negatively affect final eating quality in seabream (Ofori-Mensah et al., 2022) and rainbow trout (Yildiz et al., 2015; Jaberi et al., 2023). On the other hand, dietary hazelnut oil was reported to improve the shelf life in rainbow trout (Jaberi et al., 2023). Likewise, when vegetable oils such as linseed and sesame oils totally replaced fish oil, no negative influence was observed on reproductive success in rainbow trout (Yildiz et al., 2020; Yildiz et al., 2021).

7. Closing Remarks

It is obvious that dietary lipid resources will be one of the key elements in sustainable growth of Turkish aquaculture sector. Apparently, the usage of fish oil in aquafeeds is likely to stay stable or decrease; however, the improvement in aquaculture production will be secured by the increasing use of alternative lipid resources in aquafeeds. On the other hand, the need for the dietary oils for aquaculture species should be well projected, and especially the land use for oil seed production should be carefully planned for this purpose. It should also be realized that Türkiye naturally has a great potential to produce lipids from microalgae, land animals and other marine resources. Decreased omega-3 LC-PUFAs in fillets of farmed fish is the inevitable consequence of the fish oil substitution especially with plant oils and fats from land animals; however, using LNA rich vegetable oils in the feeds for freshwater fishes will significantly alleviate this issue. Regarding marine species, finishing diets boosted with LC-PUFAs rich resources before harvest will secure the nutritional quality, EPA and DHA content specifically, of the final products.

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CHAPTER 9

LIVE FEEDS IN AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.009

1. Introduction

Aquaculture provides high quality food for human consumption, and the sector has been constantly growing over the few decades. The total global production of aquaculture reached 87.5 million tonnes (Mt) in 2020, up around 20% from 71.5 Mt a decade ago (FAO, 2022). This increasing trend is predicted to continue, and FAO estimates overall production reaching over 100 Mt in 2030, with both an increase in the production of freshwater and marine fish. However, sustainable development of the sector will be dependent on many factors, such as availability of quality feed ingredients and success in hatchery operations to produce enough quantitity and quality of larvae and juvenile fish. Looking forward and considering the little variety in aquacultured species, the aquaculture industry is willing to expend and add new cultured species to their production. However, introducing new species and increasing the production efficiency relies on establishing a good production chain through larval rearing to broodstock management in aquaculture species.

The success of larval and juvenile production is directly affected by several factors, such as the nutritional values of live prey, the quality of formulated feed, labor, broodstock management, and the physical and chemical parameters of the culture water. The most critical issue is the utilization of high-quality live feeds during the larval period. Once marine fish larvae hatch, microalgae are applied in the larval rearing tank using a technique called the greenwater technique

(Neori, 2011). This method allows live prey, such as rotifers, to be kept alive in the larvae tank by consuming microalgae. The application of microalgae leads to an increase in larval appetite due to the shadow effect in the water column. Another beneficial effect of adding microalgae into the larval tank is a decrease in the number of pathogenic bacteria such as *Vibrio sp.*, that led to common disease outbreaks in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) cultures. This chapter summarizes the most commonly utilized live prey, including microalgae, rotifers, artemia, copepods, water fleas, sludge worms, and white worms.

1.1. Microalgae

Microalgae are an essential component of the aquatic food web chain in both freshwater and marine environments (Brown, 2002). They are the first feed of several zooplankton species commonly used in aquaculture, such as rotifers (*Brachionus plicatilis*) (Eryalçın, 2019), brine shrimp (*Artemia* spp.) (Turcihan et al., 2021), copepods (*Acartia clausi*, *Tisbe sp*., *Acartia tonsa*, *Apocyclops royi*) (Puello-Cruz et al., 2009; Rasdi and Qin, 2018), and water fleas (*Daphnia magna*) (Turcihan et al., 2022). All life stages of bivalve and crustacean species require microalgae due to their filter-feeding ability. Mollusc culture also relies on microalgae production and utilization during the larval culture period. For instance, microalgae are needed for high growth and survival in the production of sea cucumber (Shi et al., 2013) and sea urchin (Carboni et al., 2012).

Microalgae culture practices began in the early 1900s with the development of culture medium formulations based on the requirements of the species of interest. In recent years, microalgae have gained more attention in areas, such as biofuel production, feed ingredients (as essential nutrient sources, including lipids and proteins), wastewater treatments, and CO_2 emissions. The USA and China are heavily carrying out scientific investigations on microalgae (Garrido et al., 2018), and these attempts take place at the applicable industrial level. Moreover, microalgae are widely used as feed ingredients in both the terrestrial and aquatic animal feed industries (Roy and Pal, 2015; Shah et al., 2018). Some microalgae species contain a high level of protein content, such as *Spirulina platensis* (60% of dry weight) and *Chlorella vulgaris* (51-58% of dry weight). On the other hand, some are known for their high lipid contents, such as *Crypthecodinium cohnii* (40% of dry weight) (Ganuza et al., 2008; Eryalçın et al., 2015). The accumulation of lipid droplets in microalgae mainly depends on species-specific factors and varies under various culture conditions, such as myxotrophic, phototrophic, and heterotrophic culture methods. Microalgae are rich in highly unsaturated essential fatty acids (HUFAs), such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), which are essential to many

aquacultured species. For instance, *Nannochloropsis gaditana* has a high EPA (Eryalçın et al., 2015) content, while *Schizochytrium* sp. has a higher amount of DHA (Eryalçın et al., 2013). Both of these microalgae can be used to supply certain fatty acid requirements for fish larvae via the use of live prey enrichments or formulated diets. Microalgae species differ in size, shape, nutrient compositions, chlorophyll contents, polysaccharides, and pigments (Figure 1). In addition, microalgal species show a variety of carotenoids and antioxidant contents. There are several carotenoids purified from microalgae, and some of them are commercially available. The most common carotenoids include astaxanthin, α-carotene, neoxanthin, cryptoxanthin, zeaxanthin, violaxanthin, and lutein. Antioxidant compounds play an important role in the prevention of free radicals and lipid oxidation in cells. Those carotenoids are widely used in feed ingredients for chicken and salmonid diets to sustain desirable egg-yolk and flesh colour, respectively. For instance, Spirulina species are rich in antioxidant compounds such as phycocyanin and alpha-tocopherol (Vitamin E). Freshwater microalgae such as *Chlorella vulgaris* are rich in lutein pigment, which has been found to enhance chicken egg-yolk colour and digestibility (Dineshbabu et al., 2019). Marine microalgae have other carotenoids, such as astaxhantine from *Haematococcus pluvialis* and b-carotene from *Dunaliella salina*, which are the most cultured microalgae for pigment production (Borowitzka, 2013). Additionally, *Porphyridium cruentum* contains a high amount of phycoerythrin and is used in feed ingredients.

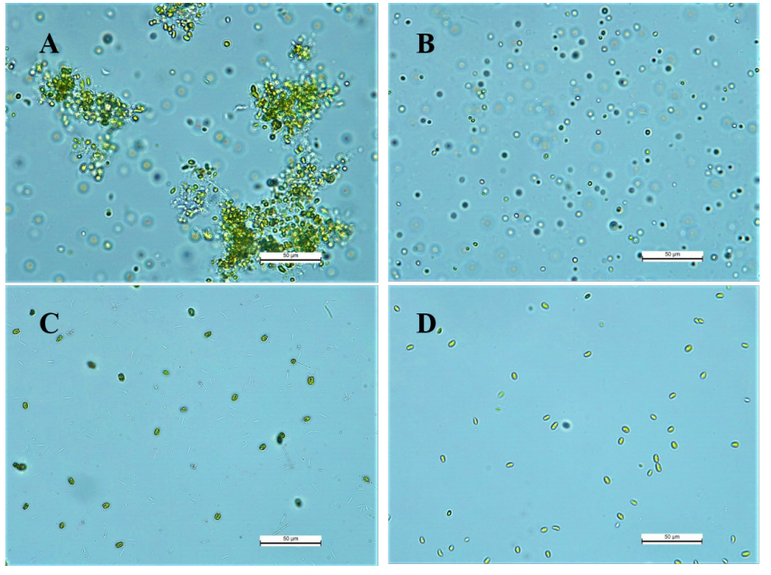

Figure 1. Microalgae species; *Chlorella vulgaris* (A), *Diacronema vlkanium* (B), *Pavlova lutheri* (C) *and Euglena gracilis* (D) (original)

Toxidity, nutrition content, and size should be taken into consideration when microalgae select the species used in aquaculture. In addition, the potential for high biomass production of algae with suitable culture methods and selection is an important consideration. Some of these criteria, along with the usage proposed are summerized in Table 1.

Table 1. Microalgae species and products (Yu et al., 2015; Dineshabu et al., 2019; Mansour et al., 2022)

	Species	***Purpose***
Feed Ingredients in Aquaculture and Poultry	*Chlorella vulgaris* *Chlorella minutissima* *Dunaliella tertiolecta* *Dunaliella salina* *Haematococcus pluvialis* *Porphyridium cruentum* *Crypthecodinium cohnii* *Nannochloropsis oculata* *Schizocytrium sp.* *Spirulina platensis* *Pavlova lutheri* *Tetraselmis chuii* *Phaeodactylum tricornatum* *Arthrospira sp.* *Scenedesmus obliquus*	Protein Lipid (DHA, EPA, ARA) Carbonhydrate Pigment source
Bivalve	*Thalassiosira pseudonana* *Pavlova lutheri* *Phaeodactylum tricornutum* *Tetraselmis suecica* *Tetraselmis chuii* *Chaetoceros calcitrans* *Chaetoceros gracilis* *Skeletonema costatum*	Feed
Rotifer	*Nannochloropsis oculata* *Nannochloropsis gaditana* *Chlorella sp.* *Chlamydomonas sp.*	Feed
Crustaceans (Artemia, Shrimp)	*Tetraselmis suecica* *Tetraselmis chuii* *Chaetoceros calcitrans* *Chaetoceros gracilis Skeletonema costatum* *Thalassiosira pseudonana*	Feed
Greenwater technique	*Nannochloropsis oculata* *Nannochloropsis gaditana* *Isochrysis galbana*	Keeping rotifer alive in culture tank, reducing pathogen bacteria such as Vibrio sp., providing low light intensity, promoting digestive enzyme for fish larvae
Molluscs such as Sea cucumber, Abalone, Sea urchins	*Nitzschia sp.* *Navicula sp.* *Amphora coffeaeformis*	Feed
Biotechnology & Biofuels	*Euglena gracilis* *Botryococcus braunii* *Chlamydomonas reinhardtii* *Odontella aurita* *Chlorella vulgaris* *Nostoc sp.* *Dunaliella salina*	

Moreover, microalgae are promising ingredients for the fish feed industry. Aquaculture is still a growing sector; however, sustainability is a major issue due to the difficulties of supplying raw materials such as fishmeal and fish oil for the production of fish feed. Therefore, there is an increasing number of research projects to replace fishmeal and fish oil with microalgae biomass (Eryalçın et al., 2013; Eryalçın et al., 2015). There are several companies and institutions around the world that produce a variety of microalgae species (Table 2). Currently, in Türkiye, there is only one company, MarinBio, which is located in the west part of Türkiye in Denizli province.

Table 2. Summary of some of the major companies and institutions using microalgae

Company name	***Country***	***Microalgae species***	***Purpose***	***Product***
Algaenergy	Spain	*Nannochloropsis gaditana* *Isochrysis galbana* *Tetraselmis suecica* *Chlorella vulgaris*	Feed Ingredients	
Algaspring	Netherlands	*Nannochloropsis* sp.	Rotifer and Artemia	Nannostar BLUE Nannostar GREEN Nannostar RED
Aquafauna Biomarine Inc.	USA	*Schizochytrium sp.*	Rotifer	AlgaMac 3050 flake Red Algamac (for Rotifers) AlgaMac ProteinPlus AlgaMac Enrich (DHA and Astaxanthin rich)
BernAqua	Belgium	*Spirulina* sp. *Thalassiosira weissflogii* *Thalassiosira pseudonana* *Tetraselmis* sp.	Fish and Shrimps	
BioMar	Norway	-	Fish	AlgaPrime DHA
Blue Biotech	Germany	*Nannochloropsis* sp. *Isochrysis* sp. *Haematococcus pluvialis*	Astaxanthin Fish coloration	
Corbion	USA	*Schizochytrium* sp.	Feed Ingredients	AlgaPrime™ DHA
Cyanotech	USA	*Haematococcus pluvialis*	Feed Ingredients	
Heliae	USA			Nymega
Innovative aquaculture	Canada	*Tahitian Isochrysis* *Isochrysis galbana* *Pavlova lutheri* *Nannochloropsis oculata*		IAP Algae Paste
June Spirulina	Myanmar	*Spirulina* sp.		
Mera Pharmaceuticals	USA		Astaxanthin for Shrimp and Salmon	AquaXan®

Table 2. Continue

Company name	*Country*	*Microalgae species*	*Purpose*	*Product*
Pacifc Bio.	Australia	*H. pluvialis*	Astaxanthin for Shrimp and Salmon	ReefAsta™
Pacific Co. Ltd.	Japan	*Chlorella* sp. *Haematococcus pluvialis*	Rotifer and Artemia	Fresh Chlorella V12 Super Fresh Chlorella V12 (DHA enriched) Super Capsule A1 Powder Chilean Astaxanthin
Pentair Aquatic Eco-Systems	USA	*Spirulina* sp. *Pavlova* sp. *Isochrysis* sp. *Thalassiosira* sp. *Tetraselmis* sp. *Nannochloropsis* sp.	Feed Ingredients	Hikari® Algae Wafers Spirulina Flake Phyto Feast® Fish Food
Phytobloom	Portugal	*Nannochloropsis* sp. *Isochrysis* sp. *Phaeodactylum* sp. *Tetraselmis* sp.		
Reed Mariculture	USA	*Nannochloropsis* sp. *Tetraselmis* sp. *Isochrysis* sp. *Pavlova* sp. *Thalassiosira weissflogii* *Thalassiosira pseudonana*		
Skretting	Norway		Artemia Fish larvae	ORI N-3 (for *Artemia*) NEPTUNE (for Green water culture) CLEAN Start (for fsh larvae)
TaiwanChlorella	Taiwan	*Chlorella* sp.	Biomass	
Tomalgae	Belgium		Feed Ingredients	Thalapure ™ Phylavive
MarinBio	Türkiye	*Schizochytrium* sp. *Chlorella* sp.		AlgomeDHA ™ AlgomeGrow ™ AlgomeDHA Oil TM

Optimal culture methods are crucial for biomass production. Microalgae are mostly cultured in a phototrophic way, where sunlight is used as the primary energy source. Phototrophic culture is the most selected culture method, and it can be set up indoors and outdoors. Higher biomass can be produced in a raceway outdoor system; however, within this method, biomass gain can be limited due to several factors, such as environmental and contamination problems. A photobioreactor system is a good option for mass production because it allows high biomass production in outdoor areas (Figure 2).



Figure 2. *Nannochloropsis oculata* culture in tubular photobioreactors (Akvatek Company, Dikili, İzmir)

Recently, more studies have focused on heterotrophic culture methods for some microalgae species, such as *Chlorella sorokiniana*, *Chlorella vulgaris*, *Chlorella protothecoides*, *Chlorella zofingiensis*, *Schizochytrium* sp., *Crypthecodinium cohnii*, *Scenedesmus* sp., *Spirulina platensis*, *Isochrysis galbana*, *Euglena gracilis*, *Tetraselmis suecica*, *Nannochloropsis oculata*, *Dunaliella* sp., *Nitzschia laevis*, and *Phaeodactylum tricornatum* (Perez-Garcia et al., 2011; Morales-Sánchez et al., 2017; Hu et al., 2018). These microalgae can be cultured using different organic carbon sources, such as glucose, acetic acid, glycerol, acetate, and ethanol, together with CO_2 utilization and without a light source under sterile conditions. In this culture method, cells can accumulate higher lipid molecules than in phototrophic cultivation and are ideal for industrial fermentors and bioreactors. However, despite the high biomass gain, this cultivation method can be suitable for only some microalgal species. Therefore, the selection of microalgal species is very important. Moreover, the expenditures on energy and basic nutrient sources are limiting factors in this technology. For any purpose, microalgae species should be maintained and kept under sterilized conditions, which is called a “pure culture room”. In this way, algae are periodically cultured in petri dishes and test tubes (Figure 3). Sub-cultures are visually and microscopically controlled before up-scaling production is started.

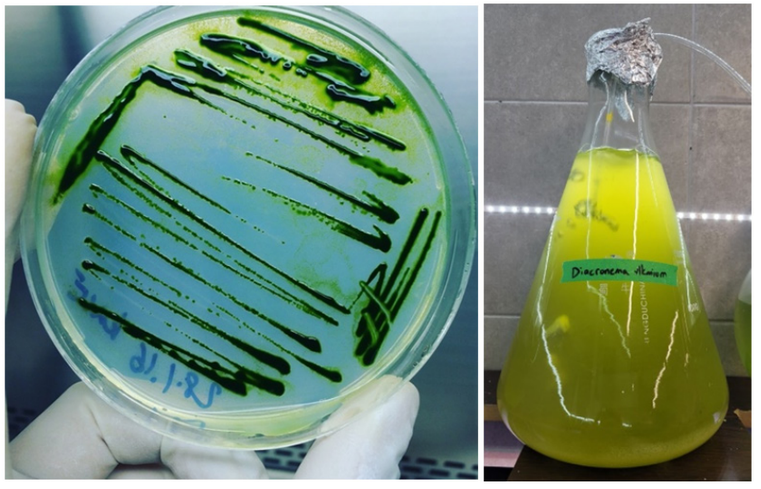

Figure 3. Microalgae culture in laboratory; petri dishes and erlenmayers (original)

In conclusion, there is still a high demand for microalgae biomass, not only for live prey culture and feed ingredients in aquaculture but also for feed ingredients for poultry and terrestrial animals as a source of protein and lipids. Therefore, in addition to phototrophic production, mixotrophic and heterotrophic cultures of microalgae species are promising production methods in the future for achieving high biomass gain. Moreover, the isolation of new microalgae species and their adaptation into culture systems are also important for the sustainable development of algae culture and biomass production.

1.2. Rotifer culture

Rotifers are the first live prey and are indispensable for fish larval culture. Due to their suitable size, all fish hatcheries should start to feed larvae with this live prey in larval production. Therefore, obtaining this live prey is very critical and vital in marine fish hatcheries. There are several types of rotifers that differ in size, such as *Proales similis* (lorica length 85 µm), SS-type (*Brachionus rotundimorfis*; lorica length 100-140 µm), and L-type (*Brachionus plicatilis*; lorica length 250 µm).



Figure 4. Rotifer *Brachionus plicatilis*

The success of rotifer culture mainly depends on the feed, chemical and physical water parameters, and labor capability in hatcheries. Rotifer numbers can be increased in a couple of days under intensive culture conditions by feeding them with baker's yeast and fresh microalgae (Eryalçın, 2019). However, rotifers lack essential nutrients such as polyunsaturated fatty acids (PUFA), essential amino acids (EAA), vitamins, and minerals (Hamre, 2016). Therefore, the nutritional composition of rotifers should be developed by the enrichment process. This process allows rotifers to be enriched with essential nutrients in a short time before being fed to fish larvae (Eryalçın, 2018). However, there are still some gaps in the cultivation and optimization of the culture process. The main bottleneck to culture success is the nutritional value of feed properties. There are various rotifer diets and enrichment products that vary in nutritional composition. This reality makes the rotifer culture fragile, and hatcheries should develop themselves according to their experiences. In the culture process of rotifers, all rotifers should be female individuals due to the high numbers of rotifers required in larval production. Each female may carry between one and four eggs. Environmental conditions, once broken down or sudden changes in some water parameters, can lead to rotifers sexual reproduction between male and female rotifers, and finally resting eggs occur (Figure 5). Marine fish hatcheries can maintain and survive a small amount of rotifer culture to keep rotifers available for the next production season.

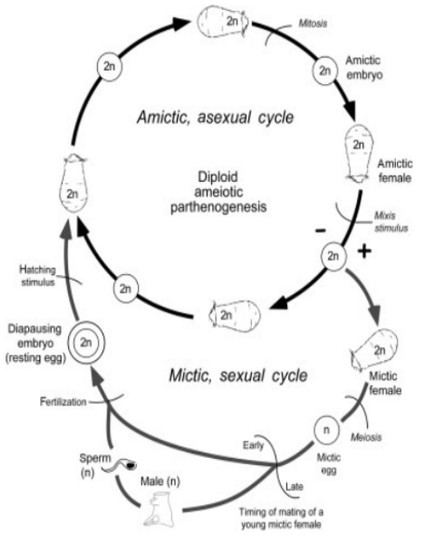

Figure 5. Life cycle of Rotifer (*Brachionus plicatilis*) (Lavens and Sorgeloos, 1996)

The top priority at commercial marine fish hatcheries is to increasing the number of rotifers in a short period of time is first priority at commercial marine fish hatcheries. However, once reaching a sufficient amount of rotifers, it is important to investigate their nutritional properties before using them to feed fish larvae. Baker's yeast is the most common diet used for feeding rotifers, but it lacks essential nutrients, which can have a negative impact on the nutritional value of the rotifers (Hamre, 2016; Eryalçın, 2018). Therefore, the selection of suitable microalgae is essential to delivering nutrients to rotifers via microalgae. While fresh microalgae can be difficult to manipulate and culture, commercial products are widely used for feed and enrichment processes, alongside fresh microalgae.

Enrichment products and application methods, including intervals and usage time, play a vital role in the accumulation of essential nutrients in rotifers. The protocols for enrichment differ among hatcheries. Additionally, cultivation type is another important factor that affects rotifer culture performance and nutritional value. Batch, semi-continuous, and high-density culture methods are applied depending on the hatchery's facilities procedure, tank shape and volumes, and the types of feed used in rotifer production. In the batch culture, harvested rotifers are separated into two parts: one part is used for new inoculation to continue the rotifer culture, and the other part is used to feed fish species. Commercial feeds are added to the

culture tank for a short time, typically 3-4 days. Semi-continuous culture differs from batch culture in that it involves periodic harvesting and washing of the rotifer cultures, which can take longer to complete. Another culture method is high-density culture, which involves the application of concentrated microalgal biomass. However, maintaining water quality and stability is a major challenge with this method. To address these issues, protein skimmers, filtering, and partial water renewals can be applied in the high-density culture method (Yoshimatsu and Hossain, 2014).

Continuous rotifer is essential in marine fish hatcheries, whether during periods of intense culture or the drying season. Rotifer feeds are also crucial, and companies can either produce them themselves via microalgal production or obtain them from commercial companies. When introducing new rotifer diets, it is important to try them out in small amounts before implementing them on a large scale. This helps to ensure that the new diets are suitable and effective before investing significant resources in their production and use.

1.3. Artemia culture

Artemia is a filter-feeding crustacean brine shrimp that is found in salt lakes throughout the world (Madkour et al., 2022). It was discovered in the early 1970s for its high nutritional value and ease of hatching cycts, making it a valuable resource for fish culture purposes (Figure 6) (Sorgeloos et al., 1977). In these years, Artemia nauplii has been widely used in the cultivation of marine and freshwater larvae and juvenile fish (Sorgeloos et al., 2001). Although artemia is rich in protein, it contains fewer lipids. Therefore, Artemia nauplii should be enriched before being fed to fish larvae, just like rotifers.

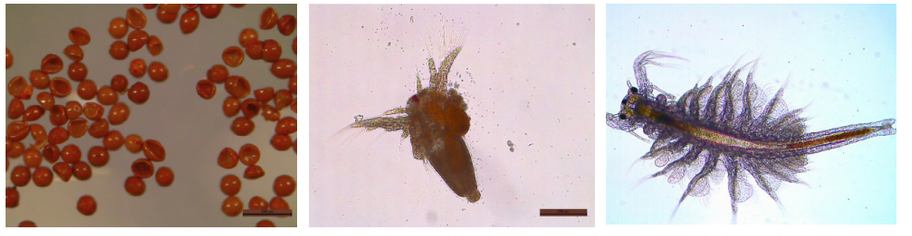

Figure 6. Artemia cysts (a), aArtemia nauplii (b) ve aArtemia metanauplii (c) (original)

Dried cycts are the inactive embryos of Artemia in the late gastrula stage. These cysts are obtained from nature using plankton nets, then dried and packed (Figure 7). However, collecting these cysts is not sustainable for further live prey utilization in aquaculture. The hatching rate of Artemia cysts varies depending on their geographic origin. Currently, artemia

cysts are mostly obtained from Salt Lake, Utah, USA, followed by China, Russia, Kazakhstan, Uzbekistan, Vietnam, Thailand, Argentina, and Brazil, respectively (Litvinenko et al., 2015).



Figure 7. Artemia cysts harvesting from nature (Great Salt Lake, UTAH, USA) (Sorgeloos and Roubach, 2021)

Fish production hatcheries require live prey that is similar in size to their natural prey, which is typically larger than 1 mm in total length, such as copepods, for feeding to larval fish. Recent studies have focused on improving the production performance of Artemia through the use of freshly cultured microalgae or commercial diets. Artemia metanauplii can reach to 2–3 mm lenght and has a high nutritional value, making it essential for the nutrition of newly cultured aquatic organisms. For example, *Artemia franciscana* metanauplii is an appropriate size for seahorse (Vite-Garcia et al., 2014), clownfish (Chen et al., 2020), anemones, crustaceans (Nelson et al., 2002), fish (Lim et al., 2001), soft corals (Tsounis et al., 2010), and for use in cephalopod culture (Guinot et al., 2013). Therefore, larger sizes of Artemia spp. are also needed for feeding new marine and freshwater larvae and juveniles.

Artemia spp. is a fast-growing brine shrimp that primarily feeds on small particles such as bacteria (Toi et al., 2013), microalgae (Zhukova et al., 1998), and organic matter in the water column (Maldonado Montiel et al., 2003). Among these, microalgae are the primary food source for *Artemia* spp. and are essential for its efficient growth (Turcihan et al., 2021).

The limited availability of Artemia cysts is a significant challenge for sustainability in larval feeding, which is why alternative live prey options must be explored for both freshwater and marine environments. Türkiye has several Artemia sources located in different regions, with İzmir Çamaltı (Saygı, 2004) and Gökçeada (Eskandari, 2014) being the most well-known salt lakes that provide Artemia cysts. However, to improve the efficiency and sustainability of Artemia production, further research is needed to optimize these sources.

1.4. Copepod culture

The primary live prey for marine fish larvae during their initial stages of development are rotifers (*Brachionus plicatilis*) and *Artemia* spp., which are suitable for their small mouth sizes. For the first 20–30 days after hatching, marine fish larvae require live prey to develop properly because their digestive enzymes are insufficient and their digestive systems are not fully developed (Kolkovski et al., 1997; Kolkovski, 2001). However, in terms of nutritional value, rotifers and *Artemia* spp. are nutritionally inadequate compared to copepods, which are the primary natural live prey for marine fish larvae in their natural ecosystems. Copepods contain higher levels of essential amino acids, fatty acids, vitamins, and minerals (Rasdi and Qin, 2016). For fast-growing specieslike grouper, successful larval culture requires the use of copepods as the sole live prey or co-feeding with *Artemia* spp. (Burgess et al., 2020; Ranjan et al., 2022).

Recent efforts have been made to isolate local copepods from the Marmara Sea, including *Acartia clausi*, *Penilia avirostris*, and *Paracalanus parvus*, which were successfully cultured under laboratory conditions by feeding them with different microalgae, such as *Chlorella vulgaris*, *Rhodomonas* spp., *Rhinomonas reticulata*, *Isochyrsis galbana*, and *Thlassiosira pseudonana*. Researchers investigated the effects of these microalgae on the survival rates and fatty acid composition of the cultured copepods. The results showed that a combination of *Chlorella vulgaris* and *Rhodomonas* species resulted in the highest survival rate for *Acartia clausi* (Eryalçın et al., 2022, unpublished data) (Figure 8). The utilization of *Isochrysis galbana* as a feed source resulted in a higher accumulation of n-3 HUFA in *Acartia clausi* compared to other microalgae species. It is evident that the survival rates and growth parameters of copepod species vary depending on the species of copepod and the specific microalgae used as feed.



Figure 8. *Acartia clausi* (A), *Paracalanus pavrus* (B), *Penilia avirostris* (10X; Leica DM1000) (original)

In recent years, there has been an increase in studies on culture techniques, optimization of culture conditions, and feeding of copepods (Chintada et al., 2022). For instance, when *Acartia ohtsukai* was cultured at different salinities and temperatures below 10 ºC, low survival

rates were observed, but survival rates were not affected by a wide range of salinities (Choi et al., 2021). In another study, the effects of different temperatures on the egg productivity of the calanoid copepod *Acartia amboinensis* were examined, and an optimum temperature of 27 ºC was determined for this species; when the culture temperature was increased to 30 ºC and 33 ºC, egg production decreased (El-Sherbiny and Al-Aidaroos, 2021). Copepod eggs are currently sold by commercial companies for marine fish larval production, and the collection and storage of these eggs require experienced labor and equipment. Several studies have been carried out to optimize egg collection, such as examining the effects of different salinities and storage temperatures on the hatching and survival of eggs in *Acartia sinjiensis*, which showed that eggs could be stored for up to 180 days at 4°C and 1 ºC (Choi et al., 2022). From these findings, it is clear that optimizing the culture temperature is crucial for successful copepod culture performance.

The aquaculture sector requires large numbers of live prey with essential nutrients, and the focus of large-scale copepod cultivation is on calanoid species due to their high egg production and culture success. However, the main challenges in this effort are the high demand for fresh microalgae and the optimization of environmental conditions such as pH, dissolved oxygen, salinity, temperature, and water exchange rate selected for the cultured copepod species (Sarkisian et al., 2019). Egg productivity is a key factor in managing high copepod biomass under controlled conditions. However, some copepod species, such as *Acartia tonsa*, exhibit low hatching rates, which are attributed to the effects of temperature and salinity. It has been observed that hatching rates of this species decreased after 8 weeks of egg storage, with varying hatching rates at a salinity of 30 ppt at a temperature of 18 ºC (Torres et al., 2021). Despite studies on culture parameters, research on the nutritional requirements of copepods is scarce in both the scientific and aquaculture areas.

In a feeding experiment on *Gladioferens imparipes,* it was observed that copepods fed with the microalgae *Isochyrsis galbana* exhibited increased egg productivity. It is well-known that survival rates and fatty acid contents directly correlated with nutrient concentrations (El-Tohamy et al., 2021). The cultivation period of *Tisbe sp*. and *Apocyclops sp*. showed that fatty acid profiles are affected by time, and it was concluded that the long-term culture of copepods increased their total fatty acids by storing more lipid (Alejos-Cabrera et al., 2022). The amount of lipid storage in copepods is known to be directly related to the feed they consume. The fatty acid composition of *Apocyclops royi* and *Pseudomonas annandalei* fed with *Dunaliella tertiolecta*, *Rhodomonas salina,* and baker's yeast was investigated. According to this study, the level of EPA was enhanced by the microalgae *Rhodomonas salina* compared

to baker's yeast. The same study showed that *Pseudomonas annandalei* fed with *Dunaliella tertiolecta* exhibited a high level of another important fatty acid, ARA (Nielsen et al., 2020).

It is possible that there is a cross-effect between microalgal diets and copepods, as some algae contain spesific long-chain fatty acids such as ARA, EPA, and DHA, which are essential nutrients for copepods. Additionally, selecting the appropriate copepod species for cultivation can also be challenging, as each species may have different preferences for certain algae and nutrients. To address this issue, several studies are being conducted to investigate the effects of different microalgal species alone or in combination. For instance, the effect of three microalgae species, *Rhodomonas salina*, *Tisochrysis lutea*, and *Pavlova lutheri*, on *Paracyclopina nana* culture was examined, and it was found that *Rhodomonas salina* was the best diet for this copepod species (Dayras et al., 2021). In another study, the copepod *A. bilobata* was fed solely or in combination with *Isochyrsis galbana, Chaetoceros muelleri*, and *Nannochloropsis oculata*, and copepods fed with *I. galbana* showed increased egg production, hatching rate, and adult individuals (Chintada et al., 2022). These studies have provided valuable data on copepod culture. Currently, copepod eggs are available commercially from some institutes and companies worldwide (https://algova.com/en/Copepod-Eggs-Cysts-Acartia-tonsa-Starter-Feed-for-Fish Larvae/COP0025M). In Türkiye, copepod culture production at an industrial level is still not common. However, some new marine species, such as the white grouper (*Epinephelus aeneus*), require a copepod mixture for their larval stage nutrition. Therefore, in order to contribute to the aquaculture sector with new species, copepod culture should be successfully managed and applied.

1.5. Water flea culture

Water fleas are freshwater cladoceran that are widely distributed in all freshwater ecosystems (Yıldız et al., 2022). They are the main target diets of fish, birds, and turtles in lakes and rainwater reservoirs (Cox et al., 2018). *Daphnia pulex* and *Daphnia magna* are the most common species that are widely used for aquaculture purposes (Ashforth and Yan, 2008; Turcihan et al., 2022) (Figure 9). Daphnia species are not only used for aquaculture but also for wastewater treatments (Ra et al., 2008), ecotoxicology, ecology (Ebert, 2022), and evolutionary biology studies (Stollewerk, 2010).



Figure 9. Daphnia individuals (A) and female daphnia with carrying eggs (B) (original)

The culture performance of Cladocerans is strongly related to diet quality, physical and chemical environmental conditions. In their natural habitat, they consume microalgae without selection. Daphnia individuals can convert organic material consumed by microalgal production from ponds and wastewaters (da Silva Campos et al., 2020). Recently, it has been shown that these cladocerans are unable to convert long-chain essential fatty acids. However, daphnia can accumulate essential nutrients through their feeds, such as fatty acids, amino acids, and minerals. For instance, the levels of oleic acid, Σ n-9, and Σ MUFAs in Daphnia biomass were correlated with their diets (Turcihan et al., 2022). Moreover, some microalgal-based powder diets can also improve Daphnia's nutritional components, including not only fatty acids but also essential amino acids (Zeybek and Eryalçın, unpublished data 2023).

Daphnia individuals are of suitable size for the first feeding of fish larval nutrition. Recent studies have revealed that daphnia can be a substitute for Artemia, which is a very limited source in nature worldwide (Chakraborty and Mallick, 2023). Due to their high protein content, Daphnia biomass can also be utilized in the form of powder as a dietary supplement in carp (Abdel-Tawwab et al., 2020; Bogut et al., 2010; Suantika et al., 2016), barramundi (Chiu et al., 2015), grey mullet (Abo-Taleb et al., 2021), and kuruma shrimp (Mona et al., 2017) diets. Therefore, the successful culture of Daphnia is more important than ever before. Growth performance in Daphnia culture is evaluated by several parameters. First of all, the Daphnia stock culture should survive and produce some females that carry eggs for future production. Sudden and extreme changes from optimum levels of culture water conditions, such as pH (optimum pH:

6–7), oxygen (O_2 >5.5 ppm), minerals (Ca^{++} is essential for the exoskeleton), and temperatures (20–22 ºC), can lead to Daphnia creating resting eggs called epiphia. This event will affect the total Daphnid production in a closed environment. Water fleas can be cultured with various microalgae with gentle aeration and daily water renovation. They reproduce parthenogenetically when physical and chemical conditions are optimal, and diets are available (Figure 10).

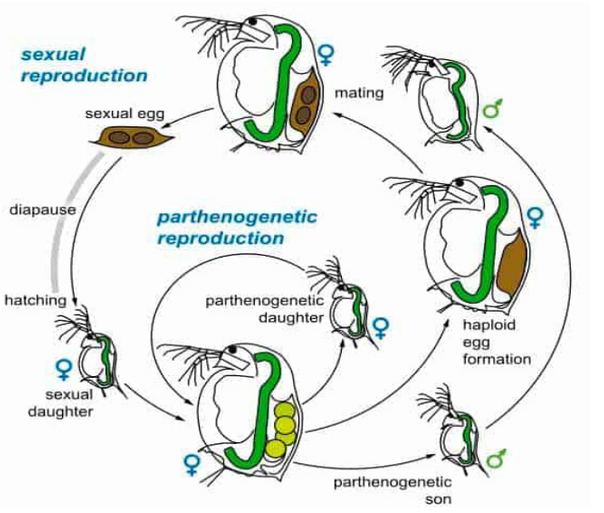

Figure 10. Daphnia life cycle (Ebert, 2005)

There is still a high demand for Daphnia culture, with large production for both aquarium and freshwater fish feeding. Moreover, recent studies have revealed that water flea species can be used as feed ingredients in formulated microdiets. Therefore, large-scale production techniques should be applied, and the private sector should produce water fleas to meet the demand for aquaculture.

1.6. Worms

The Food and Agriculture Organization (FAO) has predicted that by 2025, 1.8 billion people in countries worldwide will experience water scarcity (Van Huis et al., 2013). Therefore, the potential use of insects as a sustainable protein source for the rapidly growing population has been the subject of increasing debate. Edible insects, in particular, have gained attention due to their low requirements for feed, land, and water compared to traditional sources of protein. Furthermore, insects emit less CO_2 and greenhouse gases than cattle and small livestock, have a high feed conversion efficiency, and are easy to store on a large scale (Premalatha et al., 2011; Van Huis et al., 2013; Dobermann et al., 2017).

In recent years, there has been a growing interest in exploring alternative protein sources to meet the increasing demand for food in a sustainable and environmentally friendly manner (Rumpold and Schlüter, 2013). Yellow mealworms, in particular, have been shown to be effective in the biological transformation of organic waste, and they can convert about 1.3 billion tons of bio-waste annually (Veldkamp et al., 2012). *Tenebrio molitor* larvae exhibit a significant abundance of protein, fat, and indispensable amino acids, endowing them with great potential as a nutritional protein source for human consumption as well as animal feed. In addition, the cultivation of mealworms requires less water and land compared to traditional livestock production, and their waste products can be used as fertilizer (Lundy and Parrella, 2015). Another nutrient source, *Zophobas morio*, has been shown to consume and biodegrade plastics, such as polystyrene or polyethylene, and may be effective in waste management (Rumbos and Athanassiou, 2021). Overall, *Zophobas morio* and *Tenebrio molitor* have significant potential as sustainable sources of protein and as a means of reducing food waste and plastic pollution. However, further research is needed to fully explore their potential and to develop efficient and cost-effective methods for their cultivation and use.

Oligochaetes worms are considered one of the most cost-effective live feeds for fish and prawns (Marian and Pandian, 1984). *Enchytraeus albidus* is a small, white, soil-dwelling worm that is commonly used as a model organism in ecotoxicology studies due to its sensitivity to environmental pollutants (Schmelz, 2003; Spurgeon, 2010). In addition, it has been shown to play an important role in soil processes such as nutrient cycling, decomposition, and soil structure formation (Nielsen et al., 2020). *Tubifex tubifex*, on the other hand, is a freshwater oligochaete worm that is widely used as a live food source for fish and other aquatic organisms in aquaculture (Marian and Pandian, 1984; Hossain et al., 2012). It is a hardy and easy-to-rear species that can be cultured on a variety of organic substrates, making it an economical and sustainable alternative to conventional artificial feeds (Phillips and Buhler, 1979; Mollah et al., 2009). However, it has been found that *T. tubifex* from polluted waters can harbor human pathogens responsible for diseases such as hepatitis (Jewel et al., 2016), highlighting the importance of developing pollution-free culture technologies. Therefore, *Enchytraeus albidus* and *Tubifex tubifex* are two species of oligochaete worms that have significant ecological and practical importance. Studying their biology and ecology can provide valuable insights into the functioning of the soil and aquatic ecosystems, while their practical applications in ecotoxicology and aquaculture can have economic and environmental benefits.

1.6.1. Sludge worm (*Tubifex tubifex* Muller, 1774)

Tubifex tubifex is an aquatic oligochaete species classified in the Animalia kingdom, Clitellata class, and Tubificidae family according to the taxonomic hierarchy (*Tubifex tubifex* Müller, 1774) (Lucan-Bouché et al., 1999; Kolesnyk et al., 2019) (Figure 11). It is a benthic organism found in freshwater ecosystems worldwide, particularly in sediment-rich habitats such as rivers, streams, and lakes. It is also commonly found in sewage treatment plants and other areas with high levels of organic matter (Şahin et al., 2011).

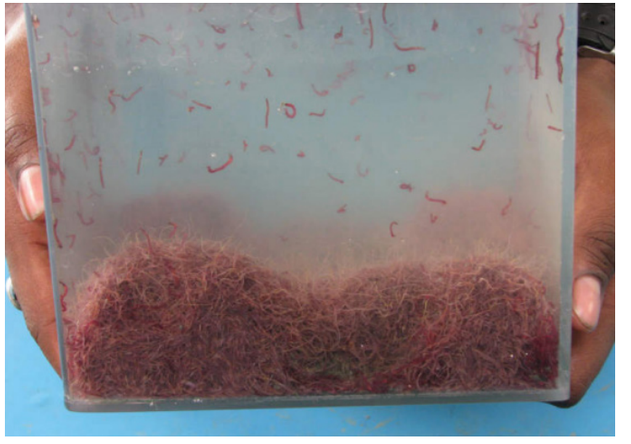

Figure 11. Sludge worm (*Tubifex tubifex*) individuals (Mandal et al., 2018)

Sludge worms, also known as Tubifex tubifex, live in silt and slime-lined tubular burrows, which gave them their name, forming large clusters in sludge-rich environments (Ikhsan et al., 2021). These pinkish-red, thread-like creatures are approximately 8 cm long and 0.6-0.7 mm thick and have four chaetae on each body segment, except for the first two segments in front of the mouth (Snimshhikova and Linevich, 1987). *T. tubifex* is a hermaphrodite species, with both male and female reproductive organs located in segments 10 and 11. However, individuals must exchange sperm with another individual to reproduce (Van Haaren and Soors, 2013). The clitellum, located in the anterior third of the body, secretes the cocoon during mating, and interpersonal sperm transfer takes place within this structure. The cocoon also provides nutrients for the embryological development of the fertilized egg until it hatches into a worm (Van Haaren and Soors, 2013). Sludge worms can produce up to four cocoons per year (Kaster, 1980). These creatures constantly swallow sludge with their front end as they reside at a depth of 5-10 cm in the soil, aiding in the mineralization of the soil by excreting simple minerals (Verdonschot, 1989; Reynoldson et al., 1991; Fedonenko et al., 2017).

Sludge worms, also known as Tubifex tubifex, are commonly used as an inexpensive live food for fish and other aquatic animals due to their high protein and essential fatty acid content. However, their exact nutrient content can vary depending on factors such as species, diet, and rearing conditions (Herawati et al., 2020). Sludge worm is characterized by its high proximate composition, specifically crude protein (52.11-65.30%), crude lipid (7.62-12.29%), crude fiber (4.07-9.55%), and crude ash (4.31-11.82%) (Herawati et al., 2020). Additionally, they have a significant content of n-3 fatty acids (18%) and n-6 fatty acids (22%). The amino acid profile of the protein in sludge worms is ideal for fish, with lysine and leucine being the most abundant amino acids. Furthermore, these worms contain carotenoids at a concentration of 15.02 mg/kg (Yanar et al., 2003).

Harvesting sludge worms in natural conditions can be risky due to their preference for contaminated waters (Reynoldson et al., 1991). To mitigate this, sludge worms should be grown in a controlled environment with steady water flow and high organic detritus. Environmental variables such as water temperature, oxygen, and substrate properties play a role in reproduction and growth, with temperature and oxygenated water being important factors (Jewel et al., 2016). The ideal temperature for sludge worm production is 22°C, and they can tolerate temperatures between 20 and 27°C but should be kept away from temperatures over 30°C. To culture sludge worms, a container with 50 to 75 mm of thick pond mud blended with decaying vegetable matter and bread can be used. The system should be inoculated with sludge worms (62.5 g/m²) obtained from muddy canals or sewage canals, and clusters of sludge worms will develop within 15 days. The most efficient production time is 20 days with a continuous mild water flow and a suitable drainage system (Fedonenko et al., 2017). Feed for sludge worms is organic matter, such as bread or manure, given once every 3-4 weeks. When mud worms are deprived of oxygen, they come to the surface (Das et al., 2012). Therefore, before feeding to fish, sludge worms must be washed and cleaned to remove accumulated pollutants and reduce the risk of disease and parasite transmission.

Overall, sludge worms have the potential to be a valuable food source for aquatic animals, but their culture requires careful attention to environmental variables and proper cleaning before use.

1.6.2.White worms (*Enchytraeus albidus* Henle, 1837)

The white worm, also known as *Enchytraeus albidus*, is a terrestrial species and is one of the first enchytraeids ever described that belongs to the Annelida phylum, Clitellata class, and Enchtraeidae family (Bunke, 1998; Erséus et al., 2019) (Figure 12). This species is found

along the coasts of northern Europe and the Arctic and is viable in both fresh and salty water as well as in soil (Stephenson, 1930; de Boher et al., 2018; Erseus et al., 2019).



Figure 12. White worms (*Enchytraeus albidus*) (https://cdn.shopify.com/s/files/1/0522/7623/2391/products/grindalworms_540x.jpg?v=1609851985)

Enchytraeus albidus is a relatively larger species within the genus, exhibiting a length ranging from 10 to 35 mm and a diameter of 0.5 to 1.0 mm. It features a pure white coloration (occasionally with a yellowish hue) and is generally transparent when viewed through a microscope (Bell, 1958) (Figure 12). The segment count of *Enchytraeus albidus* ranges from 52 to 74. The presence of a clitellum is a characteristic of adult individuals. The clitellum spans the entirety of segments 12th and 13th (Bell, 1958). The clitellum of *Enchytraeus albidus* is a specialized structure in the epidermis responsible for secreting a cocoon that serves as the site for depositing eggs and extruding spermatozoa received from a mating partner into the cocoon to facilitate fertilization (Jamieson and Ferraguti, 2006). *Enchytraeus albidus* has a hermaphroditic reproductive system, but most species show sexual reproduction (Hönemann and Nentwig 2009). During mating, the sperm is transferred from one worm to another through copulation (Jamieson and Ferraguti, 2006). The fertilized eggs are laid in cocoons that are produced by the clitellum. The cocoons are usually deposited in soil or aquatic environ-

ments, where the young hatch and develop (Maraldo and Holmstrup, 2009). The lifespan of a white worm is between 2 to 9 months, during which it can produce up to 1000 viable eggs (Hönemann and Nentwig, 2009; Fairchild et al., 2017). Egg diameter in *Enchytraeus albidus* varies between about 300 and 500 µm (Jamieson and Ferraguti, 2006). Sexual maturity is reached 5–7 weeks after hatching from the egg (Hönemann and Nentwig 2009).

E. albidus is known to contain a crude protein content that ranges from 45-70% of dry weight and a crude lipid content of approximately 15-20% of dry weight (Holmstrup et al., 2020; Dai et al., 2021). The ash content of this species is typically around 6% (Fairchild et al., 2017). White worms are known to have lower levels of n-3 fatty acids (11-23 mg/g dry weight) and higher levels of n-6 fatty acids (31-126 mg/g dry weight) (Fairchild et al., 2017). Furthermore, certain literature recognized that the total fatty acid levels are approximately 15-20% of the dry weight (Dai et al., 2021). Lysine and arginine are the most abundant amino acids, with percentages of 7.2% and 6.0%, respectively (Holmstrup et al., 2022). These worms have a high glycogen content of 20-25% of dry weight (Dai et al., 2021).

For cultivating white worms, it is necessary to maintain a temperature of 17-18°C, a humidity of 23-25%, and use slightly acidic to neutral soil. The worms are cultivated in soil with a soft texture, high porosity, and water-holding capacity in moistened plastic boxes, and poured to a height of 10-15cm (Walsh et al., 2015). White worm culture is added to the soil at a depth of 3−4 cm at a rate of 200−250 g/m^2. Following this, 2-3 ditches with a depth of 5cm are dug in the ground, where some of the food is placed and then covered with soil. In a thriving culture, the worms concentrate on the soil thickness near the bait (Springett, 1964). Different cereals, flour, bran, vegetables, roots, green herbaceous plants, berries, fruits, and yeast are used for feeding, which is carried out once a week (Walsh, 2012; Fairchild et al., 2017). To control pests such as mites and fly larvae, the culture boxes should be kept covered, and any mold-like particles in the food should be removed immediately (Fedonenko et al., 2017). The use of worm culture begins during the period of maximum increase in their biomass, that is, within 40-50 days from the moment of cultivation beginning (Springett, 1964; Fedonenko et al., 2017). It should be refreshed every six months by changing the substrate in the culture medium and creating new cultures (Fairchild et al., 2017; Fedonenko et al., 2017). For the harvest of the white worms, a container of water can be used along with a light source. The soil containing the worms is placed in the container, and the worms will gradually move to the surface and form dense tangles. The tangled worms can then be transferred to another container of water to remove any remaining soil. Sometimes special heaters are used that generate heat (Fedonenko et al., 2017).

Consumption of white worms may increase the risk of obesity due to their high glycogen content, despite their beneficial metabolic fuel properties. Hence, it is important to regulate the number of worms given to fish during feeding. Furthermore, detailed cultivation methods are necessary to improve the HUFAs content of white worms during the production process. This can help address the issue of imbalanced fatty acid composition, which is a concern associated with using white worms as fish feed.

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PART III

ADVANCES IN FISH MANAGEMENT

CHAPTER 10

BROODSTOCK MANAGEMENT AND NUTRITION

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DOI: 10.26650/B/LS32LS24.2024.005.010

1. Introduction

Broodstock management and farming practices are crucial to obtain high quality larvae and offspring in aquaculture. The main requirement for the successful mass cultivation of fish is the availability of eggs and sperm of quality. Sustainable, large quantity of healthy juveniles can only be obtained from broodstock that kept under suitable environmental and nutritional conditions (Duncan et al., 2013; François et al., 2021). To achieve successful gonadal growth, gamete maturation and spawning in captive fish, it is important to understand the reproductive physiology and the spawning processes of the target species. In order to function normally, the environmental conditions must be at optimum levels and the broodstock must be free from stress (Conte, 2004;

Berlinsky et al., 2020). However, there is still insufficient information on controlled reproduction and broodstock welfare for many aquaculture species. It is important for aquaculture success to select broodstock with good genetic characteristics and use them in production.

Genetic programs are essential aspects of broodstock management, as they are the keys for the selection and breeding of fish with desirable genetic traits. These programs involve careful genetic analysis of broodstock populations, identifying individuals with desirable traits such as disease resistance, growth rate, and tolerance to environmental stressors (Ferosekhan et al., 2021). Through selective breeding, these desirable traits can be passed down to future generations, resulting in healthier and more productive populations. In the context of aquaculture, genetic programs are critical for ensuring the sustainability and profitability of fish farming operations. By selectively breeding broodstock with desirable traits, fish farmers can produce fish that are better adapted to local environments, have improved growth rates, and are more resistant to disease. This leads to higher yields, increased profitability, and a reduced environmental impact (Senanan et al., 2015; Garber et al., 2019; Swain et al., 2022). Overall, genetic programs play a vital role in broodstock management, enabling fish farmers to produce healthier and more productive fish. In addition, nutrition has an important effect on the reproductive performance of broodstocks.

The success of obtaining high quality larvae in aquaculture is directly related to broodstock nutrition (Luquet and Watanabe, 1986; Fernández-Palacios et al., 2011). There are many fish species cultured in world aquaculture. However, research on nutrition of broodstock used in the culturing of these fish are limited. This is because large facilities are needed to hold broodstock groups and these facilities are costly to conduct long-term broodstock experiments (Izquierdo et al., 2001; Fernández-Palacios et al., 2011). Moreover, reproductive biology varies considerably among the various cultured fish species and is directly related to the nutritional needs of the species. As with other vertebrates, most of the problems in the development of fish are related to broodstock nutrition. Therefore, the composition of the diet, the amount of feed taken by the fish or the time of feeding can significantly affect the reproductive performance of the broodstock (Fernández-Palacios et al., 2011; De-Dios et al., 2022).

This chapter provides information about the source, selection, transportation, stocking density, genetic characterization, environmental requirements, and reproduction of broodstock fish. Future research avenues are also discussed, including the nutritional needs of broodstocks, feeding strategies, the need for developing an adequate broodstock diet for successful reproduction, effect of food restriction, and specific feed ingredients. In addition, research and practices related to broodstock management and nutrition in Türkiye were evaluated.

2. Broodstock Management

2.1. Sources of broodstock

Broodstock can be formed from two sources, wild caught fish or cultured fish in captivity. Wild fish should be caught with professional or advanced fishing gear in order not to be harmed. Cultured fish can be obtained from self produced stocks or from other farms. In this case, the development performance and genetic control of broodstocks in a farm may vary according to the fish source (Duncan et al., 2013).

The selection of broodstocks should be carried out starting from the juvenile stage. The following characteristics should be sought in broodstock selection (Bromage et al., 1992; Atasever and Bozkurt, 2011; Rocha and Dinis, 2017):

- Body form and color clearly reflect the species characteristic,
- No skeletal deformation,
- Fast growing and good utilization of feed,
- Resistant to diseases,
- Not exposed to infection or toxic substance,
- Having an original body form,
- Having a high reproductive potential.

The methods of obtaining broodstocks of some species cultivated worldwide are (i.e., captured from the wild and cultured in facilities) summarized below.

In the past, broodstocks used in the production of species belonging to the Salmonidae family were mostly captured from the wild. However, it was observed that broodstocks obtained from fish adapted to culture conditions were more successful in production. Today, Atlantic salmon (*Salmo salar*) (Pepper and Crim, 1996), the rainbow trout (*Oncorhynchus mykiss*) and the brown trout (*Salmo trutta*), farms mostly supply their broodstock needs from their own fish or modern facilities (Firidin et al., 2013). It is known that rainbow trout is widely cultivated in Türkiye. However, other salmonid species are also cultivated in low quantities. The broodstock supply of these fish is carried out in a way similar to the above-mentioned methods for salmonids (Salihoğlu et al., 2013). In addition, sturgeon (*Acipenser gueldenstaedti*) broodstock breeding is carried out in Istanbul University Sapanca Aquaculture Research Center.

In the early days of aquaculture, the broodstocks of marine fish such as sea bream (*Sparus aurata*) and sea bass (*Dicentharus labrax*) grown in Türkiye as well as in Mediterranean countries were obtained from wild. However, modern facilities have been established where these broodstock fish are grown today. For this reason, producers choose off-springs with high growth and development performance and obtain broodstocks with high reproductive performance and disease resistance (Çoban et al., 2004; Fırat et al., 2004). For some sparids other than sea bream, such as common, red and silver sea breams, broodstock sources are still obtained from nature together with the methods mentioned above.

In fish species such as carps, tilapias, channel catfish, grouper (*Epinephelus fuscoguttatus*), and eel, which are mostly grown on different continents around the world, the broodstock is generally obtained from fish grown under control from the fry stage (Kelly, 2004). We have seen that the broodstocks of species such as carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and grouper are also procured from modern farms in Türkiye. The turbot (*Psetta maxima*) broodstocks, which are reared in Türkiye, are obtained from the stock in the Central Fisheries Research Institute, and from time to time with additions made from the wild (Çiftci et al., 2002). The most common source of shrimp broodstock for commercial hatcheries is shrimp from farms, but it is also common for farms to seasonally source some wild broodstock from rivers and ponds (Daniels et al., 2009).

Crayfish broodstocks are obtained from aquaculture farms and from nature. Crayfish can be obtained from any pond or natural habitat as long as they are healthy and not stressed. The source of broodstocks does not negatively affect the survival or reproductive success. However, it has been reported that water temperature, quality and quantity of food sources, stocking density parameters are mostly effective on the variability in reproductive success (Thefishsite, 2010).

The supply of crab broodstocks is provided from both wild-caught and aquaculture farms. Crabs are generally collected from their natural habitats using trawl nets, bait traps, hoop and bag traps, gill nets, hand and scoop. The brootstock size used for breeding is important for reproduction studies. In general, the eyestalks of the broodstocks are removed to increase the molting frequency of the crabs for reproduction purposes and to accelerate the spawning with the development of the gonads (Azra and Ikhwanuddin, 2016).

2.2. Genetic stock characterization of broodstock

Broodstocks should have enough samples to accurately represent the genetic identity of the stock to be developed. This number is very important because the gene pool of the parent will determine the maximum genetic diversity that can be passed on to the offspring (Allendorf and Ryman, 1987; Taniguchi, 2003). Good broodstock management contributes to maximizing the transmission of genetic information to the offspring. Prolonged inbreeding increases the ratio of homozygous genotypes in fish. Since relatives are more likely to carry the same deleterious alleles, harmful genetic structures may occur in the fish. Several studies on fish species have shown inbreeding to be associated with morphological abnormalities, slow growth, or low reproductive success. For this reason, farms should periodically renew their broodstock resources to preserve the genetic richness of the fishes (Loukovitis et al., 2014; Venney et al., 2016; D'Ambrosio et al., 2019). These practices are highly important for the sustainability of a fish farm. In Türkiye, rainbow trout, sea bream and sea bass species are mostly cultured in farms. Broodstocks used in these farms are supplied from other farms at certain periods to preserve their genetic richness as stated above (Karahan, 2009; Firidin et al., 2013), which is the key one of the key factors for the successful aquaculture practices in Türkiye.

2.3. Transportation and handling

Physical injury and physiological stress during capturing, handling and transportation of broodstock might have a significant detrimental effect on spawning success. Fish should be carefully handled in suitable tanks, and optimum water conditions must be provided to minimize stress. Female fish that are ready to spawn are very sensitive to stressors. Stress and injuries can cause rapid physiological changes that can result in the breakdown (resorption) of the eggs in the gonads before the spawn. Fluctuations in water temperature and low dissolved oxygen level can accelerate the resorption of eggs. If the environmental conditions are not suitable, the broodstocks can be stressed easily. This causes failure in ovulation and lowers the egg quality (Mohamed and Devaraj, 1997; Soso et al., 2008).

There are many uncertainties in the process of broodstock transportation, such as weather conditions and delays during transportation. Negative factors during transportation may be a source of greater stress than the adaptation of fish to their new habitat. If the distance between the place where the brood fish are obtained and where they will be transported is relatively short, the stress factor will be reduced. If broodstock is grown from selected fish, it is possible to keep fish with high stock densities in tanks and ponds just before the spawning season, and to grow the broodstock in a stress-free environment (Vatanakul et al., 1997; Nnaji et al., 2009).

Fish crowded in a shipping tank can quickly become stressed by physical injury, deteriorating water quality, rapid changes in water temperature, and osmotic imbalance. Transpor-

tation tanks in transport vehicles should be large enough for the broodstock to move freely, and the tanks should not be in shape that can harm the fish. Transportation tanks are usually aerated with air stones, electric mixers, or both. A high level of dissolved oxygen is very important for broodstocks to meet their oxygen needs during capture and transportation. When the broodstock are caught from nature, oxygen should be supplied to the tanks for them to live in a healthy way and to prevent stress. For long distances, water mixers must be used in addition to oxygen to remove the carbon dioxide accumulated in the water. The potential breeders should generally be caught when the waters are cold, and although it differs according to the fish species, it is necessary to carry out their transportation early in the morning or night to reduce the physiological stress and metabolic rate of the fish. Ice can also be added to the water to prevent the increase in water temperature during transportation. Salt (0.3-1.0 percent) can be used in the transport water to minimize osmotic stress and infection. It has also been reported that anesthetics have been successfully used during the transport of fish (Orina et al., 2014; François et al., 2021).

It is extremely important to be gentle when handling fish to avoid physical injury and physiological stress. Damage to the mucus layer, scales and skin of the fish can lead to infection. It also causes excessive water intake by freshwater fish or water loss (osmotic stress) by marine organisms. When capturing fish, it is recommended to use scoops with fine mesh nets to minimize injury and scale loss. When removing fish from the water, it is necessary to minimize the number of fish in the transportation tanks and to carry out the transport process as quickly as possible. Time spent out of water with fish during transportation can make the difference between a good spawn and no spawn or death (Mohamed and Devaraj, 1997).

Although prawn broodstocks generally have similar requirements as fish, there are also prawn-specific differences. Buckets can be used to transport broodstocks over short distances. However, for longer distances, double polyethylene bags placed in insulated containers can be used. All the air in the bags must be replaced with oxygen. After the bags are inflated with oxygen, they must be tightly tied to prevent loss of oxygen and water during transportation. Using ice during the transportation of prawns decreases the temperature of the water and the metabolic rates of prawns, increases their survival rate, and reduces oxygen consumption and nitrogenous excretion (Chen and Kou, 1996). The optimum water temperature for transport should be around 19 to 20°C. But a constant water temperature during transport is also important for success (Daniels et al., 2009).

Proper handling of crayfish broodstocks is important for production success. Attention should be paid to the transportation period of broodstocks because long transportation periods

can cause damages. In addition, prolonged exposure to high temperatures, sunlight or wind can cause broodstocks to become stressed or die. It is necessary to keep the crayfish in shade and periodically wet the crayfish sacks to protect them from negativities during transportation. The limited use of ice during transport can be a good method for control of temperature and humidity. Harvesting and transporting crayfish broodstocks at dusk or at night is a convenient way to reduce stress. We should always be careful when handling animals. Rough handling can cause damage of the delicate exoskeleton or even death (Thefishsite, 2010).

Various methods have been tried to minimize the loss of life during the transport of wild-caught crabs (Stoner, 2012). The health of the animals can be compromised by various forms of things such as prolonged exposure to the air in the boat or due to stacking with other captured individuals. Usually, the shelipads are tied with jute or nylon thread for easy handling of live crabs. Gills of live adult crab from nature are immersed in fresh and clean sea water to keep them moist, and packed in a bamboo basket with wet marine plants to maintain a cool and moist environment during transport. If the transport period is long, the crabs are immersed in sea water for survival after 3-4 days. Another method is to pack adult crabs weighing 350-700 g, one crab in each polyethylene bag containing 6 liters of oxygenated sea water. Crabs obtained from nature or culture ponds are disinfected in 100 ppm formalin for 30 minutes before being transferred to broodstock holding tanks. Adult crabs are then kept in a tank containing sea water for a week for acclimatization (ICAR, 2011).

There are well-developed farms in Türkiye that produce rainbow trout, sea bream and sea bass. In addition, carp, tilapia, and some new species are produced. In case of need, the methods mentioned above are used when transporting broodstock to these farms. Since the short-term attempts on shrimp farming in Türkiye were unsuccessful, there is no aquaculture activity currently, so no information about shrimp has been provided here.

2.4. Stocking density and sex ratios

Stocking density of broodstock is directly affected by the physical, biological and chemical conditions of the water (temperature, dissolved oxygen, pH, flow rate etc.) (François et al., 2021). In this chapter, stocking density and sex ratios of broodstocks (Table 1) were evaluated within optimum breeding conditions.

Table 1. Broodstock stock density and sex ratios of fish species widely farmed in the world

Species	Stock Density	Sex Ratios (Male/Female)	References
Rainbow trout, *O. mykiss*	10 kg/m^3	1:3 – 1:8	North et al., 2006; FAO, 2023a
Seabass, *D. labrax*	10-15 kg/m^3	1:1, 2:1 or 3:2	FAO, 2023b
Seabream, *S. aurata*	10-15 kg/m^3	1:1, 2:1 or 3:2	FAO, 2023c
Catfish		1:1 - 1:4 or 2:3	Kelly, 2004
Carp	6 fish/m^2	2:1 or 3:1	Hayat et al., 2018
Tilapia	0.3-0.7 kg/m^2 in small tanks 0.2-0.3 kg/m^2 ponds	1:2 or 1:3	FAO, 2009
Prawn	30-90 prawns/m^2 in tanks	1:4 or 1:5	Daniels et al., 2009
Crayfish	5 to 16 crayfishes/m^2	1:1 or 1:2	Thefishsite, 2010; Nelson and Dandy, 1977.
Crab	2 to 6 crabs/ m^2	1:1 or 1:2	Waiho et al., 2015
Turbot, *P. maxima*	2-3 kg/m^2	1:1	Çiftci et al., 2002

For trouts, the broodstock population is sufficient for 1% of the farm's table fish production. For example, for a farm with production capacity of 100 tons, 1 ton of broodstock should be kept in tanks. Broodstocks should be kept at a stock density of 10 kg/m^3 in high quality water conditions. The male/female ratio should be 1:3 to 1:8 (North et al., 2006). Usually, males reach sexual maturity at 2 years old and females at 3 years old. Although two-year-old trout start spawning, females are seldom used for propagation before they are three or four years old. Broodstocks between the ages of 2-6 years are preferred to be used in production (Okumuş, 2002; FAO 2023a). In determining the egg production capacity in the farm, 2000 eggs were calculated for each kg of female broodstock (FAO, 2023a).

For seabass, the number of broodstocks per 1 m^3 of water varies depending on the size of the fish. Broodstocks should be stocked at 10-15 kg/m^3 water. The female-male ratio should be 1:1, 1:2 or 2:3 depending on the size, sexual maturity, and reproductive performance of the broodstock. The optimal age for female broodstock is between 5 and 8 years, whereas for males this range is 2-4 years. A flow rate of 10-20% per hour should be applied to the tanks. The water temperature should be 14-15°C. Natural seawater salinity should be used in tanks. The eggs are pelagic, so the water drainage of the tanks should be from the surface. For this, recuperator systems with 500-micron mesh size should be placed at the water drainage at the top of the tanks (FAO, 2023b).

For seabream, broodstocks should be kept at a stock density of 10-15 kg/m^3 in 4-7 m^3 tanks. Similar to seabass, the female-male ratio should be 1:1, 1:2 or 2:3 depending on the

size, sexual maturity and reproductive performance of the broodstock. The optimal age is 5 for female, and 1 for male broodstock. To obtain out-of-season eggs, optimum photoperiod conditions should be applied to indoor tanks (FAO, 2023c).

For channel catfish, 1:1, 1:2, 1:3 or 1:4 male-female ratios can be used. The probability of inbreeding increases when small populations are used. If less than 200 broodstock are used as the breeding population and only their offspring are the future broodstock, a 1:1 male-female ratio should be used to minimize inbreeding. Commercial catfish producers stock spawning ponds with 1:2 or 2:3 male-female ratios (Kelly, 2004).

For carp, the male/female ratio of 2:1 and 3:1 has a significant impact on fertility, fertilization rate, hatching rate and the total number of offspring produced. The survival of the offspring is not much affected by the male/female ratio of the broodstocks. Water quality may also be responsible for the survival of the offspring (Hayat et al., 2018). Stocking density for carp broodstocks is about 6 fish/m^2

For tilapia, the male to female ratio is 1:1-4, with the most common being 1:2 or 1:3. Stocking density for broodstocks varies from 0.3-0.7 kg/m^2 in small tanks to 0.2-0.3 kg/m^2 in ponds (FAO, 2009).

For turbot, the male to female ratio is 1:1. The initial stock density for 3-4 years old fish is 2-3 kg/m^2 while it is about 5-6 kg/m^2 for 2 years old fish (Çiftci et al., 2002).

For prawns, male to female ratios of 1:4 or 1:5 is mostly used for their successful culture. In the holding system, broodstock can be kept in tank volumes of 2 to 5 m^3 for those larger than 30 g at densities of 30 to 90 prawns/m^2 (Daniels et al., 2009).

For crayfish, the sex ratio and performance of broodstocks are important. The female to male ratio should be at least equal in stocking broodstocks. A higher ratio of females to males is also recommended, as healthy males can mate with more than one female (Thefishsite, 2010). Research with adult crayfish has shown that survival during the breeding season is not significantly different between densities of 5 to 16 crayfish/m^2 (Nelson and David, 1977).

For crabs, male to female ratios of 1:1 or 1:2 are mostly used for their culture. Research shown that using stocking density of 2 to 6 crabs/m^2 maximizes breeding output while maintaining low mortality (Waiho et al., 2015).

Rainbow trout, sea bream and sea bass are widely cultured in Türkiye, with broodstock breeding carried out under modern conditions in farms. Broodstock breeding and stocking of these species are carried out in ponds or tank systems (Çoban et al., 2004; Fırat et al., 2004;

Firidin et al., 2013; Salihoğlu et al., 2013). In addition, carp culture was mostly done in public research units in the past. For this reason, carp broodstock cultivation was also widely carried out in these centers. Recently, carp culture has decreased considerably due to the low market value. Broodstock culture continues in a few research stations. Broodstock breeding and stocking are generally carried out in concrete and earthen ponds. Similarly, tilapia broodstock cultivation is carried out locally at a few stations in pond systems. Turbot culture was carried out in the Black Sea Region between 1997-2004 in cooperation with the Japan International Cooperation Agency (JICA) and the Ministry of Agriculture of Türkiye, General Directorate of Agricultural Research and Policies (TAGEM) and since this date, TAGEM has continued this culture by itself. Turbot broodstock breeding is also carried out in this research center (Aydın et al., 2019). Stocking density and male to female ratios in the breeding of broodstocks of these species are maintained according to methods specified above.

2.5. Broodstock holding systems

Holding systems refer to the structures or containers used for housing and maintaining broodstock for breeding purposes. The appropriate holding system is important for the health and well-being of the fish and for the success of the breeding program. Holding tanks should be large enough to allow complete freedom of movement to the broodstock. Round tanks or tanks with rounded corners are preferable because they minimize injury to the fish. Holding tanks should be covered to provide shading that will help quiet excitable species and prevent the fish from jumping to their death (Aiken and Waddy, 1995; Kelly, 2004; Daniels et al., 2009; De et al., 2020).

There are several types of holding systems that can be used to keep broodstock for breeding purposes, including:

- **Tanks:** Tanks are the most common type of holding system for broodstock. They come in various sizes and materials and can be used in both indoor and outdoor settings. Tanks are versatile and can be easily controlled to maintain stable water quality and temperature.
- **Ponds:** Ponds are natural or man-made structures that provide a large, open environment for broodstock. They can be used to culture a variety of fish species and are ideal for outdoor settings.
- **Raceways:** Raceways are long, narrow tanks that are used for culturing fish. They are designed to provide a high flow of water, which helps to maintain good water quality and promote the growth and health of the fish.

- **Flow-through systems:** Flow-through systems are designed to enable fresh water to continuously flow into the holding system, which helps to maintain good water quality and minimize the buildup of waste. They can be used in both indoor and outdoor settings.
- **Recirculating systems:** Recirculating systems are designed to recycle water within the holding system. They typically use filters and other treatment systems to remove waste and maintain water quality. These systems are often used in indoor settings and can be highly controlled to maintain stable water conditions.

Each type of holding system has its own advantages and disadvantages, and the best system for a particular situation will depend on the species of fish cultured, the environmental conditions, and the goals of the breeding program.

Shrimp broodstocks are acclimatized in holding tanks for 4-7 days in the hatchery. Holding tanks should be sized to provide adequate space and aeration. Approximately 60% of the water in the tanks should be changed daily. Once the shrimps have adapted to the environment, they are encouraged to molt by adjusting the salinity of the water in the tanks (Kungvankij et al., 1985).

Adult crayfishes are benthic forms that prefer to crawl on the seafloor where light penetration is minimal. To simulate natural conditions, the inside of broodstock tanks are often painted black and covered with dark screens. The lighting in the broodstock holding unit should be at a minimum level. Crayfishes are often seen on sandy surfaces and among rocks. Crayfishes should have a layer of sand at the bottom of their broodstock tanks where they hide. Crayfish broodstock tanks should be equipped with structures that provide surfaces or crevices for attachment and shelter (Kizhakudan, 2014).

Keeping crab broodstocks healthy and stress-free is crucial for successful larval production. There should be holding and spawning tanks for the broodstocks transported to the facility. Broodstocks can be kept in maturation tanks for several weeks or longer. By keeping mud crab broodstocks under low light keeps their stress at low levels, resulting in a more successful breeding performance. For this, it is necessary to use shades or similar applications. Placing shelters in the broodstock tank creates refuge for crabs and reduces stress and fighting between themselves. Since adult crabs can easily escape from tanks, precautions should be taken to prevent their escape. Female crabs need a layer of sand at the bottom of the tanks to lay their eggs successfully (Shelley and Lovatelli, 2011).

In Türkiye, culture of marine and freshwater species is generally carried out in modern systems such as tanks, ponds and recirculating systems as stated in the previous sections. Broodstock culture is carried out under modern conditions in these holding systems. Maximum success is achieved on broodstock breeding under these conditions. There is no commercial shrimp and crayfish farm in Türkiye. For this reason, no information was given about the structural features of the farms. However, there is one farm that cultures blue crab in Milas/Muğla of the Mediterranean Region. The farm uses vertical box and earthen pond systems for crab farming. In addition, the project prepared by Tosun et al. (2023) in the Aquaculture Department of the Faculty of Aquatic Sciences of Istanbul University on blue crab culture continues. This project is supported by The Scientific and Technological Research Council of Türkiye (TUBITAK).

2.6. Environmental requirements

Environmental factors in the broodstock holding tank such as dissolved oxygen, water temperature and absence of disturbance to the fish following hormone injections are believed to play an important role for successful induced spawning. The handling stress and the physiological processes of final maturation of eggs and sperm increase the oxygen demand of the broodstock. High temperature accelerates egg maturation, resulting in an even greater oxygen demand by the fish. Elevated temperature will also increase the rate of development of pathogen organisms. However, if the temperature is too low, spawning will be delayed or in many cases completely inhibited (Schreck et al., 2000; Izquierdo et al., 2001; Bureau et al., 2002; Flores, 2014; Hill et al., 2013; Maneiro et al., 2020; Berlinsky et al., 2020; François et al., 2021; Septian et al., 2022).

Ensuring that the environmental requirements of broodstock are met is crucial for their health and well-being, as well as the success of breeding programs. Here are some of the important environmental requirements for broodstock:

- **Water quality:** The water quality for broodstock should be stable, with appropriate levels of temperature, pH, dissolved oxygen, and other water quality parameters. Maintaining good water quality helps to prevent disease and stress in the fish and can also improve the survival rate of eggs and fry.
- **Water temperature:** Different species of broodstock have different temperature requirements. Maintaining a stable temperature is important for the health of the fish and can also affect the hatching and survival rate of eggs.

- **Water flow:** Adequate water flow is necessary to ensure that the fish receive a sufficient supply of oxygen and to keep the water well-mixed and free of waste buildup. It is also important for the eggs to receive adequate water flow to ensure their survival and development.
- **Lighting:** Proper lighting is important for broodstock, as it affects their feeding, growth, and reproductive behavior. The appropriate amount of light should be provided, and the lighting regime should be consistent and stable.
- **Habitat:** Broodstock should be housed in environments that provide appropriate shelter and hiding places. The size of the tanks and ponds should be sufficient to ensure that the fish have enough room to move around and forage for food.
- **Nutrition:** Proper nutrition is essential for the health and reproductive success of broodstock. A balanced diet that provides the necessary nutrients, vitamins, and minerals should be provided.
- **Stress management:** Stress can negatively impact the health and reproductive success of broodstock. Minimizing stressors, such as handling, transport, and sudden changes in water quality or temperature, can help to keep the fish healthy and reduce stress levels.

By ensuring that these environmental requirements are met, broodstock can be maintained in optimal conditions for breeding and producing healthy offspring. It is important to regularly monitor and adjust the conditions as needed to ensure the success of the breeding program.

Although there is no scientific research on this subject in Türkiye, there are many freshwater and marine farms that fulfill the conditions mentioned above. It has been observed that environmental requirements are met in the best way during our personal visits and observations at many fish farms. However, there is a need for scientific research on the subject.

3. Reproduction

The importance of fish reproduction has increased due to the decrease of fish stocks by capture around the world. Freshwater and marine fish species show reproductive activity at different times of the year. Reproduction in fish is a continuous development process during its ontogeny and is affected strongly by environmental factors with an emphasis on temperature.

Reproduction is a biological function, in which new individual organisms are produced from their parents. Animal reproduction aims to refresh generations for a given production purpose such as meat, milk, or wool by species. To attain this aim, researchers look for a

way to control reproduction in broodfish to supply the maximum number of offspring of the required quality (Courot and Volland-Nail, 1991). Reproduction success is a very important target for all animal species. Animal species take different strategies to achieve this target (Balon, 1984). The purpose of a reproductive policy is to maximize reproductively active progeny depending on existing energy and parental life anticipation (Roff, 1992). It is known that all terrestrial animals operate internal fertilization, whereas aquatic animals perform various reproductive systems including internal fertilization with or without mating. On the other hand, many aquatic animals perform mainly external fertilization in different types such as viviparous, oviparous, and parthenogenesis (Yoshida, 2020).

Aquatic animals mostly perform external fertilization. In general, this type of fertilization occurs in aquatic environments where gametes are released into the water. The role of water is to protect the eggs from drying out during embryonic development. The gametes are released to the same location at the same time, which increases the likelihood of fertilization of the eggs eventually (Bozkurt and Bucak, 2022). Additionally, there are some strategies used by fish to ensure their offspring survive in aquatic animals. The reproductive strategies of aquatic animals are often reflected in the anatomical differences between the sexes. In this framework, aquatic animals, mainly teleost fishes, show different reproductive strategies, varying from mass spawning to parental care, from strict gonochorism to hermaphrodism, and from oviparity to viviparity (Bozkurt and Bucak, 2022).

3.1. Determination of sex

Fish species exhibit a remarkable variety of sexuality (Atz, 1964). Sexuality can be defined as gonochorism or hermaphroditism. Many teleosts are gonochoristic, which means individuals develop as males or females only and keep the same sex throughout their entire life. In many gonochoristic fishes, sex is determined genetically, which means males and females have different alleles that specify their sexual morphology (Devlin and Nagahama, 2002). However, in some cases, it can be defined by some environmental factors such as temperature, salinity, and pH (Baroiller et al., 2009). However, many teleosts exhibit natural hermaphroditism where sex changes occur gradually from one sex to the other one. In that case, different forms of hermaphroditism have been defined (Devlin and Nagahama, 2002; De Mitcheson and Liu, 2008).

Under culture conditions, male and female broodfish should be separated related to their sex and age four weeks before the reproduction period. In general, this gender separation process is performed when a specific water temperature is reached (for instance 7-12ºC in

salmonid species). Separation of gender is carried out based on typical external signs of broodfish. For this aim, broodfish are removed from the ponds via netting and placed into two pre-prepared broodstock ponds. The body structure of male and female broodfish is analyzed. In the case of male brood fish, some special body structures may develop during the reproduction period like in salmonids. During this period, in male brood trout, the lower jaw stretches forward and curls up like a hook.

3.2. Establishment of broodstock and hatchery environment

3.2.1. Broodstock

Broodstock can be defined as parents of future generations, which are selected according to certain characteristics. Broodstock is created for commercial aquaculture production and stock reinforcement as well. The first stage in aquaculture-based production is the establishment of broodstock. It can be in the form of selecting suitable broodfish grown in ponds from the existing broodstock population, or it can be in the form of capturing broodfish from natural water sources and adding them to the hatchery broodstock. In the case of the selection of broodfish from the existing fish population, it is important to adjust the breeding performance and long-term genetic management of hatchery broodstock considering the source of the fish constituting the basic population.

As aforementioned, there are many aquafarms based on the breeding of sea bream, sea bass, and rainbow trout in Türkiye. These aquafarms sometimes supply or enrich their broodstock from the existing population in their farms or capture from the wild and add to the existing broodstock when needed, for instance, in case of a decrease observed or to increase their genetic resistance.

3.2.2. Hatchery environment

Artificial fish production in hatchery conditions is an important process to meet the increasing demand for aquatic food in the aquaculture industry by providing higher gamete production and progeny viability as well. Therefore, controlled larvae or fry supply can be provided only through artificial reproduction in well-designed hatchery conditions.

Analysis of the developmental processes of hatcheries show that they can vary in structures from the kakabans, hapa systems, and earthen ponds to the modern recirculating aquaculture systems. Currently, aquaculture hatcheries have an important role in artificial reproduction processes. In modern aquaculture facilities, the efficiency of hatchery operations should be determined by the hatchery managers having a good knowledge of fish biology.

The basic conditions of aquaculture hatcheries should be well-designed to ensure the following technical requirements indicated below:

- Enclosed buildings suitable for special environmental conditions to protect broodstock against harmful external effects,

- Ensuring the optimal environmental conditions and protection for the developing embryos,

- Providing the appropriate quantity and quality of water with filtration, temperation, and oxygenation,

- Healthy working conditions for the hatchery staff,

- Approachable for transportation

In Turkish marine aquaculture, sea bass and sea bream broodstock are generally stocked in cylindrical tanks that have 5–15 m^3 volume. Both circulated seawater and closed seawater systems are used in these tanks. These tanks usually have a dark colour and are cylindrical, and their bottoms are conical. The flow rate in these tanks can vary between 10% and 20% per hour. The stocking rate of brood fish ranges between 5 to 18 kg/m^3 and the female/male rate is adjusted as 1:1, 1:2, 2:1, or 3:2 in these tanks, and the broodstock is renewed at a 30% rate every year (Saka et al., 2007). In the case of turbot aquaculture in Türkiye, the wild-caught or hatchery-bred spawners are transferred to acclimation and maturation tanks (1 m × 2 m × 0.5 m). The broodfish can be stocked at 2-4 fish per m^2 in these tanks. Over-stocking should be avoided to prevent the physical stress of broodstock. Also in these tanks, enough water exchange (roughly 900% in a day) and aeration should be provided. Furthermore, 15°C water temperature should be provided and checked daily for the gamete collection during the spawning season (Özdemir, 2007).

3.3. Gamete quality in male and female broodstock

Controlling gamete quality is an important matter for the aquaculture industry. The gamete quality may be characterized as the ability of gametes in the male and female sex to fertilize and be fertilized (Bobe and Labbe, 2010). The quality of sperm can be determined by its components: seminal plasma and spermatozoa. The standard analysis of sperm includes the examination of some parameters such as motility, density, volume, osmolarity, and pH. As previously documented, the egg size is not always related to its quality. It is clear, that eggs of different sizes can show similar developmental competence in trout (Bromage et al., 1992) and sea bass (Cerda et al., 1994). In general, it is difficult to estimate the developmental success of eggs using morphological or macroscopic parameters. However, some parameters such as sinking or white eggs in marine fish and in salmonids respectively are used to identify the non-viable eggs (Ciereszko et al., 2009). In Türkiye, studies were conducted on the effect of photoperiod on gamete quality in crayfish (*Pontastacus leptodactylus*) (Farhadi and Harlıoglu, 2019) and the relationship between weight and length on gamete quality in male individuals (Farhadi et al., 2019).

3.4. Artificial propagation

In hatchery conditions, the process of obtaining eggs from female broodfish and sperm from male broodfish by applying abdominal massage is known as gamete stripping. Broodfish may be stripped with varying frequency according to species and gender during the reproduction period. For instance, while female trout broodfish are stripped once a year, male broodfish can be stripped several times with an interval of 15 days (Brown and Gratzek, 1980). Additionally, gamete stripping provides opportunities for broodstock management such as control of reproduction and genetic improvements of fish stock bred at the aquafarms.

3.5. Hormonal and environmental applications

Fish species show many reproduction strategies that are shaped by seasonal changes since releasing of gametes is possible when conditions such as environmental, nutritional, and social are most favorable. It is well known that some factors including social (sex ratio, size structure, dominance/hierarchy), environmental (light, temperature, salinity), and nutritional (feed quality, quantity) have important roles in the synchronization of broodstock spawning. However, despite the simulation of seasonal changes in photoperiod and temperature, fish species may fail to reproduce and spawn normally due to difficulties in recreating their natural habitat in the environment. Thus, artificial propagation by applying hormonal induction is the only way to produce large amounts of eggs at the same time.

3.5.1. Hormonal treatments

In aquafarms, spawning in cultivated bred warm water fish species is usually performed with hormonal treatment to induce ovulation and increase sperm production. For this aim, several hormones such as gonadotropin hormone (GTH), human chorionic GTH, gonadotropin-releasing hormones (GnRH), and pituitary extracts containing GTH are available to induce final maturation and ovulation in captive fishes.

However, in the case of female broodstock, if eggs have not yet reached the late-vitellogenic stage, the hormonal treatment does not work. Mature fish ovulates at once, and maturing

fish requires about 2-10 days to reach maturity. For that reason, in this stage, it is necessary to apply pressure on the female's abdomen from time to time to observe whether ovulation is taking place. Various methods have been developed to determine the stage or ripeness of oocytes by using ultrasound monitoring (Shields et al., 1993) and measuring of biochemical properties of the ovarian fluid (Rime et al., 2004).

In general, research on hormone treatment is combined with artificial propagation applications in aquaculture practices. In this regard, there are some studies investigating the effect of the comparison of different hormones on fecundity in Türkiye (Arabacı et al., 2004; Arabacı, 2009). There are also studies on the effects of hormones on reproduction of crayfish in Türkiye (Harlıoğlu et al., 2018; Farhadi et al., 2020).

3.5.2. Environmental treatments

3.5.2.1. Out-of season artificial propagation

An important management tool in broodstock management is out-of-season production (Bromage et al., 1993). In this regard, broodstock exposed to phase-shifted simulated natural photoperiod and 12-month temperature regimes have been the common practice of hatcheries to produce eggs all year round. In this way, out-of-season spawning methods can provide market-sized, similar size and quality, fish demanded from the market. Additionally, all-year-round production also enables the optimal use of facilities and can ensure better utilization of manpower and technical resources in aquafarms. As a result, the creation of three groups exposed to 1-year environmental regimes shifted by 3, 6, and 9 months will probably be enough to meet the constantly growing industry demand for egg and brood production throughout the year (Carrillo et al., 1993; Taranger et al., 2010).

3.6. Incubation period

The term, "incubation period", refers to the period from egg fertilization to the completion of the embryonic stage and the hatching of the egg. The period of the embryo's exit from the fertilized egg is expressed in day-degree. The daily degree term is called the sum of the average daily water temperatures and this period changes according to fish species. For instance, gilthead sea bream eggs are usually incubated between 14 and 18ºC, and hatching of eggs lasts from 50 to 80 h depending on water temperatures.

In the Turkish marine aquaculture sector, hatcheries usually use 2000–7000 egg/l as a stocking density. Additionally, plastic or fiberglass round tanks with a conical bottom are commonly used for egg incubation by many marine hatcheries (Saka et al., 2007). Following

hatching, only the hatched larvae are moved to the clean larval tanks, and the hatching units are easily disinfected for the next egg batch. In addition, some studies were carried out on the effects of incubation time and temperature on spermatozoa extraction in freshwater crayfish (*Pontastacus leptodactylus*) experimentally in Türkiye (Farhadi et al., 2018; Aydın and Dilek, 2004).

3.7. Incubation systems

In nature, most fish species need open-flowing water to hatch their eggs. However, the incubation of fish eggs with mechanical help simulates the needs of the species being hatched in aquafarms (Watson and Chapman, 2002). Once the steps of accepting and counting the eggs are completed, it's time to get them into hatchery incubation units. As with all steps in the hatchery process, this stage also should be performed carefully by trained personnel (Troutlodge, 2019).

The three most common types of incubators used in aquaculture hatcheries are:

- Horizontal type incubators (California baskets or trays)

- Vertical type incubators (Heath trays or stack)

- Upwelling type incubators (Jars)

3.7.1. Horizontal type incubators (California baskets or trays)

There are many commercial horizontal incubator types available. These types of incubators are usually made from GRP or reinforced non-toxic plastic like the vertical incubators. The principle of a horizontal incubator is that baskets or trays are placed in a trough in an orderly (Figure 3.1). The number of baskets or trays per trough may vary according to hatchery space and water flows, but usually, 4-8 baskets or trays are placed in each trough. The base of baskets or trays is covered with a screen and sits from the bottom of the trough. At the end of each basket or tray is a partition that extends to the trough bottom. The reason is to force the water upwards through the eggs. The edges of the baskets need to fit snugly into the groove to prevent water from running down the sides of the basket, and that the baskets are orientated in the correct direction regarding water flow. The mesh size in hatching baskets or trays should be a suitable size to allow newly hatched yolk sac fry to fall through the mesh into the trough below. Once hatching is complete the baskets or trays are simply lifted out with any remaining dead eggs and eggshells. In this way, the hatched alevins can absorb the remaining yolk sac, swim up and commence feeding in the same trough (Troutlodge, 2019).



Figure 3.1. Horizontal type incubators (California baskets or trays) (Troutlodge, 2019)

Advantages of the horizontal type incubators are as follows:

- Ease of use,

- Inexpensive and can be custom-built on-site,

- Ability to see, monitor and work with eggs easily,

- Efficient use of water supply,

- Ability to hatch eggs in the same tanks for hatched alevin and first feeding

On the other hand, the main disadvantage of the horizontal type of incubators is the requirement of more space than other types of incubators.

3.7.2. Vertical type incubators (Heath trays or stack)

Different varieties of vertical incubators are used in aquaculture hatcheries. Modern vertical incubators are constructed from GRP (Glass Reinforced Plastic) and are durable, easy to clean, and disinfect.

The principle of this vertical incubators is that water enters a channel in the top tray, upwells through the egg tray, and flows over the front wall into a channel that feeds the next lower tray unit and onwards to the last tray (Figure 3.2). For hatching eggs, it is recommended that the eggs are placed in approximately 12500-15000 eggs (Troutlodge, 2019).



Figure 3.2. Vertical type incubators (Heath trays or stack) (Troutlodge, 2019)

The advantages of the vertical type of incubators are:

- Excellent use of available floor space,

- Efficient use of water supply,

-Ability to remove individual trays for monitoring or management,

- Safety of eggs and newly hatched alevins as trays are covered by a screen mesh to prevent eggs or alevins from being washed out,

- More efficient uptake of the yolk sac

The disadvantages of the vertical type of incubators are:

- Once alevins start to swim up for first feeding, they must be removed to tanks or ponds,

- Requires cleaning and management of eggs including removal of dead eggs

3.7.3. Upwelling type incubators (Jars)

Upwelling-type incubators are designed so that water flows from the bottom of the incubator to the top (Figure 3.3). In general, incubators are used for the incubation of eggs until the “eyed” stage. However, upwelling incubators can also be used for the hatching of eggs.

For this type of incubators, it is very important that this water flow be equally distributed throughout the incubator so that the upwelling water that delivers oxygen to the eggs. This can be achieved by placing some form of diffuser mechanism beneath the eggs (usually a plate, porous pad, or marbles). The upwelling incubators are placed in the rearing tanks and when the fish become more active the majority will swim out on their own while the remainder

will need to be poured out. These types of incubators maintain adequate circulation by using the water flow to partially suspend the eggs. The quantity of eggs placed in the upwelling incubators shouldn't exceed two-thirds of the total volume of the incubator. The flow rate in upwelling units should be adjusted so that eggs are suspended at approximately 50% of their static depth (Troutlodge, 2019).



Figure 3.3. Upwelling type incubators (Jars) (Aquaculture-com.net, 2023)

Advantages of the upwelling type incubators include:

- Ease of use,

- Self-cleaning and removal of dead eggs and egg shells with outflow,

- Labour saving,

- Hatches eggs in rearing tanks

Disadvantages of the upwelling type incubators are:

- Constant monitoring of flow rates is required to ensure eggs are suspended at correc level,

- Possible loss of alevins due to air bubbles or uncontrolled flow increase,

- Potentially high levels of alevin stress

3.8. Recent advances in reproductive biotechnologies

Currently, many reproductive biotechnologies have been developed for the effective control of reproduction in aquatic animals showing great differences in reproduction types. The application of biotechnology offers many advantages to animal food production through the enhancement and control of reproductive processes in aquatic animals (Bozkurt and Bucak, 2022).

3.8.1. Cryopreservation of male gametes

Cryopreservation is a process that biological materials are frozen in liquid nitrogen (LN_2) (-196°C). In this way, biological reactions creating cell death and DNA damage deactivate at these low temperatures. Thus, it is possible to preserve the biological materials as unchanged for many years following the thawing process (Bozkurt, 2018).

3.8.2. Genetical applications

Chromosome sex manipulation techniques have been widely applied in cultured fish species to stimulate uniparental chromosome inheritance (gynogenesis and androgenesis) and polyploidy (triploidy and tetraploidy) (Pandian and Koteeswaran, 1998; Lakra and Das, 1998). These techniques are significant in the development of fish farming since they ensure rapid approach to gonadal sterilization, sex control, hybrid viability improvement, and cloning.

Induced triploidy is well known as the most efficient method to produce sterile fish for aquaculture. Triploidy can be induced by subjecting the eggs to physical or chemical treatment for limited period following fertilization to hinder the second polar body from coming out (Ihssen et al., 1990). Triploid fish are anticipated to be sterile due to the inability of homologous chromosomes to synapse correctly throughout the first meiotic division. Triploidy induction methods involve subjecting of fertilized eggs to temperature shock (hot or cold), hydrostatic pressure shock, or chemicals such as colchicine, cytochalacin-B, or nitrous oxide. Another way of producing triploid species by crossing of tetraploids and diploids.

Tetraploid induction involves fertilization of eggs with normal sperm and physical or chemical treatment of the diploid zygote to suppress the initial mitotic division. Tetraploid breeding lines offer potential advantage to aquaculture by supplying a convenient way to supply large numbers of sterile triploid fish through simple interploid crosses between tetraploids and diploids (Guo et al., 1996).

Gynogenesis is the process of embryo development with special maternal inheritance. Gynogenetic individuals are important for fish breeders since a high level of inbreeding can

be supplied in a single generation. Gynogenesis can also be used to obtain all-female populations in species with a female homogamete and to reveal the sex determination in fish. It is appropriate to use all female gynogenetic lineages for sex change experiments. Methodologies combining the use of hormonal sex change with induced gynogenesis have been developed for various types of aquatic species (Gomelsky et al., 2000).

Androgenesis is the mechanism by which a progeny is produced by the male parent without any genetic contribution from the female. Stimulation of androgenesis can produce the entire male population in fish, which will have commercial application in aquaculture. It can also be used to obtain homozygous fish lineages and the recovery of lost genotypes from frozen stored sperm. Androgenetic individuals have been obtained in several species of carp, cichlid, and trout (Bongers et al., 1994).

3.8.3. Transgenesis

Transgenesis can be defined as the insertion of an exogenous gene/DNA into the host genome, resulting in its stable preservation, transmission, and expression. This technology provides an excellent opportunity to modify or improve the genetic characteristics of commercially important fish, mollusks, and crustaceans for aquaculture. In spite of important progress achieved in many laboratories around the world, there are many problems waiting solutions before the successful commercialization of transgenic broodstocks for aquaculture.

In order to become aware of the full potential of transgenic fish technology in aquaculture, some important scientific findings are required. These are i) more effective technologies for mass gene transfer ii) appropriately targeted gene transfer technologies such as embryonic stem cell gene transfer iii) appropriate promoters to guide the expression of transgenes at optimal levels during desirable developmental stages iv) identified genes with desired characteristics aquatic culture and other applications v) information about physiological, immunological, nutritional, and environmental factors maximizing the performance of transgenic fish, and vi) safety and environmental effects of transgenic fish (Danish et al., 2017).

4. Broodstock Nutrition

Broodstock nutrition is an important aspect of aquaculture that involves the feeding of adult fish used for breeding purposes. Proper nutrition is essential for broodstock to produce healthy and viable offspring. There are some key points to consider when it comes to broodstock nutrition. Broodstock have higher nutrient requirements compared to fish that are not used for breeding purposes. This is because they need to produce high quality eggs

and sperm. Essential nutrients needed by broodstocks include protein, essential amino acids, lipids, essential fatty acids, vitamins and minerals (Izquierdo et al., 2001; Bureau et al., 2002; Fernández – Palacios et al., 2011; De-Dios et al., 2022).

Broodstocks should be fed two or more times a day. This is because their digestive systems do not work as well as non-breeding fish and cannot handle large amounts of feed at once. The formulation of the broodstock feed must be carefully prepared to meet the specific nutritional requirements. High quality fishmeal and fish oil are mainly included in broodstock feeds, but some vegetable protein sources can also be used (El-Sayed, 2020).

Essential fatty acids (EFAs) are critical to the nutrition of broodstock as they are required for healthy egg and sperm production (Yıldız et al., 2020; 2021). In particular, omega-3 fatty acids play an important role in the development of the nervous and immune system of the offspring. Vitamins and minerals should be added to the feeds to ensure that the fish get all the nutrients they need in a balanced way. Some important vitamins and minerals for broodstocks include vitamin E, vitamin C, calcium, and phosphorus (Watanabe and Agius, 2003; Suloma et al., 2017).

Water quality is also important in broodstock nutrition. Poor water quality can lead to stress, disease, low feed intake and poor reproductive performance. Water temperature, dissolved oxygen and pH must be carefully monitored and maintained at appropriate levels.

There are 32 aquafeed factories in Türkiye. In most of these factories, quality broodstock feeds are produced according to the dietary needs of the different species (Ministry of Agriculture and Forestry of Türkiye, 2021). Recently, high reproductive performances have been achieved in broodstock reared with these feeds. As in developed countries, important developments are recorded in aquafeed production and especially in the production of broodstock feeds in Türkiye (Yıldız et al., 2020; 2021; De-Dios et al., 2022).

In summary, broodstock feeding is an important aspect of successful aquaculture. Providing nutritionally balanced, high quality feeds, feeding adequately, and maintaining good water quality are important considerations in broodstock nutrition.

4.1. Feeding strategies

Broodstock feeding strategies can vary depending on the species of fish, the size and age of the fish, the environment, and the goals of the aquaculture operation. Here are some common brood fish feeding strategies:

- **Continuous feeding:** This strategy involves feeding brood fish a small amount of food continuously throughout the day. The advantage of this method is that it provides

a constant supply of nutrients, which can help maintain good health and reproductive performance in the fish. The use of automatic feeders in this feeding will serve the purpose more accurately (Izquierdo et al., 2001; Bureau et al., 2002).

- **Meal feeding:** This strategy involves feeding brood fish several small meals per day at regular intervals. The meals should be timed to coincide with the natural feeding behavior of the fish, which can vary depending on the species. The advantage of this method is that it can help maximize feed intake and minimize the waste (Kaya and Bilgüven, 2015).
- **Alternate-day feeding:** This strategy involves feeding brood fish every other day. The advantage of this method is that it can help reduce the risk of overfeeding and improve feed conversion efficiency. However, it is important to ensure that the fish receive adequate nutrition for the days when they are not fed (Bolivar et al., 2006).
- **Restricted feeding:** This strategy involves limiting the amount of food that brood fish receive to promote weight loss and improve reproductive performance. This method may be used if the fish are overweight or if they are not reproducing at an optimal rate. Therefore, it is important to ensure that the fish receive adequate nutrition to maintain their health and well-being (Yokoyama et al., 2009). In addition, new studies are needed to clearly see the effect of feed restriction practices to increase the production efficiency and health of fish (Bureau et al., 2002).
- **Feeding with supplements:** In addition to regular feed, brood fish may also be given dietary supplements to improve reproductive performance. Examples of supplements include vitamin E, vitamin C, and essential fatty acids. These supplements can help improve egg and sperm quality and increase the survival rate of the offspring (Izquierdo et al., 2001; Palace and Werner, 2006, Arslan et al., 2021).

In general, the feeding strategy for brood fish can be carefully modified according to the specific needs of the fish and the aquaculture operation. Factors such as the size of the fish, reproductive status and feeding behavior of the species should be considered when developing a feeding plan.

4.2. Broodstock feeding practices

it is generally accepted that the health of a fish is best maintained by feeding it a nutritionally complete diet daily. In the recent years, the commercial broodstock diets for most cultured fish species are larger sized than on-growing diets. In practice, many marine fish

hatcheries improve the nutrition of their broodstock by feeding them fresh marine by-products along with commercial feeds. The most common fresh marine organisms used to feed broodstock fish include squid, cuttlefish, mussels, krill and small crustaceans. The use of fresh fish products often does not provide enough nutrients for the broodstock and increases the risk of disease transmission to the broodstock and offspring, including parasites, bacterial and viral pathogens, etc. The nutritional quality of formulated feeds can be improved according to the needs of the fish. These improvements significantly increase the costs of diets. However, it has more economic benefits than the cost of feed, as it increases survival. (Izquierdo et al., 2001; Kottman et al., 2020; Jiang et al., 2020).

It is generally accepted that the health of a fish is best maintained by feeding it a nutritionally complete diet daily. However, some research findings and evidence from commercial aquaculture have shown that certain deviations from daily feeding to *ad libitum* can significantly affect fish health and disease resistance (Bureau et al., 2002).

As we mentioned above (Section 3), high performance broodstock feeds are produced in many fish feed plants according to the specific needs of the different fish species cultured in Türkiye. In various freshwater and marine farms operating in Türkiye, broodstock rearing with commercial feeds is done by different methods. Thus, farms can have broodstocks with high reproductive performance.

4.3. Effective feeding periods for optimum broodstock performance

Feeding broodstock as mentioned under the title “3.1. Feeding Strategies” is important for their health, growth, and reproductive success. The feeding periods for broodstock depend on several factors such as species, age, and reproductive stage. However, some general guidelines can be followed to ensure optimum broodstock performance:

- **Pre-spawning period:** Broodstock should be fed more frequently and with a high-protein diet during the pre-spawning period. This period is the time when the fish are preparing for reproduction, and their nutritional needs increase. Feeding two to three times a day is recommended during this period (Varela et al., 2013).

In Türkiye, Yıldız et al. (2020, 2021) fed rainbow trout broodstocks two times a day until they were satiated with the high performance diets, they formulated in the pre-spawning period. The authors reported significant successes in egg and sperm quality of fish.

- **Spawning period:** Broodstock may stop feeding during the spawning period. However, they should be fed lightly before and after spawning to replenish energy reserves.

Overfeeding should be avoided during this period, as it may cause water pollution and reduce spawning success (El-Dahhar et al., 2015).

- **Resting period:** Broodstock may enter a resting period after spawning. During this time, they require less feed and can be fed once or twice a day with a lower protein diet (Ahongo et al., 2021).
- **Post-spawning period:** Broodstock require high-quality feed during the post-spawning period to recover from the energy expenditure during the spawning process. Feeding frequency should be gradually increased to four to six times per day, with a focus on high-protein and high-fat diets (Jenkins et al., 2023).

Researches on the subject are limited. Therefore, in order to obtain broodstocks with high performance, it is necessary to conduct adequate research on the feeding periods of broodfish in Türkiye as well as in the world.

4.4. The importance of dietary nutrient quality on reproduction success and gamete quality

Dietary nutrient quality is critical to the reproductive success of broodstocks. The quality and quantity of nutrients in the diet can affect the production of healthy gametes necessary for fertilization and successful reproduction. Fecundity is one of the methods used to measure the physiological maximum potential reproductive output of an individual (female) over its lifetime in broodstocks. It can be affected by nutrient deficiencies in diets. Fecundity is the total number of eggs produced by an individual fish. It has been reported that low fecundity may be caused by a low level of a component in the structure of the eggs or an imbalance of a nutrient that is effective on the brain-pituitary-gonad endocrine system. Studies have reported that diets lacking specific nutrients such as essential fatty acids, vitamins and minerals negatively affect egg and sperm quality and fertility in fish. These diets may also result in increased embryonic mortality or malformations and reduced hatchability. On the other hand, feeding broodstocks with a high nutrient value diet can increase the production of healthy, viable eggs and sperm. These diets lead to higher fertilization rates, better survival rates of embryos and larvae and, as a result, increased offspring production. The importance of dietary nutrient quality extends beyond healthy gamete production. It can also affect the overall health and well-being of broodstocks, which can affect their reproductive success (Izquierdo et al., 2001; Yıldız et al., 2020, 2021; De-Dios et al., 2022). In conclusion, the dietary nutrient quality is a crucial factor that influences the reproduction success of broodstock. A well-balanced and high-quality diet can help to ensure the production of healthy and viable gametes, improve

fertilization rates, increase embryonic and larval survival, and ultimately, improve the overall health and well-being of broodstock.

There are some studies conducted in Türkiye on determination of zebrafish (*Danio rerio*) spermatozoa motility and density (Özdemir and Ekici, 2013); reducing the bacterial load and determining the success of fertilization by sperm washing method in rainbow trout (Ercan and Ekici, 2016); different heat shock application effect on gynogenetic production of zebrafish (*Danio rerio*) (Özdemir and Ekici, 2017); the effects of dietary diludine and gametes storage at 6 ºC on the level of vitamin E and lipid peroxidation and reproductive performance of eggs in rainbow trout (Arslan et al., 2021); improvement of induction of meiotic gynogenesis in Coruh trout, *Salmo coruhensis* (Özdemir and Ekici, 2022); the effect of using magnetized water on sperm motility in dilution of the milt of Black Sea trout (*Salmo trutta labrax*) (Ahmed et al., 2022). A PhD thesis was conducted by Ahmed (2022) in Türkiye to evaluate the effect of feeds prepared using emulsifiers together with different vegetable oils on the gamete quality of zebrafish. There are also some reviews written by Turkish scientists: genetic breeding studies of marine fish farming species (Ekici et al., 2020), cryopreservation studies in aquaculture from past to present: scientific techniques, and quality controls for commercial applications (Ekici et al., 2022).

4.4.1. Protein and amino acids

Protein constitutes the highest nutrient ratio in fish eggs and is a main energy source during embryonic development of most teleost species. Proteins have an important role in fertilization and embryonic development; for example, proteins surrounding the yolk are significant for fertilization, their amino acid composition being high in proline and glutamic acid and low in cystine (Callet et al., 2017; Wang et al., 2018; Santander-Avanceña et al., 2020). Eggs of pelagic fish contain high levels of free amino acids; for example, sea bream eggs include high amounts of free amino acids, leucine, lysine, valine, isoleucine, alanine, and serine (Rønnestad et al., 1994).

Dietary protein with a balanced amino acid composition is vital for protein and amino acid supplementation of the embryo through the yolk. Therefore, it is known that the protein level and composition of broodstock diets affect egg quality. For sea bream broodstocks, a well-balanced diet with essential amino acids was reported to improve vitellogenesis (Tandler et al., 1995). It was stated that the fecundity rate in seabass fed with 51% protein was 1.5 times higher than in fish fed with 34% protein (Cerdá et al., 1994). High protein diets also had a low percentage of deformed larvae. Similar results regarding protein and amino acid nutrition were

reported for Labeo roho, *Labeo rohita* (Khan et al., 2005), catfish, *Mystus nemurus* (Abidin et al., 2006) and turbot, *Scophthalmus maximus* (Aijun et al., 2005).

Tryptophan and taurine are particularly important for fish reproduction. Tryptophan is a precursor of serotonin and can affect gonadal maturation in both males and females. Ayu (*Plecoglossus altivelis*) broodstocks fed a diet containing 0.1% tryptophan produced a significant increase in testosterone levels favoring spermiation in males, and induced female maturation (Akiyama et al., 1996). Taurine is one of the most abundant free amino acids in tissues of fish and is involved in antioxidant processes, osmoregulation, neurotransmitter modulation, calcium regulation in cells, hormone release, and formation of bile salts (Huxtable, 1992). For yellowtail (*Seriola quinqueradiata*) broodstocks, 1% taurine supplementation to the diets was reported to improve fertility, percentage of viable eggs and fertilization rate. (Matsunari et al., 2006).

Protein levels in commercial broodstock feeds in Türkiye are well-calculated according to the needs of the species. In the literature review, no information was found about different protein sources or protein levels in broodstock feeds in Türkiye. However, in a study conducted at the Istanbul University Faculty of Aquatic Sciences Inland Waters Research Center, Sapanca, Türkiye, proximate analyzes and fatty acid profile analyzes of commercial trout feeds were performed and it was reported that the results met the requirements of broodstocks (Yıldız et al., 2020; 2021).

4.4.2. Lipid and fatty acids

Lipid is the most researched nutrient in broodstock nutrition. The level of total dietary lipids and essential fatty acids significantly affects the spawning quality of fish. Dietary contents of lipids, energy, essential fatty acids and the ratios between them greatly affect fish breeding performance (Linares et al., 2016; Turkmen et al., 2019; Perera et al., 2019; Yıldız et al., 2020; 2021; Liang et al., 2022; De-Dios et al., 2022).

Few studies reported an effect of total dietary lipid on broodstock performance. However, it was stated that increased dietary energy in cod (*Gadus morhua*) increased the gonadosomatic index, resulting in increased fecundity or increased egg size. Moreover, high lipid ratio in broodstock feeds increased the fecundity and survival rate of rabbit fish (*Signatus guttatus*) larvae up to 14 days after hatching (Hara et al., 1986), while high lipid content in sea bream led to an increase larval weight and length even at 28 days post hatch (Bueno, 2001).

Essential fatty acids (EFA) in diets significantly increase the reproductive performance

of fish. It was reported that feeds deficient in EFA had a negative effect on the reproductive performance of broodstocks. The fatty acid composition of fish eggs directly reflects the fatty acid content of the broodstock diets. Polyunsaturated fatty acids (PUFAs) also regulate gonadal development such as ovulation, the production of steroid hormones, and the production of eicosanoids, which are involved in many reproductive processes. Levels of n-3 PUFAs and n-3 highly unsaturated fatty acid (HUFAs) found in sea bream eggs and gonads increased with increasing dietary amounts (Fernández-Palacios et al., 1995). Moreover, the authors observed that fecundity increased significantly with the use of n-3 HUFAs above 1.6% in the feed of sea bream. Fatty acid composition in fish eggs may vary depending on the species and even different batches of the same species (Pickova et al., 1997) or environmental conditions (Dantagnan et al., 2007). In rainbow trout fed a diet devoid of n-3 fatty acids during the vitellogenesis stage, there was only a slight decrease in the docosahexaenoic acid (DHA, 22:6n-3) content in the egg, while the eicosapentaenoic acid (EPA, 20:5n-3) concentration was highly reduced (Frémont et al., 1984; Yıldız et al., 2020; 2021). This selective retention of DHA has also been found during embryogenesis (Izquierdo, 1996; Arslan et al., 2016) and during starvation in the larval stages (Tandler et al., 1989), showing the importance of this fatty acid for embryonic and larval development (Watanabe et al., 1989).

Fatty acids are important sources of energy during embryonic development (Tocher et al., 1985; Sargent, 1995). They have an important structural function as components of the phospholipids in fish biomembranes. The ratio of unsaturated fatty acids in diets regulates the fluidity and functions of cell and organelle membranes (Takeuchi, 1997; Sargent et al., 1999). DHA is particularly important in neural tissue and retina and is effective in the development of sensory organs during the larval stage (Sargent et al., 1993; Benítez-Santana et al., 2007). It has been reported that arachidonic acid (ARA; 20:4n-6) has an active role in the reproductive performance of fish. ARA, EPA, and DHA compete for the enzymes that regulate the synthesis of eicosanoids. Therefore, a deficiency or imbalance of ARA, EPA, and DHA in the broodstock diet can have large effects on reproduction. ARA has a significant effect on the reproductive performance of broodstocks. For this reason, it should be added to the diets at the determined level (Furuita et al., 2003b). It has been stated that optimal ARA increase fecundity, egg viability, hatching rate, and larval survival (Bruce et al., 1999; Navas et al., 2001). The optimal ratio of ARA to EPA is dependent on species. For example, it was observed that the ratio is 1:1 for sea bass and 10:1 or higher for halibut and turbot (Sargent et al., 1999). In some studies, it has been reported that dietary ARA directly affects the fertilization rate in sea bream and halibut (*Hippoglossus hippoglossus*) (Mazorra et al., 2003; Fernández-Pala-

cios et al., 2005). Increasing ARA and n-3 HUFAs in diets lead to improvement of fecundity and egg and larval quality in terms of survival, stress resistance, larval weight, and inflation of the swim bladder (Watanabe et al., 1985; Tandlet et al., 1995; Fernández-Palacios et al., 2005; Yıldız et al., 2020). Numerous studies reported negative results in egg and larval quality in broodstock fed diets devoid of EFAs (Izquierdo et al., 2001; Vassallo-Agius et al., 2001; Fernández-Palacios et al., 2005; Yıldız et al., 2020, 2021). The requirement of n-3 HUFAs in sea bream broodstock varies between 1.5 and 2.5% in the diet (Watanabe et al., 1985; Fernández–Palacios et al., 2005). This value is also higher than that established for salmonids of approximately 1% n-3 HUFAs in the diet (Watanabe, 1990). The positive effect of n-3 fatty acids on spawning performance may be explained by their roles as structural components in biological membranes, where they increase fluidity (Fernández–Palacios et al., 2011, Yıldız et al., 2020, 2021).

Studies on the effects of lipids and fatty acids on reproductive performance, egg and sperm quality in different broodstock species were conducted in Türkiye. Effects of different dietary lipid resources on reproductive success and sperm and egg quality in rainbow trout (Yıldız et al., 2020; 2021); a PhD thesis on the effects of fatty acids in different vegetable oils with additive emulsifier on zebrafish reproductive performance (Ahmed, 2022); a review on the effect of fatty acids on sperm quality in fish (Harlıoğlu and Kutluyer, 2011).

4.4.3. Vitamins (Vitamin A/C/E/Thiamine)

There are 15 vitamins that are recognized essential for fish and are necessary in broodstock diets (Woodward, 1994). Vitamins C, E, and thiamine are the vitamins most often studied in fish broodstock diets. Thiamine is also often low in rough fish due to activity of the enzyme thiaminase, which is present in most fish species, and which destroys thiamine. Also, thiamine deficiency is observed in broodstocks fed with rough fish. The requirements for vitamins A, D, E, K and C were determined for some teleost species. There are few studies on the effects of these vitamins on reproductive and ovulation quality. Direct requirement studies with broodstocks are relatively rare due to the high cost of research on vitamins (Fernández–Palacios et al., 2011).

Vitamin A

Vitamin A is essential for reproduction and embryonic development of fish and must be obtained from the diets (Craik, 1985). In a study conducted with zebrafish, it was reported that vitamin A taken with diets was transported to the ovaries (Lubzens et al., 2010). The vitamin A requirement during gonadal maturation and spawning in fish is not well known. Furuita et al. (2003a) found that feeding broodstock of the Pacific sole (Paralichthys olivaceus) with vitamin A increased fecundity, viable egg percentage, and normal larvae percentage. Furthermore, vitamin A, together with vitamin C, increased the survival of 3-day-old larvae produced from largehead carp (*Aristichtys nobilis*) broodstock (Santiago and Gonzalo, 2000).

Vitamin C

Ascorbic acid also has an important role in fish reproduction. In a study by Blom and Dabrowski (1995), it was stated that vitamin C positively affects steroidogenesis and vitellogenesis in salmonids. In addition, the concentration of vitamin C in rainbow trout eggs reflects its dietary level, and it was found that appropriate vitamin C levels positively affect egg quality (Sandnes et al., 1984). In another study by Ciereszco and Dabrowski (1995), it was reported that vitamin C levels in broodstock diets affect the concentration in seminal fluid and that seminal vitamin C concentration affects sperm motility. Adding up to 1.200 mg/kg of vitamin C to rainbow trout broodstock diets has increased hatching rates (Ridelman et al., 1981). It has been reported that rainbow trout broodstocks require eight times more vitamin C than juveniles (Blom and Dabrowski, 1995). Fertility and egg quality are affected by the level of dietary vitamin C (Blom and Dabrowski, 1995) or vitamin E, as well as the ratios between these two vitamins (Silveira et al., 1996; Emata et al., 2000). Studies on vitamin C requirements are few and show significant variation in different species.

Vitamin E

Vitamin E takes the role of a natural antioxidant and prevents peroxidation of lipids in animal cells (Huber, 1988). The negative effects of vitamin E deficiency on reproduction in other vertebrates have been known since the beginning of the last century. However, the effect of vitamin E on fish reproduction was not understood until 1990 (Watanabe, 1990). Vitamin E deficiencies in carp and ayu (*Plecoglossus altivelis*) inhibited gonadal maturation and reduced hatching and survival rates of larvae (Watanabe, 1990). Watanabe et al. (1991) reported that increasing dietary vitamin E levels (over 2.000 mg/kg) in Japanese sea bream improved the percentage of floating eggs, the ratio of hatched eggs, the ratio of larvae with normal development, and larval survival. It was noted that dietary vitamin E increases α-tocopherol concentrations in maternal, embryonic, and fetal tissues and reduces congenital malformations (Simán and Eriksson, 1997). It has been reported that vitamin E levels are generally high in fish eggs and low in broodstock tissues after the spawning period (Mukhopadhyay et al., 2003). This may be the result of the transfer of vitamin E from peripheral tissues to the ovary during vitelogenesis. Similar results have been reported in turbot and Atlantic salmon (Hemre et al., 1994; Lie et al., 1994). It was observed that survival at eyed-stage embryos increased

with increasing amount of vitamin E in rainbow trout (Arslan et al., 2021). It was reported in many studies that vitamin E increases fecundity, egg fertilization rate, egg hatching rate and larval survival rate of many cultured freshwater and marine fish species (Fernández–Palacios et al., 2011; Arslan et al., 2021). In Türkiye, Harlıoğlu and Barım (2004) studied the effect of dietary vitamin E on the pleopodal egg and stage-1 juveniles of freshwater crayfish *Astacus leptodactylus*.

Insufficient vitamin E decreased the percentage of viable eggs with normal morphology in rainbow trout and gilthead sea bream (Lee and Dabrowski, 2004). Fernández-Palacios et al. (2005) also stated that in gilthead sea bream, diets deficient in vitamin E decreased the percentage of fertilized egg. The optimum dietary vitamin E requirement of broodstocks for several fish species has been investigated. In gilthead sea bream, vitamin E up to 190 mg/kg α - tocopherol greatly improved spawning quality (Fernández-Palacios et al., 2005). These levels reported to be suboptimal for turbot broodstock (Hemre et al., 1994). It was stated that increasing α-tocopherol up to 125 mg per kg feed increased fecundity of sea bream broodstock. However, increasing α-tocopherol up to 2010 mg per kg feed increased the level of α-tocopherol in eggs and decreased fecundity. In summary, insufficient or excessive use of α-tocopherol in feeds decreased egg productivity (Ortuño et al., 2000).

Thiamine

Dietary thiamine (vitamin B_1) is important for the normal development of embryos and larvae. For example, injection of thiamine into Atlantic salmon females with eggs can reduce offspring mortality (Ketola et al., 1998). Moreover, high thiamine concentration in eggs and fry with yolk sac of lake trout, rainbow trout, coho salmon and Atlantic salmon is related to a reduction in early mortality syndrome (Hornung et al., 1998; Wooster and Bowser, 2000). Thiamine injection showed some positive effect on thiamine content in the egg and hematological parameters in sterlet sturgeon, *Acipenser ruthenus* (Ghiasi et al., 2014).

4.4.4. Minerals

The most commonly used measure of nutritional status in fish is the trace element level in different tissues and organs. The levels of minerals that affect the growth and functions of fish can be different in various tissues. Feeding with low levels of minerals also lowers their concentration in the tissues of fish. This causes clinical toxicity and a gradual decline in organ function (Lall, 2002). For these reasons, it is necessary to use minerals in broodstock diets. There are limited numbers of published papers on the effect of minerals on the egg and sperm quality of fish. It was reported that rainbow trout broodstocks had poor egg quality as a result

of feeding on diets that were not mineral supplemented (Takeuchi et al., 1981). Phosphorus deficiency in Japanese sea bream (Watanabe et al., 1984a, b) and ayu (Luquet and Watanabe, 1986) broodstocks was related to a decrease in reproduction performance and an increase in the number of abnormal larvae. Studies on coho salmon, *Oncorhynchus kisutch* (Hardy et al., 1984) and Atlantic salmon (Ketola, 1985) did not show any changes in spawning quality in response to mineral supplementation.

4.4.5. Carbohydrates

Although fish do not have a definite need for carbohydrates, they serve as a basic energy source in some tissues. Several studies were conducted on carbohydrate needs in broodstock feeding. It was found that a decrease in rainbow trout fecundity when the broodstock were fed diets with low levels of carbohydrates (Washburn et al., 1990). However, Mangor-Jensen and Birkeland (1993) stated that feeding cod broodstock with increasing levels of carbohydrates slightly reduced spawning quality.

4.4.6. Carotenoids

Many fish species accumulate carotenoids in their integuments and gonads, and the salmonids have the capacity to accumulate astaxanthin in muscle. The carotenoids are orange to yellow pigments with a wide variety of functions in fish that include provitamin A, and antioxidant functions. Especially astaxanthins, are strong antioxidants that probably play roles in protecting broodstock nutrient reserves and developing embryos from oxidation (Fernández-Palacios et al., 2005; Carvalho and Caramujo, 2017). There is controversy over whether the carotene content of eggs affects egg quality in salmonids and, conflicting assessments in studies published on this subject (Craik, 1985; Torrissen and Christiansen, 1995). While some studies reported a positive relationship between egg pigmentation and egg quality in rainbow trout (Harris, 1984; Craik, 1985), other studies found no evidence on this subject (Craik and Harvey, 1986; Torrissen and Christiansen, 1995). It has been emphasized that these discrepancies may be due to differences in the methodology used in different studies, including the age of the broodstock, carotenoid content in eggs, the type of carotenoid used in the diet or determined in eggs (astaxanthin, cantaxanthin, etc.), sample size, and different assessments used to determine egg quality (Fernández-Palacios et al., 2005). However, positive effects of carotenoids in reproduction of salmonids were reported in some studies (Choubert and Blanc, 1993; Ahmadi et al., 2006). The increase in n-3 HUFAs and carotenoid levels improved fecundity and the percentage of viable eggs, hatching rate and larval survival in sea bream (Scabini et al., 2006). The use of carotenoids in broodstock feeds of several fish species had positive effects on egg quality (Fernández-Palacios et al., 2005).

4.4.7. Nucleotides

Nucleotides also affect spawning quality in broodstocks. Diets inculuding nucleotides produced an improvement in larvae survival of haddock (*Melanogrammus aeglefinus*) determined 10 days after hatching. González-Vecino et al. (2004) determined that this may be a result of better utilization of the first exogenous feed, with nucleotide-supplemented diets developing the larvae's intestine. They also stated that both halibut and haddock broodstock fed diets enriched with nucleotides had a higher fecundity than control fish.

To the best of our knowledge, there are no studies in Türkiye on minerals, carbohydrates, carotenoids and nucleotides in the literature. However, research on these topics in broodstock nutrition is of great importance. In Türkiye, it is necessary to carry out adequate research on these issues. The data to be obtained from the studies will help the farmers to achieve more successful production.

4.5. Effect of food restriction

Food restriction in brood fish can have both positive and negative effects, depending on the degree and duration of the restriction, as well as the species and life stage of the fish.

In general, short-term food restriction can be beneficial for brood fish as it can stimulate reproductive activity and increase the quality and quantity of eggs produced. This is because food restriction can cause the fish to release gonadotropin-releasing hormone (GnRH), which stimulates the production and release of reproductive hormones (Izquierdo et al., 2001).

Cardona et al. (2019) reported that higher gonadosomatic index, hatching rate, bigger eggs with less size variability and lower number of non-viable eggs can be obtained by restricting female rainbow trout. These restricted females also had a lower viscosomatic index, indicating that feed restriction is related to the effect on broodstocks. According to the results obtained from the study, the authors stated that feeding female rainbow trout broodstocks with 20% less feed did not negatively affect egg production and egg quality.

However, long-term, or severe food restriction can have negative effects on brood fish. It can lead to reduced energy stores, weight loss, and decreased immune function, which can increase the risk of disease and reduce the fish's ability to fight off infections (Azra and Ikhwanuddin, 2015). Additionally, chronic food restriction can cause stress, which can lead to decreased reproductive performance and lower egg quality.

A reduction in feeding rate was reported to cause an inhibition of gonadal maturation in several fish species, including goldfish (*Carassius auratus*) (Luz et al., 2008), European

seabass (Cerdá et al., 1994), and Atlantic salmon (Berglund, 1995). After being fed with half the usual amount of food for six months, the growth rates of seabass decreased, and their spawning time was delayed. Additionally, the eggs and newly hatched larvae from were smaller compared to those obtained from fish that were fed full rations. The negative impact of food restriction was particularly noticeable in female seabass, which had reduced plasma estradiol levels (Cerdá et al., 1994).

It is important to note that the effects of food restriction can also vary depending on the species of fish. For example, some species may be more resilient to food restriction than others, while some may require more food to maintain optimal reproductive health. In summary, while short-term food restriction can be beneficial for brood fish, long-term or severe food restriction can have negative effects on their health and reproductive performance. Therefore, it is important to carefully manage the broodstock diet and feeding regimen to ensure optimal reproductive health and egg quality (Izquierdo et al., 2001).

In Turkish aquaculture, sea bream, sea bass, turbot and some new species are farmed under intensive conditions. Feeding restrictions in broodstock feeding programs are made when necessary. There are professional farmers of broodstock feeding in many developed farms in Türkiye. However, there is not enough scientific research on this subject.

4.6. Specific feed ingredients to improve broodstock performance

Improving broodstock performance is one of the most important subjects in aquaculture operations. For this purpose, performance enhancing nutrients such as squid, soybean and krill are used in broodstock feeding. The basic information on these topics and the impact of the research are given below.

4.6.1. Squid

Squid is a good source of protein and other nutrients that can be included in broodstock diets to improve their performance and reproductive health. Squid contains high levels of essential amino acids, including lysine, methionine, and tryptophan, which are important for growth and development. It is also a good source of omega-3 fatty acids, which are essential for reproductive health, and can improve egg quality (Vassallo-Agius et al., 2001; Querol et al., 2014).

In addition to its nutritional value, squid is also highly palatable to many fish species and can stimulate their appetite. This can be particularly important for broodstock, as a healthy appetite is important for maintaining optimal health and reproductive performance (Santam-

aría-Miranda et al., 2021; Ortiz-Guillen et al., 2022).

Some researchers stated that squid contained nutrients necessary for successful spawning in seabream broodstocks (Stephanou et al., 1995; Yousif et al., 2012). Especially essential amino acids and essential fatty acids are of great importance for reproduction performance of sea bream broodstock. It was determined that egg quality was high in sea bream fed with squid meal. The high levels of protein and balanced essential amino acids found in squid meal are thought to improve egg quality. It was reported that these nutrient increases fecundity by 40% in broodstocks (Fernández-Palacios et al., 1997).

However, it is important to note that squid should be used in moderation in broodstock diets, as it contains high levels of purines, which can lead to the accumulation of uric acid in the body. Uric acid can be toxic at high levels and can lead to kidney damage and other health problems. Therefore, it is recommended to use squid as part of a balanced diet that includes other protein sources and to monitor the fish's health and uric acid levels regularly (Ishimaru et al., 2015; Zhang et al., 2016).

Overall, squid can be a valuable addition to broodstock diets as a source of protein and other important nutrients. However, as with any feed ingredient, it is important to use it in moderation and as part of a balanced diet that meets the specific needs of the broodstock.

4.6.2. Soybean

Soybean meal is commonly used as a source of protein in many animal feeds, including broodstock diets. It is a high-quality protein source with a balanced amino acid profile, which is important for the growth and reproduction of fish. It is also relatively low in cost compared to other protein sources, making it an attractive option for broodstock diets (Chen et al., 2022; Yamamoto et al., 2023). However, soybean meal should be used in moderation as the main source of protein for broodstock, as it contains anti-nutritional factors that can negatively impact fish health and reproductive performance. These anti-nutritional factors include trypsin inhibitors, lectins, and phytic acid. To mitigate the negative effects of these anti-nutritional factors, soybean meal can be treated with heat or enzymes to improve its digestibility (Kumar et al., 2019; Mravec et al., 2022).

Overall, soybean can be a valuable ingredient in a broodstock diet, but it should be used in moderation and in combination with other feed ingredients to ensure optimal broodstock health and reproductive performance.

4.6.3. Krill

Krill can be a good source of nutrition for some broodstock species, especially those that naturally feed on zooplankton in the wild. Krill are small crustaceans that are rich in protein, omega-3 fatty acids, and other essential nutrients that can promote growth, reproduction, and overall health in fish. However, it is important to note that krill can be relatively expensive and may not be readily available in all locations. Additionally, some broodstock species may not be adapted to a diet that is primarily based on krill, and may require additional supplementation with other nutrients such as vitamins and minerals. Furthermore, due to the ecological importance of krill in marine ecosystems, it is essential to ensure that any harvesting of krill for use in aquaculture feeds is sustainable and does not have negative impacts on wild populations or the broader marine food web (Mazorra et al., 2003; Saleh et al., 2018).

Krill can also be used as an alternative raw material to fish meal. It has been reported that when fresh krill is added to porgy broodstock diets, the rate of healthy eggs and hatching rate from fish is two times higher than that of those fed diets containing fish meal. It has been stated that this positive effect in krill is due to the high content of n-3 HUFAs and its high quality protein, thus being rich in essential amino acids. In addition, the pigment contained in krill increases the reproductive performance of broodstocks. However, there has not been enough research on this subject yet (Watanabe et al., 1991).

In overall, while krill can be a valuable feed ingredient for some broodstock species, it is important to carefully consider the nutritional needs of the species in question and the availability and sustainability of krill as a feed source.

In Turkish aquaculture operations, some nutrients such as squid, krill and soybean are used in order to improve broodstock reproduction performance. However, there are no studies on the use of these nutrients in broodstock feeding. Research on these issues in broodstock nutrition needs to be developed. Especially the projects and studies to be carried out by fish nutritionists of universities will make significant contributions to the farmers.

5. Conclusion and Future Perspective

In this chapter, basic information about broodstock management, reproduction and nutrition is presented in the main subjects. Broodstock management, reproductive control, and genetic improvement allow a hatchery to continuously improve the efficiency and productivity of the entire farming process. To the best of our knowledge, most of the reviews on broodstock management and nutrition are generally on only one species. However, we think that it would be more useful to present information on multi species, which was one of the objectives of this work. For this reason, this chapter will attract the attention of a wide vari-

ety of readers. In general, it can be seen that specific information about some species is still limited. This situation is due to the high cost of broodstock breeding and scientific research. However, there is definitely a need for detailed studies on broodstock management, reproduction, and nutrition. The results obtained from the researches will have a direct influence on the production performance of the farms. Therefore, we think that there is a need to increase the specific departments related to the subject at universities and research centers and to increase the number of advanced scientists in these departments. On the other hand, in order to develop the practical knowledge of the aquaculture sector on the subject, it is important to carry out joint projects with the cooperation of farmers and universities. In recent years, important studies have been carried out on broodstock management, reproduction and nutrition in Türkiye. Most of the studies in Türkiye are related to rainbow trout, seabream and seabass. However, there are some deficiencies in broodstock management, reproduction and nutrition of these species. In order for Turkish aquaculture to continue its development and growth, there is a need for extensive scientific research on broodstock breeding and nutrition. As stated in the relevant titles above, commercial shrimp and crayfish are not cultured in Türkiye. Blue crab farming is carried out in only one farm. There are Turkish scientists who do research on these species. These species, which have high economic value, should be cultivated commercially. In general, the future success of aquaculture in Türkiye will be possible with good broodstock management and nutrition.

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CHAPTER 11

GAMETE QUALITY AND PRESERVATION IN FISH: AN OVERVIEW OF STUDIES IN TÜRKİYE

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DOI: 10.26650/B/LS32LS24.2024.005.011

1. Introduction

Aquaculture in the world may have first emerged in two different geographical regions, North Africa, and China. In the old documents where production and feeding studies are located, polyculture studies using common carp and Chinese carp are mentioned in China and in addition to these culture studies, breeding studies are also mentioned that centuries ago. It is understood that production work began in 2100 BC thanks to the document explaining the method of laying eggs in carp fish (Timur and Memiş, 2011). In these production studies, selection applications made according to the morphology of the fish were used. By selection, the production of fish with the desired characteristics was carried out with the use of selected gametes, forming the basis of fish breeding practices. Over time, these issues have developed depending on needs, experience, equipment and technological developments, and the studies carried out for the purpose of gamete quality and the preservation of gametes have gained importance (Timur and Ekici, 2009).

Neuroendocrine control of reproduction in fish; it occurs as a result of the interaction of the brain, pituitary gland and gonads and is defined as the brain-pituitary-gonad axis (Karayücel and Karayücel, 2016). Gonadotropin hormones secreted from the anterior part of the pituitary gland and steroids secreted from the gonads have a direct effect on reproductive behavior. Steroids secreted from the gonads under the influence of gonadotropins perform gamete formation (Timur, 2011). The control of gamete quality is an issue that needs to be carefully addressed for successful breeding studies (Migaud et al., 2013), quality gamete production can

be achieved by implementing "broodstock management" in the breeding facility. Therefore, the first step of proper broodstock management is to determine the optimal conditions for a species to produce, the age of sexual maturity and good quality gametes (Bobe and Labbé, 2010). The first ones that come to mind from the criteria that determine the quality of the gamet; fish eggs can be fertilized by spermatozoa and complete the processes of embryogenesis (Bobe and Labbé, 2010), the successful completion of these processes depends largely on factors including the physiological characteristics of the broodstock fish and water criteria (Billard and Jensen, 1996). Moreover, gamete quality is affected by factors such as temperature, photoperiod, feed content and regimen, stress factors, hormone applications (Migaud et al., 2013). The production success of the aquacultured species; determination of the optimal incubation temperature (Saka et al., 2001; Saka et al., 2004; Polat, 2011), the quantity and quality of gametes, the hatching and survival rate (Billard and Jensen, 1996). In addition, in order to increase the production capacity, studies are carried out on many different subjects (obtaining healthy fish, reaching table fish size in a short time, obtaining only fast-growing sex, year-round gamete acquisition, obtaining quality sperm and eggs, reproductive biotechnology applications) (Aydın and Akyurt, 1993; Arslan et al., 2010; Yavuz, 2012; Ercan and Ekici, 2016; Ertekin et al., 2018; Momin and Memiş, 2018; Gelinçek and Yamaner, 2020).

Cryopreservation has become an important practice since it allows the protection of many endangered species and the long-term storage of gametes in living organisms containing commercially important species such as fish, and the use of these gametes in transfer or when necessary. In fish, sperm has been the most studied gamete in cryopreservation studies in both laboratory and culture conditions, and this is since sperm cells have small sizes and relatively high cryoresistance. In the last decade, different species-specific sperm cryopreservation procedures have been developed in fish species and studies on a wide variety of fish species are continuing today (Asturiano et al., 2017).

In the first part of this book chapter, scientific studies on determining gamete quality or factors affecting gamete quality in Türkiye are reviewed. In the second part of the book chapter, information was given about the studies carried out in Türkiye on the protection of endangered fish species and the short-term or cryopreservation of sperm, which is one of the important biotechnology applications in broodstock management in aquaculture.

2. Gamete Quality in Fish

2.1. Egg quality

Breeding studies were started with the introduction of rainbow trout (*Oncorhynchus mykiss*) eyed-egg to our country at the end of the 1960s and the use of Salmonid and Cyprinid

fish caught from nature in the same periods (Aydın and Baltacı, 2017). At the beginning of the 1970s, the incubation processes of rainbow trout eyed-eggs brought from abroad were completed and the studies on breeding (Baran, 1977) and determining the necessary conditions for a successful production started scientifically (Baran and Erdem, 1975). In the following years, especially in fish caught from nature, reproductive characteristics (such as egg diameter, fertility) were examined and production studies improved (Akyurt, 1992; Alp et al., 2003; Şahin et al., 2008; Şahinöz et al., 2011; Aydın et al., 2020).

Egg and globule diameter (Kamacı et al., 2005), blastomere morphology (Aydın, 2008), chemical and biochemical compositions (Bulut, 2004; İnanlı et al., 2019) constitute the egg quality parameters. In addition, fish egg diameters are not always related to quality parameters and that eggs of different sizes (Bromage et al., 1992) can exhibit similar developmental competence. In addition to the commercially produced species in Türkiye, the egg quality of the fish was evaluated by conducting studies on the determination of egg diameter and productivity of the endemic species (Yanar et al., 1987) (Table 1). In eggs belonging to the same species of fish culture in similar environmental conditions; differences in water quality and broodstock management practices, genotypic differences between broodstock fish can be observed due to the eyed-stage and hatching rate (Karataş and Arabacı, 2021).

The continuity of production in the culture systems is ensured by the gametes obtained from the broodstock fish in the systems. In addition to these broodstock fish, fish caught from the natural environment can also be used as broodstock individuals and the characteristics and fecundity obtained from these fish are also determined (Şahinöz et al., 2006 and 2007a). However, the quality of F_1 gametes obtained from broodstock individuals captured from nature is low (Memiş et al., 2021) and the water source influences egg quality (Yeşilayer and Bircan, 2013) and therefore on fertilization rate (Memiş et al., 2021).

Studies are carried out to evaluate the egg, embryo and larval quality of *Salmo trutta* ecotypes that spread naturally in the waters of Türkiye (Uysal and Alpbaz, 2003; Kocabaş, 2009; Kocabaş et al., 2011a, b; Kocabaş et al., 2012; Engin and Altan, 2022). It was found that there was a high correlation between the individual egg productivity of *S. trutta* broodstock height and weight, and between the egg diameter-egg weight relationships. It is stated that in each ecotype (Kocabaş, 2009) and the hybrids belonging to these ecotypes have a reproductive function (Altınok et al., 2018), and although the egg diameters of these ecotypes and their hybrids are similar, the larval weight of the Black Sea trout (*S.t. labrax*) (Başçınar et al., 2010) and individual egg productivity are at the highest level. In addition, hatching and the transition period to free swimming stage was realized in a short time and the best growth in freshwater size was again determined in Black Sea

Table 1. Egg quality parameters in fish species some studied in Türkiye

References	Scientific name	Feed Additive	Egg diameter (mm)	Egg weight (g)	Fecundity (nmbrs/kg)	Total Fecundity (nmbrs./ind.)	Fertilization rate (%)	Eyed-stage (%)	Hatching Rate (%)
Karataş, 1990	*S.t.macrostigma*		3.1±0.5 - 4.9±0.6		2783±8 - 3203±40				
Tabak et al, 2001	*S.t.labrax*		4.5-5.8		2428±162 (FW) 2543±131 (Sea)				
Şahin et al., 2008			1.56 ± 0.025			171.4 ± 109x10^3	17.2 ± 15.7		51.5 ± 27.6
Sonay, 2008	*S.t. labrax*		5.09 ± 0.03 – 5.3 ± 0.04	0.074±0.001 -0.085±0.003	2638.5	2844.1	97 ± 1.52 – 98.5 ±1.06		74.41 ± 2.26 – 83.42 ± 3.75
Hara et al.,2002	*Psetta maxima*						39.5		32.5
Ciftci et al., 2002	*Psetta maxima*		1.08 – 1.21		510				
Aydın and Şahin, 2011	*Psetta maxima*					2329 ±1260x10^3	30.6 ± 25.4		17.9 ± 16.1
Kurtoğlu, 2002	*S.t.labrax* *S.t.labrax*.(marine)		5.47 ± 0.308 5.45 ± 0.411		1020-3199 711-6263				68-92 (FR and HR)
Aydın et al., 2020	*Psetta maxima*				2.4 ± 1.71x10^6 2.3±1.75x10^6		59.2 ± 17.07 67.1 ± 18.87		67.3 ± 30.67 (Wild) 61.3 ± 30.85 (Hatchery)
Demir et al., 2010	*S.t.macrostigma*		3.51 – 3.78		1840 - 3200				32 - 57
Gelinçek and Yamaner, 2020	*S.t.labrax*		4.3±1.8	416±165	4.4 ± 1.6	4362±1638			

Table 1. Continue

References	Scientific name	Feed Additive	Egg diameter (mm)	Egg weight (g)	Fecundity (nmbrs/kg)	Total Fecundity (nmbrs./ind.)	Fertilization rate (%)	Eyed-stage (%)	Hatching Rate (%)
Gelinçek and Yamaner, 2020	*S.t.labrax*	Mealworm larvae	4.5±1.4 – 4.5±1.8	323.6±140-368±105	3.4±1-3.8±1.1	3436±1016-3797±1130			
Özdemir and Ekici, 2022	*S.coruhensis*							80.4±3.3 – 87.8±3.8	76.4±4.8-83.5±7.7
Erbaş and Başçınar, 2013	*S.t.labrax*		4.55±0.5 – 5.12±0.5	0.076 - 0.082			93.3±16.3 – 99.5±1.34		
Doğu et al., 2013	*Capoeta trutta*		1.52-1.65						
Uysal and Alpbaz, 2003	*S.t.abanticus*		5.01±0.2 – 5.06±0.2	0.080 – 0.081			95.1- 53	93.2-51.5	91.1-46.5
Uysal and Alpbaz, 2003	*O.mykiss*		5.20±0.2 – 5.1±0.3	0.085 - 0.110			88 - 85	82- 78	76 - 72
Canyurt and Akhan, 2008	*O.mykiss*							84.1±2.39	
Canyurt and Akhan, 2008	*O.mykiss*	E vitamine						82.4±1.55-85.1±2.05	
Kocabaş, 2009	*S.t.abanticus*		4.91±0.4	60.13±56.9	1871±742	623±515	99.88±0.2	67.19±46.6	54.9±35.3
Kocabaş, 2009	*S.t.macrostigma*		4.52±0.5	11.78±7.9	2403±953	193±123	82.85±4.6	48.39±10.99	43.9±42.4
Kocabaş, 2009	*S.t.caspius*		4.23±0.3	24.81±10.1	4000±1092	505±206	85.47±16.9	58.47±38.6	50.4±20.6
Kocabaş, 2009	*S.t.fario*		4.59±0.53	106.2±102.3	1988±865	1179±669	83.47±10.4	48.82±2.89	41.3±9.01
Kocabaş, 2009	*S.t.labrax*		4.67±0.5	112.02±92.02	2314±858	1476±1043	96.92±7.5	67.99±22.18	47.8±18

Table 1. Continue

References	Scientific name	Feed Additive	Egg diameter (mm)	Egg weight (g)	Fecundity (nmbrs/kg)	Total Fecundity (nmbrs./ind.)	Fertilization rate (%)	Eyed-stage (%)	Hatching Rate (%)
Yıldız et al., 2020	*O.mykiss*	CD	5.4±0.1	0.077±0.001	1497.5±38.1				
		LO	5.4±0.1	0.079±0.002	1468.8±21.2				
		SO	5.3±0.1	0.076±0.002	1468.0±33.4		89.2±5.8	87.3±5.3	81.2±4.3
		FO	5.4±0	0.076±0.003	1453.3±5.9		92.1±4.9	84.1±4.4	78.3±3.4
Yıldız et al., 2021	*O. mykiss*	CD					90.3±1.7	83.2±2.3	75.8±2.0
		FO					96.2±1.8	88.2±3.3	83.0±3.0
		LO					94.3±1.8	89.0±2.5	86.2±3.2
		SO					95.3±1.8	89.1±2.7	85.0±3.8

CD: Control diet, LO:Linseed oil, SO: Sunflower oil, FO: Fish oil, nmbrs: numbers, ind.: individuals, FW: fresh water

trout (Kocabaş, 2009). While larval hatching times are similar in some studies (Erer, 2004; Başçınar et al., 2005; Kocabaş, 2009) there are also studies where there is a difference (Kurtoğlu, 2002). Significant relationships have been identified between fecundity and height-weight in S. *trutta* populations captured from nature (Arslan and Aras, 2007; Kocabaş and Başçınar, 2016). In Anatolian trout (*S. t. macrostigma*), egg diameter increased as the fish age increased, and fecundity decreased (Karataş, 1990). It is stated that there is a relationship between broodstock weight and absolute egg productivity (Yeşilayer and Bircan, 2013) and fish length and egg diameter (Ekingen, 1983). It is stated that there is a significant negative relationship between the number of eggs and the egg diameter in rainbow trout (Köprücü and Gür, 1999). It has been reported that the egg diameters of *S. trutta* depending on the size of the fish (Tatar, 1983; Kurtoglu, 2002). While a positive correlation was determined between fertilization rate and egg size in Abant trout (*S. t. abanticus*), a negative correlation was determined between fertilization rate and egg productivity (Bozkurt et al., 2006a).

The spawning success of rainbow trout; the number of eyed-eggs and larval growth performance are greatly influenced by various biotic and abiotic factors. Photoperiod, which is one of these factors, is important in initiating or delaying gonadal maturation as well as reproductive time that varies according to species. Thus, ovulation and spermiation processes can also be taken early period (Atasever and Bozkurt, 2015). The quality of gametes obtained from broodstock fish exposed to photoperiod may vary from that of gametes supplied during the normal breeding season and a decrease in fertilization rate may be observed (Çoban et al., 2011; Momin and Memiş, 2018). In addition, the prolongation of the retention time of broodstock fish in light and dark environment shows a positive effect on the survival and growth rates of the larvae (Engin, 2022).

During embryogenesis and larval development of most fish species, growth and energy supply depend on endogenous yolk reserves transferred by broodstock (Bromage and Roberts, 1995; Zengin and Akpınar, 2006). Dietary ration and ratio of nutrition used for feeding broodstock fish; it significantly affects fecundity, egg maturation and egg size (Bromage and Roberts, 1995; Okumuş, 2002). It has also been identified as lipid and fatty acid composition, gamete quality, and major factors determining the survival of offspring (Fernandez-Palacios et al., 2011). Astaxanthin, vitamin E (Izquierdo et al., 2001), and phospholipids containing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been cited as the most important components affecting egg quality (Bromage and Roberts, 1995). However, the addition of vitamin E to the rainbow trout feed ration does not affect fertilization rates (Canyurt and Akhan, 2008), as well as the inclusion of n-3 series essential fatty acids in the diet positively affects egg quality and related parameters (Özgür, 2009). The total amount of lipids and fatty acids, which reached the highest level during the ovulation period, showed a decrease after the ovulation period (Metin and Akpınar, 2000). Fish egg (Yavuz Keskin, 2017; Baki et al., 2021), embryos and fry (Zengin and Akpınar, 2006), in studies where the amount of fatty acids were examined, it was stated that the fatty acids in the egg were used as energy sources and structural components in the embryogenesis and yolk-sac stage (Zengin and Akpınar, 2006). Although the use of vegetable oil as a source of lipid in fish feeds does not show a negative effect on egg quality (Yıldız et al., 2020) and reproductive performance in rainbow trout (Agh et al., 2019; Yıldız et al., 2020) the fatty acid profile of eggs and spermatozoa is influenced by lipid in the diet (Yıldız et al., 2020, 2021). In addition, it is stated that there is no change in the egg amount and diameter of the Black Sea trout broodstock fed with mealworm larvae (*Tenebrio molitor*) (Gelinçek and Yamaner, 2020), but the low frequency of feeding of the broodstock increases the egg diameter (Erbaş and Başçınar, 2013).

In a limited number of studies on the short-term preservation of fish eggs; using glucose (Bozkurt et al., 2010) and ovarian fluid in carp (*Cyprinus carpio*) (Bozkurt and Yavaş, 2012; Bozkurt, 2019); in rainbow trout, fertilization success has been achieved by using commercial medium (İnanan, 2020).

2.2. Sperm quality

The success of sperm in fertilizing the egg and allowing the development of a normal embryo is expressed as sperm quality and this quality is affected by factors such as stress and environmental factors (temperature, photoperiod, salinity) (Bobe and Labbe, 2010). In addition, the feed content of broodstock fish (Gelinçek and Yamaner, 2020; Yıldız et al., 2020 and 2021) and reproductive time (Seçer et al., 2004) also affect sperm quality.

Spermatozoa motility affects fertilization success (Bozkurt and Seçer, 2006a). However, studies to evaluate the macroscopic and microscopic parameters of sperm in Türkiye started in the 2000s (Çevik, 2000). Tekin et al. (2003) states that spermatozoa are directly related to motility time and live body weight, fish size and sperm amount. However, regardless of the increase in age, it is stated that the sperm amount and sperm motility of young broodstock individiuals that develop well in length and weight are better than older broodstock individual (Tekin et al., 2003). Similarly, sperm motility parameters of 2-3-years-old masculinized females were found to be better than those of older males (İnanan and Yılmaz, 2018). As the age of fish increases, the amount of sperm (ml) (Tekin et al., 2003; Doğu et al., 2014), motility (%), survival time (sec), total number of spermatozoa ($x10^9$) increase but spermatozoa density ($x10^9$sp/ml) decreases (Tekin et al., 2003). The age of the fish as well as the time gets sperm from the broodstock fish also affects the quality parameters (Aral et al., 2004a and 2007; Şahinöz et al., 2007b). In mirror carp (*C. carpio*); sperm amount, motility, duration of viability, density, and total number of spermatozoa (Bozkurt and Seçer, 2006b), as well as spermiating, pH, and sperm concentration in young rainbow trout showed a significant increase in the middle of the reproduction season (Aral et al., 2005). However, while sperm volume and sperm motility time did not differ (Aral et al., 2005), Şahinöz et al. (2008) stated that semen pH values were significantly related to sperm concentration and volume. In *S. t. abanticus*, there is a positive correlation between fertilization rate and spermatozoa motility (Bozkurt et al., 2006a). In *S. t. fario* (Bozkurt et al., 2006b) and scaly carp (*C. carpio*) (Bozkurt, 2006a), a low and meaningless relationship was found between fish size and sperm quality parameters. In addition, it has also been noted that there is no significant correlation between live body weight and total body lenght and spermatologic characteristics (Aral et al., 2004b). In sea bream (*Sparus aurata*), it was determined that there was no relationship between broodstock weight and length and sperm volume-concentration-velocity-preservation times (Engin et al., 2018). It is stated that there is a significant negative relationship between sperm amount and spermatozoa motility and sperm amount and density in rainbow trout (Köprücü and Gür, 1999) and there is also a correlation between fertilization and hatching rates (Bozkurt, 2006b).

The water source where the broodstock fish was kept affects the sperm motility parameters and therefore the fertilization rate (Memiş et al., 2021) and the variable water temperature during the gamete production period positively affected the sperm motility parameters in the Çoruh trout (*S. coruhensis*) (Tunçelli and Memiş, 2021).

Even among species in the same family as well as in different families, the duration of sperm motility varies. The duration of spermatozoa motility in crucian carp (*Carassius carassius*) was 118.57±16.3 sec (Doğu et al., 2015), while in grass carp (*Ctenopharyngodon idella*) it was 69.5-77 sec (Bozkurt et al., 2009a) and in mirror carp it was 571 sec (Akçay et al., 2004). It was 190.30±11.25 sec in *Carasobarbus luteus* and in crucian carp was 107.30±12.03 sec (Aral et al., 2014). The sperm motility duration in wild-caught Mesopotamian spiny eel (*Mastecembulus mastecembulus*) 175.80±17.00 sec (Şahinöz et al., 2007b), 90.80±10.4 sec (Aral et al., 2007) in rainbow trout, and in Salvelinus fontinalis was between 38.30±4.26 sec and 60.70±4.70 sec (Köse and Şahin, 2015a,b), in flounder (*Platichthys flesus luscus*) 25.4±4.20 min (Aydın et al., 2011) and 22.0±1.5 min (Şahin et al., 2012); in the Russian sturgeon was 304±135 sec (Yamaner et al., 2015) and was measured as 80.9±1.5sec in leaping mullet (*Liza saliens*) (Engin et al., 2020). The density of spermatozoa is $17.88±5.48x10^9$/ml in grass carp (Bozkurt and Öğretmen, 2012), in Russian sturgeon (*Acipenser gueldenstaedtii*) $3.03±1.1x10^9$/ml (Yamaner et al., 2015), in crucian carp $17.84±3.44x10^9$/ml (Doğu et al., 2015), in *Salvelinus fontinalis* 9,10±1,16-$12.9x10^9$/ml (Köse and Şahin, 2015a,b), in rainbow trout $12.71x10^9$/ml (Çevik, 2020), in flounder $2.8±0.72x10^9$/ml (Aydın et al., 2011) and 2.7±0. $16x10^9$/ml (Şahin et al., 2012), 0.16-$5.35x10^9$/ml in gilthead sea bream (Engin et al., 2018) and $14.0±8.9×10^9$ in leaping mullet (Engin et al., 2020) and $4.5±1.8x10^9$/ml in Çoruh trout (Özdemir and Ekici, 2022).

The methods, instruments and equipment used in the determination of sperm quality parameters differ between studies. Spermatocrit (%) (Aral et al., 2004a; Ölçülü et al., 2022) or haemocytometer (Babaoğlu, 2019) is used for sperm density determination. In the measurement of sperm motility, 5 scale method (Yamaner, 2012) or Computer Aided Sperm Analysis system is used (Yamaner et al., 2018; İnanan and Kanyılmaz, 2020; Tunçelli and Memiş, 2021; Hassan Ahmed et al., 2022). In addition to the use of different methods and devices in the evaluation of quality parameters, the motility parameters obtained may vary according to the characteristics of the slides used in the evaluation of kinematic parameters of sperm motility (Yamaner et al., 2020).

Hormonal applications; in addition to providing ovulation (Kayım et al., 2010) and spermiation, it is also stated that it increases the VCL value, which is one of the kinematic param-

eters of spermatozoa (Yamaner et al., 2018). In addition, it is stated that the type of steroid hormones used for sex transformation also affects sperm motility parameters (İnanan and Acar, 2021) and various solutions are used to mature the testicular sperm obtained from these fish (Hacısa and Arslan, 2021).

It was also determined that increasing the amount of vitamin E in the rainbow trout feed ration increased the sperm quality parameters (concentration, motility, spermatocrit ratios) (Canyurt and Akhan, 2008), and that the Black Sea trout broodstock fed with mealworm larvae increased sperm volume, but there was no change in sperm kinematic parameters (Gelinçek and Yamaner, 2020). It was determined that the use of vegetable oil as a lipid source in fish feeds did not make a difference on sperm kinematic parameters (Yıldız et al., 2021) and that the low feeding frequency positively affected sperm motility (Erbaş and Başçınar, 2013).

The success of sperm in fertilization the egg varies depending on the content of the solution used in sperm activation (İnanan et al., 2018). For this reason, studies are carried out to add various substances to the activation solution to improve sperm motility parameters. To the activation solution; boric acid (*S. rizeensis*- Kutluyer and Kocabaş, 2017; *S. coruhensis*, *O. mykiss*-Kocabaş and Kutluyer, 2017a), L-tryptophan (*S. coruhensis*, *S. rizeensis*, *O. mykiss*-Kutluyer, 2018a), L-arginine (*O. mykiss*- Danabaş et al., 2019; S.*coruhensis*- Kocabaş et al., 2019a), cobalt ($CoCl_2$) (*S. coruhensis*-Kocabaş and Kutluyer, 2017b, spirlin-*Alburnoides bipunctatus*-Kutluyer et al., 2019a), zinc (*S. coruhensis*, *O. mykiss*-Kocabaş and Kutluyer, 2017c), N-(2-Merkaptopropionyl)-glycine (*O. mykiss*-Çakır Salihli et al., 2019) positively affected sperm motility parameters. In addition; the adding to plant extracts (*Tribulus terrestris*, *O. mykiss*-Ölçülü et al., 2022) in the activation solution, the duration and percentage of sperm motility increases with the addition of cystein (*S. coruhensis*-Kutluyer Kocabaş, 2022) as an antioxidant.

Different diluent contents are being composed for the activation of the sperm of each fish species. In this activation solution, pH, osmotic pressure, ions, and their concentrations are important (Cosson, 2004; Billard and Cosson 1992, Ciereszko et al., 2002). In addition to ion composition, oxidative damage and andioxidant level also affect sperm motility, and the oxidative stress level also differs between fish species (Kutluyer Kocabaş et al., 2022a). In the sperm motility parameters of the Black Sea trout; the highest motility value was found in the non-ionic 300 mM sucrose solution, while the addition of $CaCl_2$ to iso-osmotic ionic and/or non-ionic solutions also increased motility (Yamaner et al., 2022). The fact that the activation solution contains 1 mM $CaCl_2$ and 103 mM NaCl in sperm activation in brown trout positively affects sperm kinematic parameters (Özgür, 2018). It was observed that the sperm of masculinized females had higher Mg^{+2}, Na, K and Cl^{-} concentrations compared to

normal males, but the pH was lower (İnanan and Yılmaz, 2018). The maximum percentage and duration of sperm motility of shabout (*Barbus grypus*) spermatozoa was determined in diluents with an osmolality value of 56 mOsmol kg^{-1}, containing 5 mM K and Ca^{+} free (pH 9) (Öğretmen et al., 2014). Bozkurt et al. (2008) found that Na and Ca^{+} ions in grass carp sperm showed negative correlation with spermatozoa motility and K ion showed positive correlation. In mirror carp, a negative correlation was observed between Ca^{+2}, K and Mg^{+2} ions and spermatozoa motility (Bozkurt et al., 2009b). In scaly carp semen, K and Ca^{+2} ions were positively correlated with spermatozoa motility; Na and Mg^{+2} ions were negatively correlated with motility (Bozkurt et al., 2009c). In *S. t. macrostigma*, Ca^{+2} and Mg^{+2} ions were positively correlated with sperm motility and Na, K and Cl^{-} ions were negatively correlated with motility (Bozkurt et al., 2011a).

pH (Öğretmen et al., 2016; Kutluyer, 2018b) and osmotic pressure, as well as ovarian fluid, are effective in sperm activation and ovarian fluid can also lead to suppression of sperm motility. It was found that in ovarian fluids that do not show activating properties, K, Ca^{+2} and Mg^{+2}, protein, cholesterol, glucose, and enzymatic activity concentrations are at higher levels and, Na and pH levels are lower compared to ovarian fluids with activating properties (İnanan and Öğretmen, 2015).

In Türkiye, studies have been carried out on the biochemical contents of seminal plasma belonging to various fish species and ecotypes and the relationship between these parameters and sperm quality values (Bozkurt, 2008). In addition, the amount of sperm and Packed Cell Volume (PCV) values in the blood are also shown to be affected by age. A negative relationship was found between sperm motility percentage and Red Blood Cell (RBC), which is a hematologic parameter (Doğu et al., 2014). The seminal plasma of masculinized female rainbow trout was found to have higher Total Antioxidant Capacity (TAC) values and protein concentrations and lower levels of lipid peroxidase (LPO) levels compared to male trout without steroid hormone treatment (İnanan et al., 2016). Güllü et al. (2015) observed that the chemical composition levels, osmolality and pH, sperm volume and density of shabout seminal plasma affect sperm motility and duration. Although there is no exact relationship between the biochemical and spermatological parameters of the sperm of rainbow trout, it is stated that higher Na content has a positive effect on semen volume (Seçer et al., 2004). The Na and K content in Abant trout (Bozkurt, 2008) sperm and the ion composition of the activation solution in scaly carp sperm influence the survival rate and duration of the sperm (Bozkurt et al., 2011b). In *S. t. fario*, a negative correlation was found between body weight-length and spermatological and biochemical parameters (apart from protein and cholesterol)

(Bozkurt et al., 2006b). It is stated that it can be used to determine the relationship between haematological parameters and sperm quality characteristics (Şahinöz et al., 2006).

Pesticides, herbicides, insecticides, and other contaminants; it acts at levels that can cause the death of living creatures in the terrestrial environment (insects, rodents, plants) as well as aquatic organisms. Fish sperm is used to understand as marker how these pollutants released into the aquatic environment affect the health of aquatic organisms. Non-lethal doses of cypermethrin (O. *mykiss*-Kutluyer et al., 2016; *S. coruhensis*-Kutluyer et al., 2018a); malathion (*S. coruhensis*-Kocabaş et al., 2018) and chlorpyrifos (*S. coruhensis*-Kutluyer et al., 2019b) have been found to cause oxidative stress in sperm. Low concentrations of bisphenol A (spirlin-*A. bipunctatus*-Kocabaş et al., 2021; chub-*Squalius orientalis*, padanian barbel-*Barbus plebejus*-Kutluyer et al., 2022) and glyphosate (*O. mykiss*-Akça et al., 2021) have been shown to act on spermatozoa even at low doses; similarly, lambda-cyhalothrin has been found to reduce sperm motility and affect oxidant and antioxidant status (*O. mykiss*-Kutluyer et al., 2015). Aroclor 1254 adversely affected sperm kinematic parameters as well as fertilization and larval hatching rates (*O. mykiss*-Kocabaş et al., 2022). The use of antioxidant substances (ascorbic acid) to reduce the negative impact caused by various pollutants (arsenic) has shown a positive effect on oxidative stress and fertility as well as reducing the negative effect on sperm cells (Kutluyer Kocabaş et al., 2022b). It has been determined that boron (50 and 100 mg L^{-1}) has a negative effect on the DNA integrity of tilapia (*Oreochromis niloticus*) sperm (Acar et al., 2018) and leads to changes in the oxidative balance of spermatozoa cells and adversely affects the quality of spermatozoa (Acar et al., 2022). In addition to these studies, studies are also carried out to examine the effects of metal bioaccumulation on gonad tissues (Danabaş et al., 2020). In addition, environmental conditions in which fish living in their natural habitat are located also affect the antioxidant level of sperm (Kutluyer et al., 2018b; Kocabaş et al., 2019b).

3. Sperm Preservation Studies in Türkiye: Short-Term Storage and Cryopreservation

Cryopreservation has become an important practice since it allows the protection of many endangered species and the long-term storage of gametes in living creatures containing commercially important species such as fish, and the use of these gametes in transfer or when necessary. In fish, sperm has been the most studied gamete in cryopreservation studies in both laboratory and culture conditions, and this is since sperm cells have small sizes and relatively high cryoresistance. In the last decade, different species-specific sperm cryopreservation procedures have been developed in fish species and studies on a wide variety of fish species are continuing today (Asturiano et al., 2017).

So far in Türkiye, a large part of past research has focused on the cryopreservation of sperm cells of some fish species, mainly salmonids, carp, sturgeon, catfish, and in recent years significant research has been conducted on marine species and various inland finfish species. At the same time, short-term storage, which is another application of sperm preservation, has been studied on fish species belonging to Türkiye and the details of all studies (including short-time storage and cryopreservation) on a family basis are compiled below.

3.1. Inlandwater fish species

3.1.1 Salmonidae

Cryopreservation is a procedure that requires the use of the extender and cryoprotectant substance, which is responsible for protecting the sperm cells from shock, which must be added with its effect on the seminal plasma. The addition of cryoprotectant, freezing and thawing are applications that cause damage to sperm cells and cause a decrease in fertilization rate. For this reason, in sperm cryopreservation, it is essential to comprehensively evaluate the different extender and cryoprotectant and to develop an optimal cryoproservation procedure for each species (Hu and Tiersch, 2011). For this reason, fish sperm cryopreservation studies in our country as well as in the world are focus on the variety and dilutiation rate of extender and cryoprotectant that are important during and after freezing.

Rainbow trout, (*O. mykiss*) is one of the most important fish species in the Türkiye due to reasons such as high aquaculture potential, economic value, widespread consumer demand and the elimination of all kinds of unknowns in aquaculture studies. Rainbow trout, which is one of the most cultured species (165.683 tons in 2021) (TUİK, 2022) in Türkiye, is also the most emphasized species in sperm cryopreservation studies. In sperm cryopreservation studies on this species, extender and cryoprotecant diversity and dilution rate have become the main target and the studies have been designed to find the optimum equation for these substances.

DMSO (Dimethyl sulfoxide) and DMA (Dimetilasetamid), which are among the most widely tried cryoprotectant in both research and application areas that have proven their effectiveness in sperm freezing studies in many fish species. In our country, sperm cryopreservation studies of rainbow trout have focused on the availability of these substances. In one of the studies that can be considered as one of the first studies on freezing rainbow trout sperm, DMSO was used in sperm cryopreservation, and it was reported that the percentage of fertilization obtained from frozen-thawed sperm samples with 10% DMSO (glucose supported) was like the results obtained in the fertilization study with fresh sperm (Tekin et al., 2003b). With the knowledge that the freezing procedure and extender differences should be evaluated

by taking into consideration, there is a study reporting that 15% DMSO (sugar-base) can be used in rainbow trout sperm cryopreservation (Bozkurt et al., 2005b). Four different diluents (0.3 M glucose, 0.6 M sucrose and Erdahl and Graham's) and four different equilibration times were tried, and the authors reported that the best result was achieved with an equilibration time of 0-5 minutes for each diluent for rainbow trout (Aral et al., 2009).

In sperm cells that have undergone the freeze process, the main factors of cell damage are intracellular ice crystal formation, changes in antioxidant defense mechanisms, cold shock, osmotic stress, production of Reactive Oxygen Species (ROS) and elements of these. Antioxidants have been proposed in numerous studies to counteract the harmful effects of ROS on spermatozoa during the freezing process (Amidi et al., 2016). In addition to the selection of extender, which is one of the most important components of sperm cryopreservation studies, studies on the addition of different antioxidant substances to the selected diluent so that sperm cells are minimally affected by the shock of the procedure are also common. In a study conducted with rainbow trout, it was suggested that the motility parameters of sperm cells cryopreserved with 11 different antioxidant-containing extender changed and antioxidant substances should be used (Kutluyer et al., 2014). In another study conducted in rainbow trout, it was found that the use of taurine as an antioxidant substance as 50 mM positively affected all motility parameters (Ekici et al., 2012). In addition, it is known that the addition of ascorbic acid to the sugar-based extenders improved frozen-thawed rainbow trout sperm motility and fertility (Yavuz and Bozkurt, 2020).

In addition to the use of chemicals as extenders in sperm freezing studies, there are studies that try the use of natural substances that are protective and can be used as an energy source such as glucose for sperm cells after thawing (Betsy et al., 2015), and one of these studies was carried out with rainbow trout in our country. Grape molasses, which is one of the traditional products of Türkiye and obtained from grape juice, the substances tried in sperm cryopreservation studies. In the study conducted by Doğu et al. (2016) with rainbow trout, grape molasses tried various ratios (5, 7, 10%) and it was revealed that 10% grape molasses could be successfully used in sperm cryopreservation.

Antibiotics are used for the elimination of sexually transmitted diseases. It has been reported that penicillin and streptomycin are also used to prevent contamination during the preservation of the sperm of farm animals (Salvetti et al., 2006). Antibiotics are biologically active substances which are thought to affect cell function. Streptomycin, one of the antibiotics used in laboratory studies, is used in mammals for sperm washing, and in cryopreservation media to control fungal and bacterial growth (Magli et al., 1996; Khaki et al., 2008). In our country,

the effect of antibiotic use of rainbow trout in sperm cryopreservation has been examined and it has been revealed that the presence of streptomycin in the diluent has a negative effect on motility percentage and motility duration (Ekici et al., 2013).

Brown trout (*S. t. macrostigma*), a member of the salmonidae family that spreads in the inland aquatic habitats of Southern Europe, West Asia, North Africa, and Anatolia, is a species of fish that is critically endangered in inland waters due to illegal fishing, overfishing and environmental changes, including pollution in general and pollution from hydroelectric power plants in particular. Brown trout, which is produced and researched aquaculture conditions to increase natural populations, is also a species used in hybrid studies in our country. Hybridization studies of Salmonidae species are being carried out in our country, and besides these studies, there are studies in which frozen sperm is used in hybrid studies. It has been reported that the use of frozen brown trout sperm in fertilization rainbow trout eggs, the fertilization percentage obtained at a rate of 42.5±1.4% was obtained by cryopreserved sperm with carbohydrate-based extender (Bozkurt and Yavaş, 2014a).

In addition to DMSO and DMA studies, there are studies that have tested cryoprotectants known as extracellular cryoprotectants. In the study conducted with Brown trout sperm freezing was conducted with egg yolk, which is an extracellular cryoprotectant, and it was revealed that it had a positive effect on motility after thawing (with ionic extender) (Bozkurt et al., 2012). Apart from cryoprotectants, there are studies that recommend the addition of other substances that can help cryoprotectant. In a study with Brown trout, it was revealed that the addition of docosahexaenoic acid (DHA), one of the PUFAs, to the extender was effective and it was stated that sperm samples frozen with tris-glucose supported DHA (7.5 ng mL^{-1}) added extender resulted in fertilization with a rate of 52.6% (Bozkurt et al., 2021a). In another study conducted on brown trout, it was revealed that the addition of vitamin E as an antioxidant substance in sperm freezing had a positive effect on motility parameters, fertilization, and DNA integrity (Bozkurt et al., 2021b) and addition of boron an increase in the fertilization rate (Bozkurt et al., 2019a).

In addition to cryopreservation sperm using liquid nitrogen, it is also one of the commonly used techniques to store it at 1-9°C. The process, which is called "short-term preservation", can be used in many applications in aquaculture. In genetic studies that require time, to eliminate the low quality of the egg, in cases where the sperm needs to be transferred and in order to use the high-quality sperm, the storage of the sperm for a few days makes it possible to perform the above-mentioned applications (Jawahar et al., 2020). Short-term storage studies, which are widely used in aquaculture studies, have been tried in many fish species in our

country. It is known that this application, the usability of which has been demonstrated in practice and theoretically, is especially important in aquaculture when gamete quality parameters are not equivalent.

In the short-term storage study conducted with Abant trout sperm, one of our endemic species, it was reported that the rate of reaching the eyed stage was 80.4% by storing the sperm with glucose-added solution for 24 hours (the highest rate obtained in the study) (Hatipoğlu and Akcay, 2010).

Another study on short-term preservation is for the sperm of brook trout (*Salvelinus fontinalis*) and rainbow trout to be stored at 4°C temperature in Dimethyl sulfoxide (DMSO) for 12 days with L-tryptophan supplementation. And the authors have revealed that L-tryptophan can be used effectively in short-term storage for both species (Kocabaş et al., 2019a). In the study conducted with rainbow trout, it was reported that the highest motility (64.4±5.27%) and fertilization (94.3 ± 0.58%) rate were obtained by storing sperm with glucose-based extender for 72 hours (at 4°C) (Şahin et al., 2013b). There are studies showing that rainbow trout sperm maintains its motility for 6 days as a result of short-term storage with glucose DMSO-based extender in a ratio of 1:3 (Aksu et al., 2018). Also, it has been reported that spermatozoa which short term preserved without using any extenders or additives between 0-4°C in laboratory were included in the scope of minimum motility categories class (Babaoğlu, 2019).

3.1.2. Cyprinidae

Cyprinid fish, especially carp (*C. carpio*), is important for the future in countries that are mostly focused on the culture of carnivorous species, for the sustainability of aquaculture. With the acquisition of fish meal and oil, which are essential for the nutrition of carnivorous fish species, from natural fish and the notification that natural fish stocks are in danger of collapse by various authorities, increasing the cultivation of omnivorous and herbivorous species in addition to carnivorous species is important for the sustainability of both human food supply and natural fish stocks. Although cyprinide species are listed as the most produced species in general aquaculture worldwide; in our country, which is among the top ten in aquaculture (FAO, 2022), the production of cyprinid species has been disrupted. At the end of the 1980s our country, carp fish constituted approximately 70% of the total aquaculture, while carp production in 2020 was reported to be 0.04% of total production. According to TUİK data, in 2021, 171 tons of carp fish were cultured in our country (TUİK, 2022).

Another family that includes fish species where sperm cryopreservation studies are more common in our country is the Cyprinidae species. And in carp fish species, the most emphasized

component in sperm cryopreservation was the cryoprotectant to be used during freezing. Egg yolk is one of the most common cryoprotective components of extender used to freeze and store sperm cells, especially those belonging to mammalian species and fish species. Due to this feature of the egg yolk, in the study conducted with the egg yolks of three different poultry species in carp sperm, duck eggs were classified as the best cryoprotectant due to it contained (Avlar and Bozkurt, 2022). However, since the yolk, contains pathogens, and increases the transmission in the freezing study, the studies have led to the discovery of an alternative that may be extracellular but does not contain pathogens. It has been suggested that replacing egg yolk as a non-permeable cryoprotectant in cryogenic expanders with plant-based LDLs, such as soybean lecithin, may reduce sanitary risks and improve the quality and fertility of cryopreserved sperm (Gil et al., 2003; Akhter et al., 2012). Soybean lecithin is currently used in commercially prepared extenders such as sound Andro-Med (Andromed, Minitube, Germany), Biociphos plus and Bioxcel l (Aires et al., 2003; Moussa et al., 2002), which have been successfully used for the cryopreservation of beef, waffin and ovine sperm. The first comparative research on soybean lecithin as an alternative to egg yolk in cryopreservation of sperm in fish was conducted in Türkiye. In a study with carp, it was concluded that the animal protein-free extender, which contains 10% soybean lecithin, has similar cryoprotective effects to the traditional egg yolk-based extender against frost damage and fertilization (Yıldız et al., 2013).

As in the Salmonidae family, it is also present in studies on sperm cryopreservation in cyprinid species and in studies where the use of antioxidant substances during freezing is applied. In the study that examined the effect of taurine (sulfonic amino acid), vitamin C (ascorbic acid) and bovine serum albumin (BSA) on motility, viability, and fertilization ability after thawing, it was concluded that taurine was the most effective antioxatidant material for carp sperm (Yavaş et al., 2014). In the study conducted with amino acids, another additive added to the extender to protect the cell from damage in sperm cryopreservation, a fertilization rate of 97 ±1.73% was obtained by using cysteine (20 mM) in the sperm cryopreservation procedure of carp (Öğretmen et al., 2015). Different cholesterol-loaded concentrations of cyclodextrin were tried by Yıldız et al. (2015) for cryopreservation of carp sperm. And the authors concluded that cholesterol-loaded cyclodextrin for carp sperm cryopreservation significantly improved cell cryosurvival and fertilization. In addition, Öğretmen and İnanan (2014a) stated that the presence of butylated hydroxytoluene (BHT) in the ratio of 0.001–0.1 mM in the extender in the sperm freezing of carp increased the success of the cryopreservation procedure.

In the study conducted with another cyprinide species, Tigris scraper, (*Capoeta umbla*) (Pisces: Cyprinidae), it was stated that the use of 1 mM L-tryptophan influenced motility after

freezing-thawing but did not seem motility with 2 mM L-tryptophan (Kutluyer et., 2019c). In the sperm cryopreservation study conducted with longspine scraper (*Capoeta trutta*), another cyprnid species that is a dominant species in the Atatürk dam lake fish fauna and is considered one of the important species in sport and commercial fishing, it was stated that DMSO is the best cryoprotectant for this species and that these studies should be expanded for this species in the future (Şahinöz et al., 2018). In addition, DMA (15%), which is used intracellular cryoprotectant, can be used effectively to freeze carp fish sperm with sugar-based extender (Akçay et al., 2004; Bozkurt et al., 2005c). In the sperm cryopreservation study conducted with grass carp, it was stated that glycerol, which is used as a cryoprotectant, should be used in the ionic extender at a rate of 10-20% (Bozkurt et al., 2011c). In addition to these studies, there is a study reports that sugars belonging to different sugar groups are used in the sperm cryopreservation of carp and that the best result is obtained with maltose and trehalose (disaccharide) (Bozkurt et al., 2016).

In addition, the investigation of the possibilities of using α-lipoic acid (ALA) in the cryopreservation of fish sperm was first tried in Türkiye and on carp. İnanan and Kanyılmaz (2020b) used ALA (0.025, 0.05, 0.1, 0.5, 1, 2, 5 and 10 mM) in carp sperm in both short-term storage and cryopreservation study, and they reported that the use of 0.5 mM (in short-term storage) and 1 mM ALA (in cryopreservation), was most suitable for carp sperm.

The first study on the use of honey in the extender in the cryopreservation of fish sperm was tried in our country on carp. In sperm cryopreservation using pine honey in various concentrations (100-500 mg ml^{-1}), it has been stated that hatching is seen in the fertilization study after thawing and that honey can be successfully used in cryopreservation carp sperm (Öğretmen and İnanan, 2014). The other substance whose protection is investigated in sperm cryopreservation in carp is propolis. It has been reported that propolis used in the extender protect the integrity of the sperm cells during the cryopreservation of carp sperm and when evaluated by its content, propolis is a suitable cryoprotective agent in the cryopreservation of fish sperm (Öğretmen et al., 2014b).

The other species of cyprinid used in sperm cryopreservation studies is goldfish (*Carassius auratus*), an important aquarium species. In a sperm cryopreservation study with goldfish, it was revealed that the use of 4 mM taurine in the freezing procedure caused an increase in motility after thawing and a decrease in DNA damage (Kutluyer et al., 2016b). In a comprehensive sperm cryopreservation study with the same species, the addition of L-methionine to the extender reduced DNA damage and that the pellet method could be used in the sperm cryopreservation procedure for goldfish (Kutluyer et al., 2015b). In another study conducted

with goldfish, sperm freezing was performed in sperm samples taken from male individuals exposed to boron (via water and feed), and it was reported that the use of boron, which is necessary for each organism in essential amounts, feed-borne B was beneficial to increase the resistance to cold shock in sperm cells (İnanan and Yılmaz, 2018b).

Short-term storage, which is another application that allows sperm cells to be stored without losing their vitality, has also been tried in cyprinid species. In the study conducted with grass carp, it was stated that sperm cells can be stored effectively in a glucose-based diluent at 4°C for 8 hours (Bozkurt et al., 2009d). It has been reported that sperm samples in the mirror carp (*C. carpio*) can be stored at 4°C for 72 hours before fertilization experiment (Bozkurt and Secer, 2005a). In addition, the effects of tryptophan, phenylalanine and cysteine use on DNA integrity, lipid peroxidation, viability, and motility in short-term storage of carp fish sperm have been examined and it has been reported that cysteine and phenylalanine have more harmful effects than tryptophan and that these amino acids are not beneficial for the storage of carp spermatozoa at 4°C (Kanyılmaz and İnanan, 2020).

Sperm cryopreservation studies on fish are based on the variety and dilution rate of agents to determine the various agents responsible for protecting the cell during freezing. However, sperm cells can be damaged during the freezing procedure, as well as during thawing, which is essential for the cryopreserved cells to be used during fertilization. In the light of this scientific data, sperm cryopreservation studies are carried out on the variety of sperm freezing and thawing procedure as well as the chemicals used. In the study conducted with on the subject, it was stated that the thawing temperature and duration of the frozen sperm directly affected the fertilization rate, and the ideal thawing temperature and duration for grass carp were 35°C and 30 sec (Yavaş and Bozkurt, 2011) and 30°C and 30 sec (using 1.5 mL straws) for carp (Bozkurt and Yavaş, 2017a).

In sperm cryopreservation studies, sperm cells treated with extender are commonly subjected to slow freezing (liquid nitrogen vapor and liquid nitrogen or programmable device). The other freezing procedure, which has recently found wide application in sperm freezing methods, is vitrification (Magnotti et al., 2018). The basic sperm vitrification procedures are to suspend the spermatozoa in a vitrification solution and then plunge the sample into liquid nitrogen to obtain a vitreous transparent state. Vitrification has been applied for fish primordial germ cells, oocytes, eggs, testicular tissues, and embryos (Xin et al., 2017). The use of vitrification in fish sperm is a new practice. Several scientists have tested vitrification on fish sperm, focusing mainly on various concentrations, administration times, toxicity, and temperatures of permeable cryoprotectants (Magnotti et al., 2018). In our country, sperm vitri-

fication has been tried in carp and it has been claimed that carp sperms can be frozen directly in liquid nitrogen despite the low percentage obtained in fertilization in sperm samples where vitrification is applied with various cryoprotectants (Bozkurt et al., 2014b).

As mentioned earlier, sperm cryopreservation has harmful effects on sperm cells and these effects are related to the loss of motility, which is of great importance in fertilization, changes in plasma permeability, changes in mitochondrial integrity and DNA structure of the sperm cell (Cabrita et al., 1998). All these parameters that determine the success of the sperm cryopreservation procedure are the parameters that can be measured by post-thawed of the sperm. However, the measurement of these parameters also differs methodically. Methods to detect damage to the DNA of sperm cells, which have a particularly important place in sperm cryopreservation have been studied in boar, ram, equine and fish (Cabrita et al., 1998; 2005), and studies are still underway to improve the methods. One of these studies is the study with carp fish in our country. In the study where three different Comet analysis methods (also known as single-cell gel electrophoresis) with different chemicals and application times were tried in the detection of DNA damage of sperm cells in the cryopreservation of carp fish sperm, it was reported that all three methods showed similar success in detecting undamaged and total damaged DNA, but there may be differences in the identification of DNA damage classes (İnanan et al., 2016b).

3.1.3. Acipenseridae

Sturgeon is one of the most economical species in aquaculture because of the value of its meat and caviar. Sturgeon (Acipenseriformes), one of the oldest primitive fish, is called living fossils by many authorities. Sturgeon, which has survived the ecological changes that have continued throughout the ages, has disappeared from nature due to the greed of human beings and is happening. The intensive fishery of sturgeon fish, which dates to 200-250 years ago, in order to obtain their especially valuable caviar, has brought about many conservation studies in order to protect these fish. International Union for Conservation of Nature (IUCN) listed all commercially utilized sturgeon species worldwide in Annex II of the CITES regulations, thus requiring internationally agreed trade quotas to promulgate the protection of these highly endan-gered species (CITES Export Quotas, 2012). A Red List update by the IUCN Sturgeon Expert Group in 2022 (21 July) revealed that sturgeon populations have declined further. As a result, the cultivation of sturgeon fish has started to increase every year all over the world, especially to obtain caviar. While there are a total of 27 sturgeon species in the world, it has been determined that about 13 of them are cultured and the most widely aquaculture species is Siberian sturgeon (*Acipenser baerii*).

In our country, especially in the Black Sea and the rivers flowing here, there were plenty of sturgeon in the past, but today they have disappeared due to the destruction of the habitats of these species and overfishing. Thus, in our country, it is out of the question to obtain caviar from sturgeon obtained through fishing. As a result, the relevant institutions have included sturgeon fish within the scope of alternative species that should be cultured in our country. However, according to the TUİK report, 14 tons of sturgeon were produced in our country in 2020 (TUİK, 2022).

Both to support the declining population in nature and due to its enormous economic importance, the sturgeon has become one of the most important fish families in practical and theoretical applications in aquaculture studies. In addition, in recent years, cryopreservation has played an important role in preserved gametes to protect fish of high economic and biological value, and sturgeon fish is the most important species in cryopreservation studies due to their economic and biological importance. Among sturgeons, these include beluga (*Huso huso*), sterlet (*A. ruthenus*), pallid (*Scaphirhynchus albus*), Siberian (*A. baeri*), European sturgeon (*A. sturio*), also known as the Atlantic or common sturgeon, Russian (*A. gueldenstaedtii*) sturgeons and the shortnose (*A. brevirostrum*) (Magnotti et al., 2018).

Sturgeon aquaculture studies, which started with the contributions of both the private sector and universities in our country, have accompanied scientific studies. In our country, sturgeon sperm cryopreservation studies have been carried out and studies on the reproductive biology of sturgeon species are continuing. One of the species in which sperm cryopreservation study is carried out in our country is the Russian sturgeon. In his study, Aydın et al. (2012) stated that DMSO (8%) was used as a cryoprotectant in 5 different diluents in the cryopreservation of Russian sturgeon sperm and that the best motility result was obtained with a solution containing NaCl, KCl, $NaHO_3$ and HCL. In the study in which the addition of glutathione was tried for the first time in the cryopreservation of Russian sturgeon sperm, (DMSO at two different rates) and stated that the highest motility was seen in 15% DMSO and solution without glutathione added (Yamaner et al., 2015).

3.1.4. Other species

In addition to salmonide, carp and sturgeon species in our country, there are also sperm cryopreservation studies with various freshwater fish found in the waters of Türkiye. One of these species, *Barbus grypus* (Heckel 1843), known as the shabout, is a river species also found in estuaries and reaches a maximum size of about two meters and over 50 kg. In the sperm cryopreservation study conducted by Doğu (2012) with this species, DMSO (10%) was

used as a cryoprotectant, and a fertilization percentage of approximately 37% was obtained. In another study, results showed that oxytetracycline can be used as an antibiotic additive in extender, providing better sperm freezing- thawing, without decreasing semen quality and antioxidant and increasing the oxidant, DNA damage and apoptotic sperm level of the shabout (Doğu et al., 2022a).

Another species in which short-term preservation of sperm is studied is the leaping mullet (*Liza saliens*) species. In the study conducted with this species, where a decrease in natural stocks was seen due to overfishing and destruction of reproduction areas, the survival time of the sperm reported to be 6 minutes in fresh sperm, 6.5 minutes in HEPES, 5-20 minutes in BSA, 3-20 minutes in Tris and 8.5 minutes in DMSO at 4°C (Engin et al., 2020a).

In the sperm cryopreservation study conducted with Mesopotamian spiny eel (*M. mastacembelus*), another freshwater fish species that has been stated to be considered in culture studies in recent years, an increase in motility and fertilization rate after thawing was reported in sperm samples cryopreserved with the extender, briefly expressed as ArShaDo (Doğu et al., 2022b).

Again, one of the species that are the subject of sperm cryopreservation studies in our country is nile tilapia (*Oreochromis niloticus*) and it has been reported that glycerol and methanol are more effective cryoprotectant than DMSO in the sperm cryopreservation procedure of nile tilapia (Bozkurt et al., 2019b).

In a sperm cryopreservation study with Mesopotamian catfish (*Silurus triostegus* H., 1843) it is concluded that the glucose solution with low osmolality had a harmful effect on the spermatozoa (Şahinöz et al., 2020). In another sperm cryopreservation study with mesopotamia catfish, it was reported that the use of inositol increased the success of the sperm cryopreservation procedure, in addition to increasing inositol levels, increasing spermatozoa motility and duration (Doğu et al., 2021). In another study with wild African catfish (*Clarias gariepinus*), different concentrations of glycerol were tried in the sperm freezing procedure and it was reported that the highest motility was achieved with 15% glycerol after thawing (Bozkurt and Yavaş, 2017b).

3.2. Sea-water fish species

Although the support for the breeding production of new species continues in our country, the most cultivated species today are trout, sea bream and sea bass. Gilthead seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*) production continued to increase in the period of 2010-2019. Gilt-head seabream aquaculture production, which was 28.2 thousand metric tons in 2010, reached

133.4 thousand metric tons in 2021 and seabass aquaculture production, which was 50.8 thousand metric tons in 2010 reached 155.1 thousand metric tons in 2021 (TUİK, 2022).

To date, sperm cryopreservation studies have been carried out in many freshwater fish species and in addition to this, sperm cryopreservation studies have been tried in about 40 sea fish species. One of the most important reasons why sperm cryopreservation studies are carried out more in freshwater fish is that fertilization in marine fish takes place in a completely natural environment (tank, pond ext.) and it is difficult to get egg and sperm process of these fish as artificial (Ekici et al., 2020). Nevertheless, there are studies on sperm cryopreservation in sea fish in the world and in our country.

In the sperm freezing study with sea bream, the effect of DMSO on sperm cells at different rates was examined and it was revealed that 10% DMSO (10% egg yolk, buffer solution) gave the best result (Engin et al., 2020b). In another study, it was reported that the highest motility and fertilization rate was achieved with the use of Mounib and Ringer extender in sea bream sperm cryopreservation with four different diluents [Mounib, Salty Mounib, Ringer, and sea water (1% NaCl)] using 10% DMSO as cryoprotectant (Tırpan et al., 2016). In addition, it was revealed that sea bream sperms can be stored at 0°C for 126 hours without adding any diluents (Engin et al., 2018).

Another economic marine species in which sperm cryopreservation studies are carried out in Türkiye is turbot (*Scophthalmus maximus*; also known as *Psetta maxima*) and attention is drawn to turbot as a potential source of high market value, stock expansion, and aquaculture in Türkiye (Aydın, 2021). In the study with turbot, the effect of various antioxidants on the sperm cryopreservation procedure was investigated and it was reported that the highest motility (77.41±2.90%) and fertilization rate (81.67±2.52%) was obtained in solution containing 25 µg/ml rosmarinic acid (Polat and Kurtoğlu, 2023). In another study, the effect of different freezing procedures (cooling rates) on sperm parameters in cryopreservation sperm of turbot was examined and it was found that the fertilization rates obtained with sperm samples applied to three different freezing procedures were similar (65.3-75.6%) (Aydın et al., 2022).

4. Conclusion

When the studies on gamete quality and cryopreservation in our country are examined, it is understood that the studies on *O. mykiss* species, which is the first species in our country where breeding studies in fresh waters have begun, have been intensified. The reasons for emphasizing this species can be shown as the fact that the production of this fish species has been carried out for many years in the research facilities within many universities in our

country and that production and scientific studies are carried out as a widespread species in the world. In the last 10-15 years, studies on gamete quality and cryopreservation have been carried out in different regions of our country and it is possible to carry out these studies on fish species in the water resources in these regions. Although the history of fish production in our country is new compared to other countries, Türkiye is technologically, methodologically, and commercially in a position to have a say in aquaculture today. When the researchs are examined, in addition to determining the gamete characteristics of different species in our country, there is also the presence of an increasing researcher profile day by day. With this review, the variety of species about which gamete quality and cryopreservation studies have been carried out in our country has been determined. Moreover, it is believed that by providing information on researchers working on this issue, it can increase collective studies in the country. In this review prepared in honor of the 100th anniversary of the establishment of the Republic of Türkiye, it is tried to reveal the general evaluation and development of the breeding studies in our country, which started with the introduction of eyed fish eggs at the end of the 1960s, on gamete quality and cryopreservation until 2023.

Acknowledgements: While preparing this book chapter, researchers who have worked on gamete quality and cryopreservation in Türkiye were reached by using the “google scholar” search engine. As a key word, “sperm”, “egg”, “gamete”, “cryopreservation”, “short-term preservation”, “fish” were used. The sources accessed using these keywords were also checked in the literature reports and these citations were also utilized. The CV files of the people were examined, and the articles were researched one by one. However, if the full article cannot be reached, unfortunately those articles cannot be included in this section. A telephone interview was also made to confirm the first studies conducted in our country on this subject. In this section, only articles whose experimental studies have been conducted in our country are included. As a result of our research, it is likely that there is literature that we have overlooked. For this reason, we owe our apologies to the valuable researchers.

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CHAPTER 12

SCIENTIFIC STUDIES ON STURGEON CULTURE IN TÜRKİYE

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DOI: 10.26650/B/LS32LS24.2024.005.012

1. Introduction

The production of fish through aquaculture plays a vital role in satisfying the significant demand for protein in human diets. Aquaculture, which has a long history, is positioned as the fastest-growing sector among the agricultural sectors. Since 2013, the amount of product obtained by aquaculture has been higher than the amount of product obtained by natural fishing, and it is foreseen by the competent authorities that this situation will continue.

As reported by FAO in 2022, the global production of aquaculture achieved an unprecedented milestone in 2020, reaching 122.6 million tonnes. Out of the total aquaculture production, which includes fisheries, approximately 49% was derived from aquaculture methods. According to the same report, 37% of the total aquaculture production belonging to inland waters is production made in inland waters (FAO, 2022). One of the most important species in the breeding of inland water fish to meet the demand for both its meat and caviar, and most importantly to reduce the pressure on natural stocks, is the Sturgeon (Acipenseriformes), one of the oldest primitive fish, is called living fossils by many authorities. Sturgeon, which has survived the ecological changes that have continued throughout the ages, has disappeared from nature due to the greed of human beings and is happening. Species of Acipenseriformes found in the Atlantic, Pacific, Mediterranean and Black Seas are also known to spread in rivers, lakes and inland seas above the 30th parallel of the Northern Hemisphere (Huang, 2002).

Within Türkiye, there have been documented records of six sturgeon species found in the Black Sea region and the rivers that drain into the Black Sea. These species have been reported as Beluga (*Huso huso*), Danube or Russian sturgeon (*Acipenser gueldenstaedtii* Brandt and Ratzeburg 1833), Common sturgeon (*A. sturio*), Ship sturgeon (*A. nudiventris* Lovetsky, 1828) and Stellate (*A. stellatus*) and Sterlet sturgeon (*A. ruthenus*) (Çelikkale, 1994). It is widely recognized that these particular species, which are found in the Black Sea basin, make reproductive migration to large rivers such as Sakarya, Yeşilırmak, Kızılırmak and Çoruh. It is known that sturgeon has an important fishing potential in the Black Sea (Çarşamba, Bafra, Karasu) and in the Istanbul region (Çelikkale, 1994; Tırıl Ustaoğlu and Memiş, 2018; Memiş et al., 2020). Although it has been reported in the past that *A. sturio* and *H.huso* are the most fished species in Turkish waters, *A. sturio* of these species is no longer present in our and other's world big river waters (Chandra and Fopp-Bayat, 2021).

In this section, information will be given about the current status of scientific and commercial studies conducted on sturgeon, which are also found in Turkish waters and have economic and biological importance, and about the aquaculture opportunities and problems that exist from past to present. Information about sturgeons in this section is given only about the family Acipenseridae. Paddlefish (Polyodontidae) are excluded. Although paddlefish are not found in the waters of Türkiye, they are not included in the Codex Alimentarius as a species where caviar is produced. Extensive information on paddling fish may be found in Jarić et al. (2019). In addition, the most comprehensive data on sturgeon fishing and caviar production in Türkiye is found in the article published by Memiş in 2014 (Memiş, 2014).

2. Global Sturgeon Fisheries and Aquaculture

The fisheries of sturgeon, known as fossils living today, go back 200-250 years (Billard and Lecointre, 2001). When the sturgeon itself and the value of the products obtained are examined historically, it is known that in addition to the increase in commercial value since the Middle Ages, the visibility of these fish has increased. In addition, it is known that sturgeon was depicted on coins in the region known today as Tunisia in 600 BC (the region known as Karthago at that time), that the swimbladders of sturgeon were used in wine production and that these fish were called "noble fish" at that time. Caviar, which is identified with sturgeon today; it is pictured as a product included in the classification of luxury food and obtained from sturgeon; Nonetheless, there have been reports indicating that the term "caviar" originated from the Persian expression "mahi-i-xavi-yar'," which essentially translates to "egg-bearing fish." Initially, this term encompassed all types of fish eggs. However, as time passed and traditional caviar production developed, along with the growth of local

markets, caviar derived from Ponto-Caspian sturgeon species acquired a reputation as a luxurious product. Thus, the reasons why both sturgeon and their products are commercially and socially important, regardless of the region and time in which they are located, have made these fish the most valuable product in fisheries worldwide. In the study published by Bronzi et al. (2011), the maximum number of sturgeon caught was reported as 32078 tons with the catch in 1977 (that has never been reached again). In the commercial fishing of sturgeon, it has been reported that there was a sharp decrease in the number of fish caught between 1990 and 2018, however, this decrease decreased in 2019 and even the number of fish increased slightly. In 2020, a total global catch of 260 tons was recorded (Degani and Yom Din, 2022).

From that day until the present, there have been significant fluctuations in global fisheries. Sturgeon fishing remains important for both trade and social reasons. However, this importance has led to a decline in the sturgeon population due to poaching and overfishing. To combat this, national, regional, and international organizations have implemented measures to reduce the decline of sturgeon populations in natural waters. Sturgeon is believed to be the most endangered group worldwide, with 27 species and over 85% of those species classified as endangered or critically endangered (IUCN, 2017). A major step was the listing of the sturgeon species on Annex II of CITES in Harare 1997The International Union for Conservation of Nature (IUCN) included all sturgeon species that are commercially exploited worldwide in CITES regulations. As a result, internationally agreed trade quotas were established to ensure the protection of these critically endangered species (CITES Export Quotas, 2012). CITES issued and incorporated a declaration regulating the trade of sturgeon species and products with its regulations. A Red List update by the IUCN Sturgeon Expert Group in 2022 (21 July) revealed that sturgeon populations have declined. Furthermore, the common sturgeon (*Acipenser sturio*) has become extinct in the Guadalquivir, Rhine, Elbe, and Thames Rivers. This unfortunate outcome can be attributed to the compounding effects of overfishing, obstructed spawning migrations, and degradation of their habitats (Chandra and Fopp-Bayat, 2021). After that, Romania was the first country to ban sturgeon fishing in the Caspian and Black Seas in 2006. However, it is known that legal sturgeon fishing in designated limited quantities is still allowed in countries such as Russia, Iran, Kazakhstan, Canada, and the USA (Bronzi et al., 2019). Türkiye signed the CITES agreement on 22 December 1996 and all sturgeon fisheries were banned after 1 April 1998.

Although it is protected by various authorities; natural sturgeon populations are known to continue to decline. For this reason, it is important to produce these fish under aquaculture conditions in order to meet the demand for products such as meat and caviar obtained from

these valuable fish and thus reduce the pressures on natural stocks. Today, the aquaculture of sturgeon is studied in two ways. This classification of controlled propagation refers to the controlled production made to support natural stocks, and commercial production, which is made to meet the needs of the consumer market (especially caviar). Although it was defined as a luxury food item in the past, today the breeding of sturgeon fish has increased greatly due to the intense interest of middle-class consumers (Sicuro, 2019) and sturgeon aquaculture; it has replaced sturgeon fishing. It was reported that a total of 2329 commercial sturgeon farms operated in the world in 2017, and the production of sturgeon biomass was four times higher than the fishing harvest in the 1970-1980s. Currently, in an effort to counter the decline of wild sturgeon populations, caviar production is exclusively carried out through aquaculture methods. However, the economic sustainability of a sturgeon farm depends on the presence of a market for sturgeon meat (Bronzi et al., 2019).

It is known that the culture of sturgeon dates back to ancient times. It is accepted that the first production of sturgeon in culture conditions began with the discovery of the breeding ground of the species Sterlet near the Volga river in 1869 by Professor Ovsyannikov. Following this discovery, it is known that Ovsyannikov and Peltsam carried out studies to fertilize the egg of the sterlet species in artificial conditions, and that these studies were developed by Straganov in the 1940-50s (Bronzi et al., 2011; Bronzi and Rosenthal, 2014; Bronzi et al., 2019). However, The first numerical data on sturgeon aquaculture was recorded in 1984 as 150 tons. After 1984, special techniques have been developed in the production of sturgeon, in response to the high market demand for caviar, the sturgeon aquaculture sector experienced significant growth. Sturgeon farming, which has been gradually increasing since 1984, has thus become the fastest-growing aquaculture sector since the end of the 20th century (Bronzi et al., 2011; 2019; Chandra and Fopp-Bayat, 2021).

In the last 20 years, the biggest reason for the rapid increase in sturgeon production in the world is seen as the production of sturgeon in China. It is known that the total production of sturgeon in 2002 was 4,100 tonnes, half of which took place in Russia and the rest in the EU (FAO, 2022). In 2003, global sturgeon production witnessed a significant increase, more than tripling its previous levels, primarily due to China's reported production of over 9.000 tonnes. By the year 2020, the total global sturgeon production reached 123.476 tonnes, with China contributing 84% of the production (104.280 tonnes). Russia accounted for 4% of the global sturgeon production (4.836 tonnes), followed by Armenia at 3% (4.200 tonnes). In 2020, sturgeon production is followed by EU countries (3.081 tonnes), Iran (2.640 tonnes), Vietnam (2.410 tonnes), USA (1.166 tonnes) and Others (863 tonnes) after Armenia (EUMOFA, 2023).

If we look at European countries, the country with the highest sturgeon production was Italy with 1051 tonnes in EU producers in 2020. In the last decade, Poland and Bulgaria have established themselves as the second and third largest (EU producers) producers of sturgeon, respectively. However, it has been reported that there was a decline in Poland's production in 2020 compared to 2019 and that this may be related to the COVID-19 pandemic. In 2020, Bulgaria produced 376 tonnes of sturgeon, 13% more than its 2019 production. Another country with a high production of sturgeon is France. The country, which produced 400 tonnes of sturgeon in 2020, increased its production this year by 39% compared to the previous production. In the production ranking made as European Union countries after France comes to Netherlands/Spain (150 tonnes); Germany (149 tonnes); Lithuania /Romania (85 tonnes), Hungary (67 tonnes) and others (118 tonnes) respectively (EUMOFA, 2023).

It is known that the most commonly produced feathers by aquaculture are Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*) and White sturgeon (*Acipenser transmontanus*), followed by Beluga sturgeon (*Huso huso*), Sterlet (*Acipenser ruthenus*), Persian sturgeon (*Acipenser persicus*), Stellate sturgeon (*Acipenser stellatus*) and some hybrid species (Lopez et al., 2020). That the meat obtained from sturgeon is best quality obtained from White sturgeon; the most famous and valuable caviar is reported to have been obtained from Beluga (*H. Huso*), Osetra (*A. gueldenstaedtii*), and Sevruga, (*A. stellatus* and *A. persicus*) (Lopez et al., 2020). In addition, nearly all caviars on the market today are harvested from farmed sturgeon (EUMOFA, 2023).

According to export data from CITES and production data from FAO covering the period from 2016 to 2020, Italy was found to be primarily producing white sturgeon, accounting for 47% of their sturgeon production. This was followed by Russian sturgeon at 28% and Siberian sturgeon at 23%. In the case of Poland and Bulgaria, their production was mainly focused on Siberian sturgeon, with percentages of 56% and 22% respectively, along with significant production of Russian sturgeon (44% and 73% respectively). France, on the other hand, predominantly produced Siberian sturgeon (96%) and to a lesser extent, white sturgeon (3%) (EUMOFA, 2023).

If we look at the consumption of sturgeon, the main reason why the meat of sturgeon is valued as having an incredible taste is reported that as the fact that the meat of sturgeon contains glutamic acid (%18,1) (Kaya et al., 2008). Furthermore, sturgeon meat contains a wealth of vitamins, including niacin, pyridoxine, vitamin B12, essential amino acids, as well as important minerals like potassium, magnesium, and phosphorus. Additionally, the flesh of sturgeon contains two long-chain omega-3 fatty acids, namely docosahexaenoic acid (DHA) (ranging

from 3.8% to 11.1%) and eicosapentaenoic acid (EPA) (ranging from 4.9% to 6.8%) (Pelic et al., 2019). In Eastern European countries like Bulgaria, Ukraine, Serbia, and Romania, as well as in China and Russia, sturgeon has been a cherished part of traditional cuisine for centuries, and it continues to enjoy popularity on dining tables to this day (Raposo et al., 2023).

Many tissues and organs of the sturgeon, such as caviar, meat, skin (leather) and air bladder, have economic benefits. For example, one of the byproducts is sturgeon glue (Isinglass), which is made from the sturgeon's air bladder's inner membrane, as a consolidant and adhesive (Petukhova and Bonadies, 1993). Sturgeon served as a source of sustenance, offering meat, oil, and caviar that could be consumed directly, preserved, or traded (Holzkamm and Waisberg, 2005).

3. Sturgeon Conservation Efforts

Of the four billion species estimated to have evolved over the past 3.5 billion years on Earth, about 99% have disappeared (Barnosky et al., 2011). This shows how widespread extinction is. Of course, these extinctions are normally balanced by the development and reproduction of new species. But sometimes in world history, the balance has been so shaken that several times it has had higher extinction rates and has been awarded "mass extinction" status five times. These are the "Big Five" mass extinctions. Given the known loss of species over the past few centuries and millennia (Hughes et al., 1997; Dirzo and Raven, 2003), scientists now suggest that a sixth mass extinction may be on the way (Barnosky et al., 2011). The sixth mass extinction is due to a number of factors as a result of human activities such as loss of natural habitats due to human activities, habitat degradation, climate change, overfishing and pollution. In this process, ecosystems deteriorate, the number of species decreases and the number of species at risk of extinction increases. The sixth mass extinction, unlike other mass extinctions, is due to the influence of human beings.

Unfortunately, sturgeon also has a place in the adventure of extinction of species due to this human influence. Sturgeons are an important part of marine and freshwater ecosystems. The disappearance of these fish can cause an imbalance in the ecosystem and make it difficult for other species to survive as well. Therefore, it is important to conserve the sturgeon species.

As mentioned before, if we examine the sturgeon fish protected by the construction of barriers and dams in the rivers where they make reproductive migration, the destruction of river beds, pollution and overfishing in terms of the protection strategy; these may consist of:

- Restrictions on production: To stop overfishing of sturgeon, countries might impose specific restrictions. For instance, age restrictions or catchy conditions.

- Protection of habitat: It's critical to safeguard the ecosystems in lakes and streams that support sturgeon fish. This focuses on issues including waste management and the conservation of water resources.
- Scientific research: Studies are carried out to learn more about sturgeon populations, life cycles, and habitats. The planned use of this knowledge can make conservation efforts more successful.
- Campaigns for education and awareness: To raise public awareness of sturgeons and their conservation, campaigns for education and awareness might be established.

There are some well-known non-governmental organizations in the world about the protection of sturgeons. These organizations include the World Sturgeon Conservation Society (WSCS), The Fraser River Sturgeon Conservation Society, the IUCN Sturgeon Specialist Group (SSG), and the WWF Sturgeon initiative and Mersin Balıklarını Koruma ve Yaşatma Derneği (MERKODER).

A nonprofit organization called the World Sturgeon Conservation Society (WSCS) is devoted to the protection and conservation of sturgeon species and their natural habitats. The WSCS strives to promote sustainable management methods for these species and to increase public awareness of the challenges to sturgeon populations, including habitat degradation, overfishing, and pollution. To aid in sturgeon conservation efforts worldwide, the society also conducts research and works with other groups. After the following activities-initiated the 4th International Symposium on Sturgeon in 2001, the World Sturgeon Conservation Society is established in 2003. The Society aims to serve as a worldwide venue for scientific exchange for anybody interested in relevant sturgeon-related topics, while also looking for chances for close international cooperation.

"Mersin Balıklarını Koruma ve Yaşatma Derneği (MERKODER)" which was established in 2004, is one of the leading non-governmental organizations for the protection of sturgeon in Türkiye (Ustaoğlu, 2006).

Moreover, awareness campaigns on the conservation of sturgeon may include promoting the vital needs of these species and the challenges to their conservation. These may include:

I. Social media campaigns: Raising awareness by posting pictures, videos or animations that provide information about sturgeon conservation.

II. Exhibitions and panel talks: Raising public awareness by organizing events where information about sturgeon is presented.

III. Educational programmes: To provide information on sturgeon and their conservation in schools and environmental education programmes.

IV. Advertising and brochures: Raising public awareness by publishing advertisements and brochures containing information about sturgeons.

4. Sturgeon Aquaculture in Türkiye

The six sturgeon species—*Acipenser gueldenstaedtii, A. nudiventris, A. stellatus, A. sturio, A. ruthenus*, and *Huso huso*—are the native fish species in Turkish rivers around the Black Sea coast. Despite its importance in the local fishing industry and its cultural significance, the sturgeon has faced significant challenges in recent years, including overfishing, habitat degradation, and disease. Thus, in our country, it is out of the question to obtain caviar from sturgeon obtained through fishing. As a result, the relevant institutions have included sturgeon fish within the scope of new species that should be cultured in our country.

According to official data from the Ministry of Agriculture and Forestry (BSGM, 2023), the approved sturgeon production capacity in Türkiye is 1028 tons per year (Table 1). However, these capacities are not fully utilized. According to the FAO (2022), Türkiye produced 15 tons of sturgeon in 2020 (Figure 1).

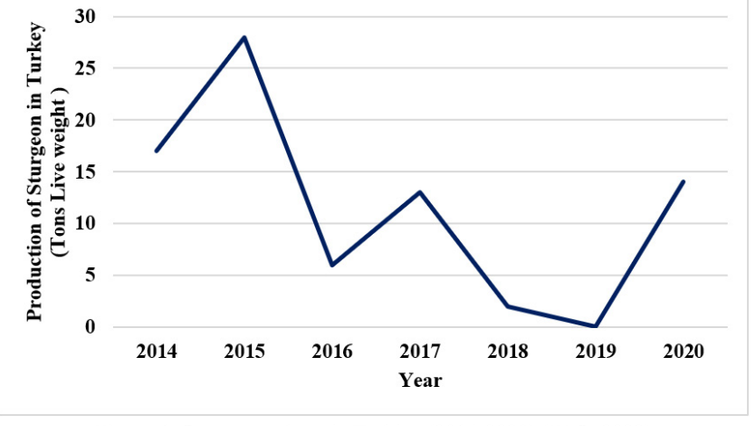

Figure 1. Sturgeon culture in Türkiye (2014-2020) (FAO, 2022)

Table 1. Sturgeon project capacity approved by the Ministry of Agriculture and Forestry in Türkiye (BSGM, 2023)

Facility Owner's Name	City	Production Type	Region	Facility Type	Species
MEHMET TOSMUR	ADANA	Cages	MEDITERRENIAN	Growing	The Siberian sturgeon (*Acipenser baerii*) (25000 Kg, 0 Individual)
OMNİA NİŞASTA SANAYİ VE TİCARET ANONİM ŞİRKETİ	ADANA	Concrete Ponds	MEDITERRENIAN	Hatchery + Growing	The starry sturgeon (*Acipenser stellatus*) (9600 Kg, 30000 Individual), The Siberian sturgeon (*Acipenser baerii*) (9600 Kg, 30000 Individual), The Russian Sturgeon (*Acipenser gueldenstaedtii*)(9800 Kg, 40000 Individual)
AYFİSH SU ÜRÜNLERİ SANAYİ TİCARET LİMİTED ŞİRKETİ	ELAZIĞ	Cages	EASTERN ANATOLIA	Growing	The "Bester" Hybrid Sturgeon (*H. huso* x *A. ruthenus*) (350000 Kg, 0 Individual), The Beluga Sturgeon (*Huso huso*) (600000 Kg,0 Individual)
MAVİ DAMLA SU ÜRÜNLERİ SANAYİ VE TİCARET LİMİTED ŞİRKETİ	KARABÜK	Concrete Ponds	BLACK SEA	Hatchery + Growing	The starry sturgeon (*Acipenser stellatus*) (14999 Kg, 1 Individual)
AYFİSH SU ÜRÜNLERİ SANAYİ TİCARET LİMİTED ŞİRKETİ	ŞANLIURFA	Cages	SOUTHEASTERN ANATOLIA	Growing	The "Bester" Hybrid Sturgeon (*H. huso* x *A. ruthenus*) (29000 Kg, 0 Individual)

5. Scientific Sturgeon Studies in Türkiye

To better understand this species and create conservation and management plans that can help to assure its long-term survival, scientific research has been done in response to these difficulties. This research has shed a significant lot of light on the biology, genetics, and behavior of the sturgeon species, offering insightful knowledge about the life cycle and ecological needs of the species.

The significance of habitat protection for sturgeon survival is one of the major results of these investigations. The survival of the species depends on certain spawning and feeding environments, and scientists have identified these locations and made recommendations for protecting and maintaining them. To assist preserve the sustainability of the species, this has led to the establishment of sturgeon-protected areas and the application of fishing laws.

Sturgeon research has contributed significantly to both the preservation of the species' environment and our understanding of the reproductive biology of the species. Sturgeon conservation efforts have been greatly aided by research into the life cycle of the species as well as approaches for hatchery production and stock improvement.

The effects of illnesses on the species have been a topic of more sturgeon research. In addition to developing vaccines and disease management techniques, researchers have investigated the origins of diseases and created measures to lessen their effects on sturgeons. The scientific research about sturgeon has been essential in bringing this ancient species' full potential to light and in ensuring its long-term survival. By continuing to invest in research and conservation efforts, we can work towards a brighter future for the sturgeon and the ecosystems it depends on.

When we examine the studies originating in Türkiye by region, we see that the Black Sea comes first with 44% (Figure 2). This is probably due to the fact that the Kızılırmak, Yeşilırmak and Sakarya rivers flowing into the Black Sea are breeding and living areas for sturgeon.

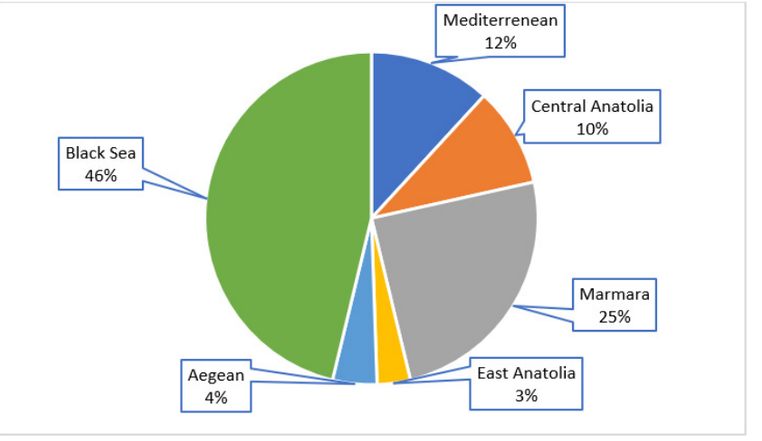

Figure 2. Distribution of articles from Türkiye about sturgeon by regions

When we look at the distribution according to the subjects of the studies originating in Türkiye, it is seen that the most studied subjects are feeding and growth studies (Figure 3).

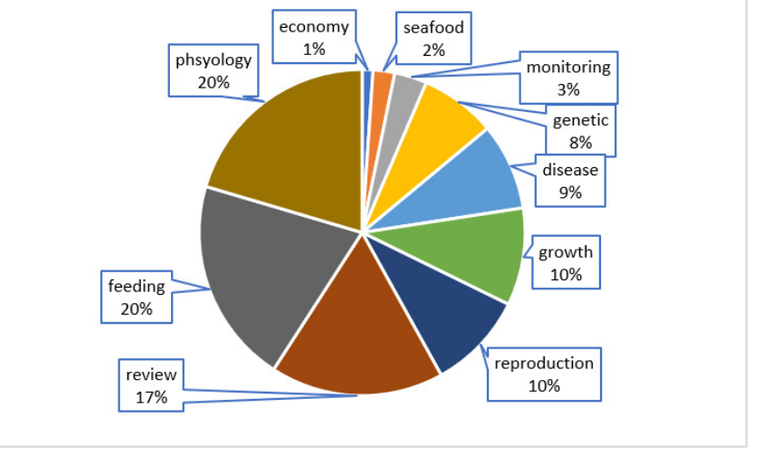

Figure 3. Distribution of articles from Türkiye about sturgeon by subjects

5.1. Monitoring and conservation studies

The most important study topic about sturgeon fish is the studies carried out to monitor these fish in natural stocks. The history and status of sturgeon species in Turkish waters have been reviewed by Geldiay and Balık (1996), Çelikkale (1994), Ustaoğlu and Okumuş (2004), Bat et al. (2005) and Akbulut et al. (2011a).

The tagging process is important in the monitoring of these fish. In the study conducted on the subject, Acipenseridae samples accidentally caught in fishing nets in the Black Sea region were tagged with plastic T-bar and released into the sea after recording all information such as weight, length, capture locations. As part of collaborative efforts with Fisheries Cooperatives and fishermen in the Black Sea region, a series of activities were conducted. During this initiative, a total of 99 fish were tagged and released into the sea (9 in 2006, 22 in 2007, 48 in 2008, and 20 in 2009). Out of these tagged fish, five were recaptured and subsequently released again after recording the information found on the tags (Ustaoğlu Tiril et al., 2011). A follow-up study was conducted with direct observations along the Black Sea coast of Bulgaria, in the area up to Georgia, where commercial trawl fishing was intense between 2005 and 2009, and along the three major rivers (Yeşilırmak, Kızılırmak and Sakarya) where sturgeon populations spawn, found that along the southern Black Sea coast, sturgeon species feed mainly on benthic and benthopelagic macrofauna. In this study conducted in 2013, both the gonad development of individuals caught in the river mouths and the samples obtained in the rivers reported that the population still entered the Yeşilırmak and Sakarya rivers for spawning (Zengin et al., 2013). In the monitoring study conducted in the Karasu region of the Sakarya River, fish fauna was investigated at 4 different stations (April-Semptember 2014) on the river and a few *A. gueldenstaedtii* juveniles were observed at the mouth of the Sakarya River near the Black Sea, while only one young *A. stellatus* (YOY) (W: 25g; TL: 28cm) was reported to have been captured from the Lower Sakarya River (Memiş et al., 2019).

5.2. Adaptation studies

The study (Çelikkale et al., 2002) involved juvenile Russian sturgeon weighing 4.77±0.55 g, which were raised under hatchery conditions in open water heated to 18-22 ºC. The sturgeon was gradually acclimated to colder water (12.8ºC) in tanks before being transported to the Sakarya River, where they were released on March 27th, 2001. The goal of the study was to help restore juvenile sturgeon populations along the Black Sea coast of Türkiye. In the study on the adaptation of juvenile beluga fish caught on the Black Sea coast to breeding conditions, the fish were tagged individually and fed with whiting (Akbulut et al., 2010) and anchovy (Akbulut et al., 2011b). As a result, it is established that whiting and anchovy can be used routinely in the feeding and adaptation of young beluga caught from the natural environment to its cultural conditions (Akbulut et al., 2010; 2011b). Memiş et al. (2011) investigated the acclimatization conditions, monitoring, condition factor and mortality rates of three different sturgeon species caught from the Black Sea coast *(Huso huso*, Linnaeus 1758; *Acipenser stellatus*, Pallas, 1771; *Acipenser gueldenstaedtii*, Brand). They reported that stellate sturgeon, beluga, and Russian sturgeon, respectively, were the most sensitive sturgeon species observed.

5.3. Reproduction, gametes, and biotechnology studies

In a study with sturgeon sperm, the effect of sperm quality and temperature after diluting sperm motility and survival time was investigated. It was stated that the motility of sperm cells was 50% up to the 8th minute at 14°C and therefore Russian sturgeon sperm could be used up to the 8th minute after dilution (Aydın et al., 2012a).

One of the species in which sperm cryopreservation study is carried out in our country is the Russian sturgeon. In his study, Aydın et al. (2012b) stated that DMSO (8%) was used as a cryoprotectant in 5 different diluents in the cryopreservation of Russian sturgeon sperm and that the best motility result was obtained with a solution containing NaCl, KCl, $NaHO_3$ and HCL. In the study in which the addition of glutathione was tried for the first time in the cryopreservation of Russian sturgeon sperm, (DMSO at two different rates) and stated that the highest motility was seen in 15% DMSO and solution without glutathione added (Yamaner et al., 2015).

Since sexual dimorphism does not appear in the separation of male and female in sturgeon, and since gonad development within both sexes is gradual, various methods have been developed to determine gender separation and gonad development in these fish. In the study conducted by Memiş et al. (2016), approximately 210 Russian sturgeon individuals were examined in terms of gender discrimination and gonad development using an ultrasound technique and these fish were taken into production in the following years. Another study with the determination of sex and the stage of gonad development was conducted on Russian sturgeon and Siberian sturgeon. In the study, it was stated that gender separate was made by examining blood parameters as well as ultrasound technique (Ak and Kurtoğlu, 2017).

It is a common practice to apply hormones to sturgeon fish in culture conditions. And it is desirable that the hormone to be used is effective in obtaining sufficient and high-quality gametes (Zohar and Mylonas, 2001). In the first reproductive period of Russian sturgeon, the effect of luteinizing hormone-releasing hormone analog (LHRHa), which indirectly affects gonads, and carp pituitary (CPP) that directly affects gonads on sperm parameters was examined, and it was found that both hormones were successful in obtaining sperm in Russian sturgeon and only the parameter called VCL (Curvilinear velocity of spermatozoon) was affected by the hormone used (Yamaner et al., 2017).

5.4. Feeding, growth and culture studies

According to Köksal et al. (2000), the growth and feed conversion of the Siberian sturgeon were investigated using concrete raceways, which are a type of flow-through system used in rainbow trout farming. The purpose of the study was to explore the potential for introducing

the Siberian sturgeon to Türkiye as a viable option for aquaculture. The results indicate that the growth performance of the Siberian sturgeon is comparable to, or even better than, the commercially farmed species in Türkiye. Furthermore, the study suggests that the Siberian sturgeon can be easily raised in concrete channels, which are commonly used in rainbow trout farming. In their study, Rad et al. (2003) explored the impact of varying daily feeding rates on the specific growth rate and food conversion ratio of Siberian sturgeon, initially weighing an average of 1736 ± 37 g. The research concluded that the ideal daily feeding rate for Siberian sturgeon of that weight was found to be 1% of their body weight per day, while maintaining a temperature range of 19-22°C.

According to a study (Çelikkale et al., 2005) conducted in freshwater Sapanca Lake, the growth performance of juvenile Russian sturgeon was examined in experimental net cages with two different stocking densities of 12 and 8 individuals per cubic meter. The results showed a fourfold increase in biomass density for both groups, and the specific growth rate was 2.69% day^{-1} in both groups. After the experiment, it was determined that the mean condition factor of sturgeon was 0.36 ± 0.05 in the high-density group and 0.41 ± 0.05 in the low-density group. The mean feed conversion ratio was found to be 5.7 in the high-density group and 5.8 in the low-density group. Based on these findings, the study concluded that sturgeon juveniles can be effectively raised in cages, with no significant differences observed in terms of growth performance and mortality between the two stocking densities that were tested.

In one study, the effects of feeding young Russian sturgeon feed with various sources of fat on growth performance and fatty acid composition were examined. They reported that soybean oil or sunflower oil can be substituted with fish oil to some extent in sturgeon diets (Şener et al., 2005). In another study (Şener et al., 2006), the effects of different feed compositions on growth performance and body composition of Russian sturgeon (*Acipenser gueldenstaedtii*) fry were investigated. As a result, it was observed that when vegetable protein and vegetable oil were used instead of fish meal and fish oil, it did not influence the growth performance of Russian sturgeon, but the accumulation of body and liver fat content.

To assess the impact of different diets on the growth performance of Russian sturgeon, Memiş et al. (2006) evaluated weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and body composition, including protein, lipid, and dry matter. The study compared the effects of a commercial trout feed, consisting of 45% crude protein and 12% crude fat, with a carp diet, containing 35% crude protein and 10% crude fat. The findings showed that Russian sturgeon growth performance appeared to be slightly better when fed a diet of trout food than when fed a diet with carp food. Body composition and growth performance (WG, SGR, and FCR) did not statistically differ between the two groups.

Memiş et al. (2009) describe the artificial incubation and rearing of Russian sturgeon larvae. The larvae were hatched in a waving system at 14-15°C and were fed *Artemia nauplius*, tubifex, and artificial diets at different stages of development. At the end of the feeding trial, the larvae reached a length of 12 cm and a weight of 5.25 g. They were then fed only commercial trout diets five times a day for 33-75 days. The survival rate of the Russian sturgeon was 27% at the end of 75 days. However, during the first 7 days of incubation, the mortality rate of fertilized eggs was approximately 69% of the total number.

In Memiş et al. (2011), the effects of reduced daylight over shaded ponds on the growth of adult Russian sturgeon were examined. The experiment lasted for one year, during which the fish were externally marked and fed a commercial diet. The final mean weight of fish in non-shaded ponds was 6710 g (±1670) and in shaded ponds was 6404 g (±1291). The specific growth rate was lowest in January-March (0.07 in non-shaded and 0.06 in shaded ponds) and highest in September-November (0.3 in both groups). The feed conversion ratio was better in shaded ponds. The growth performance did not differ significantly between the two groups, but the color of fish in non-shaded ponds was darker when exposed directly to sunlight.

In another study with Russian sturgeon, the growth performance of fish for 5 years was determined, and the problems encountered in the growth stage were investigated. Fish samples (2750.88±351.18 g, 80.18±6.49 cm average±SD) were stocked equally in fiberglass tanks, fed for 5 years. At the end of the study, it was reported that slow growth was detected in fish and that there was a need for a comprehensive evaluation of the data obtained from field studies and laboratory studies (Kocabaş et al., 2015).

Ustaoğlu Tiril et al. (2016), aimed to investigate the effect of replacing 50% fish oil with canola oil or safflower oil in the diets of Russian sturgeon on growth performance and muscle fatty acid composition. The fish were fed two diets for 15 weeks and there were no significant differences observed between the groups fed with canola oil and safflower oil diets in terms of weight gain, feed conversion ratio, and protein efficiency ratio. The muscle composition of protein, lipid, ash, and moisture content was not affected by the oil replacements. However, the total n-6 fatty acids were significantly higher in the safflower oil group compared to the canola oil group. The study concluded that replacing 50% fish oil with canola oil or safflower oil had no negative effect on the growth performance, feed efficiency, and fatty acid composition of Russian sturgeon muscle.

Emre et al. (2018), the researchers investigated the effects of replacing fish meal with soybean meal and cottonseed meal on the growth, body composition, and blood parameters

of Russian sturgeon. The results showed that sturgeon fed the diets with cottonseed meal had a higher specific growth rate and lower feed conversion ratio compared to those fed soybean meal and the control diet. There were no differences in the percentage of blood cells among groups, but the alkaline phosphatase levels were significantly lower in the soybean and cottonseed meal groups than in the control group. The researchers concluded that cottonseed meal could be used to enhance the growth of juvenile sturgeon.

Ak et al. (2019), the response of Siberian sturgeon and rainbow trout in mono-culture and duo-culture conditions was evaluated in culture tanks. The culture groups were planned as mono-culture sturgeon, duo-culture sturgeon, duo-culture trout, and mono-culture trout. The study found that neither species showed significant differences in specific growth rate and condition factor in mono-culture or duo-culture conditions. There were also no significant differences in final biomasses among the groups.

Karabulut et al. (2019) conducted a study to examine the impact of hazelnut meal (HM) at different levels (0%, 15%, 30%, and 45%) on the growth performance and body composition of Siberian sturgeon. The experiment lasted for 90 days and included 3 replicates. The control group, which did not receive any HM, exhibited the highest weight gain, followed by the 15%, 30%, and 45% HM groups. The control group also demonstrated the best performance in terms of specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). Adding 15% HM to the diet had a positive effect on fish growth, including growth performance, feed conversion efficiency, meat quality, and body composition. However, from an economic standpoint, it was determined that the HM level could be increased up to 45%. In another study focused on Russian sturgeon, the researchers investigated the use of hazelnut meal in the fish's diet. They found that the optimum dietary level of hazelnut meal for growth and feed conversion ratio, as determined by quadratic regression, was 15%. However, when considering economic factors, the optimal dietary level was found to be 45% hazelnut meal (Karabulut et al., 2017).

Karabulut and Osmanoğlu (2019), aimed to investigate the use of whiting meal (WM) in the feed of Russian sturgeon and its impact on the fish's growth performance and body composition. Four different experimental diets containing 0%, 10%, 20%, and 30% of WM were fed to the fish for 90 days. The results showed that the group fed with 20% of WM had the highest weight gain compared to the other groups. The differences between the WM 20 group and other groups were statistically significant in terms of weight gain, feed conversion ratio, and protein efficiency ratio. The study concludes that the use of 20% of whiting meal in sturgeon fish feed can significantly contribute to fish growth performance.

Mazlum et al. (2020) aimed to examine the effects of body and meal sizes on the gastric evacuation of Siberian sturgeon using the radiography technique. The researchers used sturgeon of different sizes ranging from 1.5 kg to 5 kg and fed them with meal sizes ranging from 4.6 g to 47.6 g, composed of either commercial pellets or live food. The study found that the course of gastric evacuation in sturgeon was best described by the square root model, with the effects of body mass and temperature on gastric evacuation rates described by power and exponential models respectively. The study also found that gastric evacuation rates were different for sturgeon fed on live food and commercial pellets. The researchers proposed models to estimate the time required for a meal to be evacuated from the stomach of the sturgeon or to determine the stomach fullness at postprandial time. The results of this study can be useful in planning feeding regimes for Siberian sturgeon to avoid overfeeding or underfeeding. In their research, Mazlum and Alabdullah (2020) aimed to examine the impact of body and meal sizes on gastric evacuation (GE) in farmed Russian sturgeon, considering their consumption of live food or commercial pellets. Radiography was employed to monitor the movement of food from the stomach to the intestine following feeding. The square root model was deemed suitable for describing the process of GE in Russian sturgeon, regardless of the type and size of the meal. The influence of fish size on GE was accurately represented by a simple power model. These findings contribute to the estimation of stomach fullness in Russian sturgeon at a specific time after feeding, which can be valuable in devising feeding strategies to minimize feed wastage and optimize fish growth.

In a 90-day experiment conducted by Duman (2020), the effects of two different culture systems, namely concrete ponds and net cages, on the growth and hematological indices of Siberian sturgeon were investigated. The study involved 300 fish in net cages and 276 fish in concrete ponds. The findings indicated that the fish reared in net cages exhibited higher growth rates, specific growth rates, and increases in body weight, while demonstrating lower food conversion rates compared to those in concrete ponds. Moreover, there were no significant differences observed in terms of condition factor or survival rate between the two systems. However, when analyzing hematological parameters, there were no differences found during the initial 30-day and 60-day sampling periods. In contrast, statistical differences were observed in the values of RBC, MCV, MCH, and WBC between the ponds and net cages during the 90-day sampling period. Overall, the study concluded that the culture of Siberian sturgeon in net cages offered more advantages in terms of growth parameters compared to concrete ponds.

Karabulut et al. (2021) aimed to investigate the effects of fish diets containing 30% hazelnut meal and different portions of phytase enzymes on the growth performance of Siberian

sturgeon. The experiment groups were fed with feeds supplemented with 0.25 g kg-1, 0.50 g kg-1, and 1.00 g kg-1 phytase enzyme, and a control group with no enzyme added. The trial lasted for 90 days, and growth performance was evaluated based on weight gain, protein efficiency ratio, feed conversion ratio, and specific growth rate. The study found that adding phytase enzyme to feeds with hazelnut meal had a positive effect on growth performance, and the group with 1.00 g kg-1 phytase enzyme showed the best results. Additionally, the enzyme-added groups had a higher total digestion rate than the control group.

In a study conducted by Kurtoğlu et al. (2021), the focus was on examining the impact of various stocking densities on water quality and blood parameters during the transportation of Siberian sturgeon. The experiments were conducted within the fish tanks, with three different stocking densities tested: 50, 100, and 150 kg/m^3. The duration of the transportation experiment was 20 hours. Water and blood samples were collected to measure ammonium nitrogen, nitrite nitrogen, erythrocyte, haematocrit, hemoglobin, cortisol, and sodium ion levels. Results showed that water quality deteriorated as stocking density increased, and the highest stocking density (150 kg/m^3) could threaten fish welfare and health after the 16th hour of transportation. However, at a stocking density of 50 kg/m^3, fish could be safely transported for 20 hours at 15°C water temperature. Blood parameters did not differ significantly between the experimental groups.

In another study, which examined growth parameters and cannibalism by stocking individuals of different sizes of Russian sturgeon, it was found that the highest cannibalism was in the group in which the individuals were not homogenized (Ak, 2023).

5.5. Physiology, histopathology, and ımmunology studies

According to Kolayli et al. (2011), the extensive utilization of dithiocarbamate pesticides could contribute to increasing the susceptibility of the Russian sturgeon to pollutants.

Dinçer et al. (2016) conducted a study with the aim of characterizing and purifying an α-carbonic anhydrase (CA) enzyme from the gill of the endangered sturgeon species Acipenser gueldenstaedtii. The CA enzyme was purified to a 66-fold using a Sepharose-4B-l-tyrosine-sulfanilamide affinity column, exhibiting a specific activity of 222.2 EU/mg protein. Kinetic values for the gill CA enzyme were determined as 2.5 mM for Km and 5×10^6 μM/min for Vmax, using p-nitrophenol acetate (p-NPA) as the substrate. The study also found that the CA enzyme was inhibited by sulfanilamide and acetazolamide, with IC50 values of 13.0 μM and 0.1 μM, respectively. Furthermore, the enzyme was inhibited by Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Ba^{2+}, with IC_{50} values of 0.2 mM, 1.7 mM, 1.2 mM, 1.1 mM, respectively.

Kurtoglu et al. (2016) investigated the histopathological effects of cement mixed with water on rainbow trout and Siberian sturgeon. Both fish species were exposed to concentrated cement at concentrations of 125 mg/l and 500 mg/l for a duration of 96 hours. The study determined LC_{50} values and revealed that both fish species exhibited an intolerance to cement-related contamination. Multiple deformations were observed in the gills of both species, with higher rates of hyperplasia observed in sturgeon compared to trout. Trout liver tissue displayed multiple fat granules, while melanomacrophage centers and necrosis were observed in sturgeon liver tissue treated with 500 mg/l of cement.

Akayli et al. (2017) aimed to describe the development of the thyroid gland and the initial functional activity of the thyroid gland and hormones in larvae of two sturgeon species, *Acipenser gueldenstaedtii* and *A. stellatus*, using immunohistochemistry. The study observed that the initial development of the thyroid gland occurred on the 3-4th day post-hatching in *A. stellatus* and 4-5th day post-hatching in A. gueldenstaedtii. Melanomacrophage centers were also observed around the thyroid follicles in sturgeon larvae. The study revealed a similarity in the early development of the thyroid gland between the two sturgeon species, but *A. stellatus* exhibited faster functional development and earlier hormone production compared to *A. gueldenstaedtii*.

In a physiology study conducted with Siberian sturgeon, a grading method was developed in which the physical measurement of scoliosis encountered in fish could be made and it was revealed to what extent the health of fish with scoliosis was affected (Duman, 2019a).

In a study conducted by Baba (2021), immune parameters in skin mucus samples were examined in four fish species: *Oreochromis niloticus*, *Oncorhynchus mykiss*, *Acipenser baerii*, and *Dicentrarchus labrax*. The analysis included measurements of alkaline phosphatase, lysozyme, myeloperoxidase, bactericidal activity against *A. hydrophila*, total protein, and immunoglobulin concentration. This study offers initial insights into the immune function of skin mucus, which serves as a crucial component in the immune systems of various fish species in aquaculture.

In a study conducted with Russian sturgeon (*Acipenser gueldenstaedtii*), the reference ranges of hematologic parameters of Russian sturgeon fry and adult individuals in the breeding environment were determined. In the study where hematological parameters were measured, it was determined that some values differed depending on age and some values (erythrocyte, hemoglobin, and hematocrit) did not differ (Ak et al., 2023).

5.6. Genetic studies

It is present in the study conducted to determine the genetic difference between sturgeon species sampled from the Turkish coast of the Black Sea (Eroğlu, 2009). In the genetic studies

conducted with sturgeon in Türkiye, there are genomic studies and studies on the determination of appropriate methods for the separation of species, especially in fish caught from nature. In the study conducted by Çiftci et al. (2013a), three species of sturgeon (*A. stellatus*, *A. gueldenstaedtii* and *H.huso*) were used from the Trabzon region. As a result of the study, it was emphasized that multiplex PCR application is an inexpensive and effective method of identifying sturgeon species in a short time. In another study with the same 3 species, the phylogenetic relationship, collected from the Turkish coast of the Black Sea between 2005 and 2007. The result of the study showed that all *A. gueldenstaedtii* samples were replaced in the Black Sea Lineage Group and were separated into 2 clades (A and B). It is also noted that the samples of *A. stellatus* are divided into 2 clades, but the specimens are *H.huso* are not separated (Çiftci et al., 2013b). Furthermore, Ciftci et al. (2013) conducted a study to examine the genetic variation in three sturgeon species, namely *A. stellatus*, *A. gueldenstaedtii*, and *H. huso*, found along the Turkish coast of the Black Sea. The researchers focused their investigation on the analysis of tandem repeat polymorphism and heteroplasmy. They conducted amplification and sequencing of the tRNApro and D-loop segment of the mitochondrial DNA (mtDNA) in the studied species. Subsequently, they calculated the quantities and frequencies of the repeats. The results indicated that all three species displayed varying mtDNA length variants, ranging from 2 to 6 copies. These variants were attributed to different numbers of copies of an 82-84bp repeat sequence. Around 9.9% of the sturgeons exhibited heteroplasmy, characterized by the presence of three to five repeat variants. A higher level of genetic diversity within individuals was observed in *A. gueldenstaedtii* and *A. stellatus* in comparison to H. huso. The repeat region, responsible for the variations in length and heteroplasmy, was situated near the end of the D-loop and control region, with only a few nucleotides separating it from the tRNApro gene (Çiftci et al., 2013a).

In the study conducted with three sturgeon species, namely *Huso huso*, *Acipenser stellatus*, and *A. gueldenstaedtii*, the researchers investigated the genetic coding of gonadotropin-secreting hormone (GnRH), insulin-like growth factor receptor I (ILGFRI), and androgen receptor (AR) genes. Polymerase chain reaction (PCR) analysis and DNA sequencing were employed for this purpose. The results of the study indicated a significant level of diversity within and between the same species. Furthermore, it was suggested that these three genes could be valuable tools for species identification and characterization (Albayrak et al., 2014).

5.7. Disease studies

A study by Timur et al. (2010) reported that in Russian sturgeon, *Aeromonas hydrophila* alone or in the form of mixed infection with *Flavobacterium hydatis* caused low mortality in fish by inducing bacterial hemorrhagic septicemia.

Extensive research on disease diagnosis and treatment methods has been conducted in sturgeon. In a study aimed at identifying the causes of losses in cultured Russian sturgeon during the period of 2007-2008, genetic analysis was employed to confirm the genetic identity of the isolated organism. Sturgeon affected by this organism exhibited symptoms such as ulceration, bleeding, and superficial skin erosions, primarily on the ventral side, including the pectoral and pelvic fins. Similar findings have been documented in other studies (Karatas et al., 2010). In another study, the types of bacteria that cause health problems in Russian and Siberian sturgeon were investigated. Fungal, parasitic, and bacterial pathogens found in these two species have been studied until the fish reach a weight of about 3 Kg (3 years). At the end of the study, several pathogens of bacterial diseases (*Acinetobacter radioresistance*, some species of *Aeromonas* and *Pseudomonas*, and *Bacillus mycoides*) and the parasite Trichodina sp. and the fungus Saprolegnia sp. have been identified (Kayiş et al., 2017). Other types of bacteria isolated from sturgeon has been reported as *Aeromonas hydrophila* (Ture et al., 2018) and *Citrobacter gillenii* (Türe et al., 2022).

In another study conducted with the same two species (*Acipenser gueldenstaedtii*; *A. baerii*), the resistance profiles and resistance genes to antimicrobial agents of a total of 37 strains isolated from fish in the period up to 3 kg in weight were investigated. And the antibiotics to which bacteria are most resistant were found to be sulfamethoxazole and ampicillin (97.3%) (Terzi, 2018). In another study, the immunostimulant effects of rosehip (*Rosa canina*) on hematologic and nonspecific immune parameters in *Mycobacterium salmoniphilum*-infected sturgeon were investigated. The findings indicated that feeding fish with rosehip at a concentration of 15% significantly enhanced the hematological and immune response when challenged with *M. salmoniphilum* infection. As a result, the study determined that 15% rosehip supplementation was an effective dose in fish, suggesting that rosehip could serve as an alternative to the currently recommended immunostimulants (Duman and Şahan, 2018).

5.8. Anesthesia studies

In Russian sturgeon larvae, clove oil's anesthetic potency was assessed using four anesthetic concentrations ranging from 0.20 to 0.75 g L (Akbulut et al., 2011c). They showed that Russian sturgeon larvae may be effectively anesthetized with clove oil, and that a higher dose of clove oil lengthened the recovery period. Another study found that in the anesthetic use of clove oil in juvenile Russian sturgeon, the dose used and the duration of exposure to the dose affected the duration of recovery (Akbulut et al., 2011d). Akbulut et al. (2012a) compared the effects of feed intake after anesthesia on Siberian sturgeon fry and determined the ideal dose and amount of time needed to achieve a stable level of anesthesia using clove

oil and benzocaine. In the results, they found that the effects of both anesthetics on feed intake lasted for four hours in fish treated with clove oil and benzocaine, whereas feed intake occurred within ten minutes of treatment. Also, clove oil had a less significant impact on feed intake than benzocaine. Duman, (2019b) aimed to investigate the effect of 2-phenoxyethanol on reducing stress response during transportation of Russian and Siberian sturgeons. Two transportation experiments were conducted, and the fish were divided into groups that received 2-phenoxyethanol or not. The results showed that the anesthetic-free groups had higher levels of plasma glucose and cortisol during transportation. Red blood cells and hematocrit decreased in the anesthetic-free groups, while neutrophils and white blood cells increased. The use of 2-phenoxyethanol reduced mortality rate and physiological stress in sturgeons during transportation. Ak, (2022) aimed to investigate the anesthetic efficiency, hematological, histopathological, and echocardiographic effects of clove oil and 2-phenoxyethanol (2-PE) in adult Russian sturgeon (*Acipenser gueldenstaedtii*), which undergo unavoidable handling and surgery procedures in broodstock management. The fish used in the experiment were subjected to varying concentrations of clove oil and 2-PE, and the time taken for induction and recovery was measured. The findings indicated that concentrations of 250-500 µl/L of 2-PE were ineffective in inducing anesthesia, whereas a concentration of 750 µl/L resulted in anesthesia within 290 seconds. The ideal concentrations of clove oil were determined to be between 40 and 80 µl/L, resulting in anesthesia within 180-120 seconds.

5.9. Processing studies

Kaya et al. (2008) looked at how the fatty acid and amino acid content changed in hot-smoked sturgeon (*Huso huso*, L. 1758). In a study with beluga (*H.huso*), the chemical composition of raw and smoked sturgeon meat was determined. At the end of the study, the beluga meat was described as color, smoke aroma, flavor, texture, sensory parameters of general acceptance for chewing and smoked sturgeon, excellent and the flesh tasty (Şengör et al., 2010).

The study aimed to investigate the effects of boiling, grilling, and cold storage on the levels of florfenicol residues in sturgeon muscle tissues. The researchers used 16 sturgeons, ten of which were given a single dose of florfenicol, and the remaining six were not. The analysis was conducted through HPLC. The results showed that boiling and cold storage processes significantly reduced the initial florfenicol level in fish muscle tissues, but there was no reduction in the florfenicol level due to grilling. Additionally, the study found that the florfenicol levels were higher in the boiling juice than in the muscle tissue. The study provides useful information on the impact of different cooking and storage methods on the levels of florfenicol residues in fish muscle tissues Gürbüz et al. (2021).

5.10. Other studies

In addition to the studies that are tried to be detailed above, there are also review studies on sturgeons. These are reviews containing information about fish in general (Akbulut, 2002; Ustaoğlu and Okumuş, 2004; Üstündağ, 2005; Ercan, 2011; Ustaoğlu Tırıl and Memiş, 2013; 2015; Akbulut et al., 2007, 2011a, Ateş, 2009; Memiş et al., 2020), conservation strategies (Ustaoğlu, 2006; Tırıl Ustaoğlu and Memiş, 2018; Zengin and Dağtekin, 2019), gamete and gamete preservation (Yamaner et al., 2015), nutrition (Akbulut et al., 2012b) and caviar (Özden et al., 2018).

Also, Yıldırım, (2021) is about the historical importance of the Azov Sea basin as a major supplier of fish and caviar in the international trade market since ancient times. In the Middle Ages, various fish species such as cod, herring, sprat, and sturgeon were caught in abundance in the fresh and cool waters of the Kuban and Don rivers that flow into the Azov Sea. These fish were traded regionally as well as internationally, in fresh, dried, and salted forms. Caviar and fish glue were also important commercial products traded in the region, with the black caviar of sturgeon being considered a luxury item. The text focuses on the trade routes of fish and caviar from the Azov Sea to the Black Sea coastal cities, Constantinople-Pera, Mediterranean islands, and Europe, based on historical sources such as Latin texts and travel notes.

6. Conclusion

Some Sturgeon species is an anadromous species and its populations living in the Black Sea enter the rivers flowing into the Black Sea to breed. Therefore, events such as pollution, overfishing and global climate change in the Black Sea directly affect the fish living here. The total coastal length of the Black Sea, including the Sea of Azov, is 4,869 km. 3,456 km of this is the coasts of Ukraine (1,756 km, 36.1%) and Türkiye (1,700 km, 34.9%). There are three major rivers (Sakarya, Kızılırmak and Yeşilırmak) flowing into the Black Sea from Türkiye. The protection of these ecosystems is very important for the survival and welfare of sturgeon species.

So far, many scientific and non-governmental studies have been carried out to protect sturgeon and instill this awareness in the public. However, it is seen that the momentum of these studies is decreasing, and more efforts are needed to keep people's interest in this subject alive. Therefore, a great task falls on the aquaculture sector, where people will increase their economic benefits and provide sturgeon-based economic gains.

Acknowledgement: In this book section, only studies conducted in Türkiye are reviewed. Studies by Turkish scientists abroad or studies of foreign origin in which Turkish scientists participated as authors were not included. Additionally, only articles were compiled, and

symposium presentations, graduate theses, and similar academic activities were excluded. The literature review was conducted using the WOS system, Google Scholar, and by carefully scanning the CVs of scientists working with sturgeon. We apologize if any studies were inadvertently overlooked during this scan.

We would like to express our gratitude to the esteemed scientists and teachers who have contributed to the aquaculture studies of sturgeon in Türkiye, a fish species with historical significance in our country. We appreciate their efforts in expanding these studies and their ongoing projects in this field.

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CHAPTER 13

RECIRCULATING AQUACULTURE SYSTEMS IN AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.013

1. Introduction

1.1. Purpose and scope of the chapter

This chapter aims to provide a comprehensive overview of the recirculating aquaculture technology and its applications in the aquaculture industry with a focus on Turkish aquaculture. The purpose and scope of this chapter include the following:

- Definition and background: A clear definition of RAS and an overview of the history and development of the technology.
- Components and design: A description of the key components of RAS and an explanation of how RAS systems are designed and constructed.
- Advantages and disadvantages: A discussion of the key advantages and disadvantages of RAS, including environmental sustainability, cost-effectiveness, improved yields, and the reduced risk of disease and parasitism.
- Future directions: A discussion of the future directions of RAS, including the potential for further technological development and the continued growth and acceptance of RAS as a sustainable and environmentally friendly way of growing aquatic species.
- RAS in Türkiye

2. Definition and Background

2.1. Definition of recirculating aquaculture systems (RAS)

Recirculating aquaculture systems (RAS) are a type of aquaculture technology that are used to rear aquatic species, including fish, crustaceans and mollusks, in a controlled and sustainable environment, usually within indoor tank-based systems. They are typically characterized by their closed-loop design, which allows for the efficient use of precious water resources by the means of water treatment systems and reduces the environmental impact of water discharge.

In a RAS, pumped water is circulated through various filtration and treatment units including suspended solid filters, biofilters, protein skimmers and UV sterilizers before being returned to the rearing tanks. This complex process helps to properly maintain a consistent and high-quality water environment for the aquatic species to thrive in. The water treatment units are designed precisely to remove waste solids, ammonia, nitrite, and other contaminants from the water, thus reducing the risk of disease and ensuring the health and survival of the aquatic species (Helfrich and Libey, 1991; Ebeling and Timmons 2010; Martins et al., 2010; Murray et al., 2014).

A favorable culture environment is critical for aquatic animals and plants. When designing recirculating systems, the key unit operations to maintain a healthy environment are solid removal, ammonia removal/conversion and aeration/oxygenation. Waste solids, including uneaten food and metabolic by-products of reared fish, must be removed promptly. If left in the aquatic system, these solids can increase oxygen demand and produce carbon dioxide and ammonia nitrogen during bacterial decomposition.Accurate control of the water parameters allows for the cultivation of aquatic species beyond their natural climate, enabling farmers to focus on production targets based on factors such as market demand, regulations and resource availability. RAS technology can be beneficial in situations where ideal locations are unavailable, such as when there is limited land or water space, water is scarce or of poor quality, temperatures fall outside the optimal range for the species, or the species is exotic. And also, RAS technology can be utilized when there are more rigid environmental regulations regarding effluent control and biosecurity, or when low-cost energy sources are accessible. Generally, “intensive” or “fully-recirculating” RAS have a water replacement rate of less than 10% per day, while systems with higher replacement rates are referred to as “partial-replacement” systems. Partial-replacement systems are commonly utilized to increase rainbow trout production in raceways and tanks and require minimal, often modular water treatment facilities, leading to lower capital investment compared to intensive RAS. (Twarowska, 1997; Wheaton, 2002).

Recirculating aquaculture systems represent a remarkably efficient and sustainable technology for rearing aquatic species. With their closed-loop design, they allow for efficient use of precious water resources, reduced environmental impact, and improved year-round control over the growing environment, leading to higher yields and improved product quality.

2.2. Brief history and evolution of RAS

Recirculating aquaculture systems (RAS) have been used for rearing aquatic species like fish, crustaceans and mollusks, for several decades. The gradual development of modern RAS technology can be traced back to the 1970s, when researchers first began to scientifically investigate closed-loop systems for rearing fish in controlled environments. In the early days of RAS development, the technology was primarily used for research purposes, as the complex filtration and treatment units were too expensive and difficult to operate satisfactorily for commercial applications. However, as technology improved and costs came down, RAS began to gain broader acceptance as a viable option for commercial aquaculture (Masser and Lossordo, 1999; Murray et al., 2014).

The historical trend towards more intensive aquaculture practices is driven by the need to increase production, which is being limited by factors such as the scarcity of water and land, as well as concerns about environmental impact from water discharges. One of the key drivers of RAS growth represents the specific need for more sustainable and environmentally friendly aquaculture systems. Traditional aquaculture systems, which typically rely on extensive quantities of water, often lead to the discharge of waste and other pollutants into the sensitive environment, causing significant harm to the ecosystem. Recirculating aquaculture systems sufficiently address these ecological issues by conserving water and controlling the water quality in a closed-loop system, predictably leading to improved production efficiency and reduced disease risk, significantly reducing the excessive amount of water required and the environmental impact of aquaculture. These systems additionally have a smaller footprint, making it easier to establish facilities in urban areas with limited land. Ultimately, recirculating aquaculture technology offers a sustainable solution for fulfilling the increasing demand for seafood (Guiterrez-Wing and Malone, 2005; Goldburg et al., 2001; Aich et al., 2020).

In the late 1990s and early 2000s, modern RAS technology advanced significantly, with the gradual development of more efficient filtration and treatment units, and improved understanding of the water treatment and management processes required for rearing diverse species. This naturally led to increased interest in RAS for commercial applications, particularly for farming high-value species, such as high-value fish, shellfish and ornamental species. Over

the preceding decade, RAS has continued to evolve and improve, with the economic development of more advanced and efficient water treatment systems, and improved understanding of the best practices for growing different species in RAS environments. As a result, RAS has become a widely used and accepted technology for rearing aquatic species in a sustainable and environmentally friendly manner (Xiao et al., 2019; Espinal and Matulic, 2019; Ramli et al., 2020).

In conclusion, the development of recirculating aquaculture systems has remained a gradual process that has taken place over several decades. Driven by the need for more sustainable and environmentally friendly aquaculture systems, modern RAS technology has evolved and improved over the years, becoming a widely accepted and widely used technology for farming aquatic species in controlled environments.

2.3. Overview of the significance of RAS in the aquaculture industry

The expansion of conventional cage-based and flow-through aquaculture systems is hindered by several key challenges, including limited space for growth and development due to competition with other land uses, scarce access to fresh water, and environmental pollution concerns. These systems allow for a high degree of water recycling, up to 90-99%, through the use of various components (Badiola et al., 2012). The greater control over the water quality and environmental parameters offered by RAS systems creates optimal conditions for fish cultivation. Recirculating aquaculture systems (RAS) have become increasingly significant in the aquaculture industry in recent years, offering numerous benefits over traditional aquaculture methods such as;

Sustainability: The rapid expansion of aquaculture has led to growing concerns about its environmental sustainability. This industry has been linked to numerous negative environmental impacts, like habitat destruction, water pollution and eutrophication, depletion of native species, transmission of diseases and parasites, and greenhouse gas emissions. Intensive aquaculture practices have also been accused of allegedly causing antibiotic pollution, eutrophication, land occupation, and other environmental hazards. The controversial introduction of non-indigenous fish species in some aquaculture systems inevitably has the destructive potential to negatively impact biodiversity and sensitive ecosystems. Coastal aquaculture, particularly shrimp farming, has been shown to unintentionally cause significant harm to mangrove forests. Salmon aquaculture in marine environments has faced criticism for various environmental impacts, including water pollution, pathogen transfer to wild salmon and other fish, and other negative effects. RAS are much more sustainable and environmentally friendly

than traditional aquaculture methods. RAS systems enable fish farming in a land-based, indoor, and controlled environment, which minimizes the direct impact of production processes on the environment. RAS recirculate and treat water, considerably reducing the amount of water required and the environmental impact of aquaculture. This naturally makes RAS a more sustainable and responsible way of rearing aquatic species (Naylor et al., 2000; Paez-Osuna, 2001; Lebel et al., 2002; Ford and Myers, 2008; De Silva and Soto, 2009; De Silva et al., 2009; Bush, et al., 2010; Hall, 2011; Midilli et al., 2011; Badiola et al., 2012; Hamilton, 2013; Richards and Friess, 2016; Thomas et al., 2017; Ahmed et al., 2019;)

Control: RAS provides a high degree of control over the culture environment, which reduces the risk of disease and parasitism and ensures aquatic species receive optimal rearing conditions. This makes RAS particularly useful for growing high-value species, such as high-value fish, shellfish and ornamental species, which require specific conditions to thrive (Ebeling and Timmons, 2010).

Improved yields: RAS allows for consistent and optimum rearing conditions, which can naturally lead to higher yields and improved quality of the final product. Additionally, the efficient use of precious water resources and the reduced risk of infectious disease and parasitism result in improved overall production efficiency (Ngoc et al., 2016a; Ngoc et al., 2016b).

Cost-effectiveness: Although modern RAS systems can be more expensive to set up than traditional aquaculture methods, the long-term cost-effectiveness of RAS is often greater due to reduced water usage and improved production efficiency. Use of renewable energy is advised instead of fossil based fuels (Badiola et al., 2018).

Land-use efficiency: RAS allows for the rearing of aquatic species in land-locked areas, where traditional aquaculture methods may not be possible. This naturally makes RAS particularly useful for urban areas and regions with limited water resources (Bregnballe, 2022).

Recirculating aquaculture systems have undoubtedly made a significant impact on the aquaculture industry, offering numerous benefits over traditional aquaculture methods. With their sustainable and environmentally friendly approach, improved yields, cost-effectiveness, and land-use efficiency, RAS are an increasingly important technology for profitably growing aquatic species in controlled environments.

3. Components of RAS

Recirculating aquaculture systems (RAS) comprise complex systems that involve several key components to maintain the water quality and environment for cultured aquatic species. The following are some of the fundamental components of a typical RAS system;

3.1. Tank systems

The primary component of any RAS system is the tank where the aquatic species are maintained and play a critical role in the cultivation of aquatic species. Tank systems can be designed for a wide range of species, including finfish, crustaceans, and mollusks, and can be adapted to meet the specific water quality and production requirements of each species. The tank can be made of concrete, fiberglass or plastic (Figure 3.1), and can be designed to support freshwater or saltwater species (Masser et al., 1999; Bregnballe, 2022).



Figure 3.1. Samples of tanks used in recirculating aquaculture systems (Original photo, Deniz D. TOSUN)

A wide variety of tanks are used in recirculating aquaculture systems;

- Culture tanks: Culture tanks are the primary rearing units in RAS, where aquatic species are kept for growth and production. Culture tanks can be designed for a variety of species and can range in size from compact tanks for individual fish to large tanks for group housing. Small or juvenile aquatic species are reared in larval rearing/nursery tanks. Larval rearing/nursery tanks are designed to provide a safe and secure environment for juvenile fish to grow and develop (Ebeling and Timmons, 2010; Perumal et al., 2015).

- Reconditioning/Holding tanks: Reconditioning tanks are typically used to acclimate newly introduced aquatic species to the water conditions in RAS. New arrivals are held in reconditioning tanks for a period of time before being moved to the culture tanks. These tanks are typically designed to maintain the water quality and to keep the fish in a stress-free environment (Ebeling and Timmons, 2010; Perumal et al., 2015).

- Quarantine tanks: Quarantine tanks are used to isolate new arrivals or sick individuals in RAS. Quarantine tanks are used to reduce the risk of disease transmission and to monitor the health of new arrivals before they are introduced to the culture tanks (Ebeling and Timmons, 2010; Perumal et al., 2015).

- Filtration tanks: Filtration tanks are used to house water treatment equipment, such as mechanical filters, biofilters, and chemical addition systems. The water in RAS is pumped through these tanks for treatment and to maintain water quality. Settling tanks are used to separate solid waste from the water in RAS. Solid waste can be removed from the water by gravity or through the use of mechanical filtration devices (Ebeling and Timmons, 2010; Perumal et al., 2015).

- Harvest tanks: Harvest tanks are used to collect and store aquatic species at harvest time. Harvest tanks are typically designed to maintain the water quality and to prevent stress to the fish during harvest (Ebeling and Timmons, 2010; Perumal et al., 2015).

- Water storage tanks: Water storage tanks are used to store water for use in RAS. Water storage tanks can be used to store freshwater, saltwater, or treated water, and can be designed to maintain the water quality and prevent contamination (Ebeling and Timmons, 2010; Perumal et al., 2015).

The specific tank systems used in RAS will depend on the species being cultured, the water quality requirements, and the desired outcome of the system. The material used to construct tanks in recirculating aquaculture systems (RAS) is an important consideration as it affects the water quality, water chemistry, and overall system performance. Some of the most commonly used materials for tank construction in RAS include:

1. Concrete: Concrete is a durable and long-lasting material that is commonly used for tank construction in RAS. Concrete tanks (Figure 3.2) are easy to maintain and can be easily modified to meet changing needs.



Figure 3.2. Concrete tanks used in RAS, Denmark (Original photo, Deniz D. Tosun)

2. Fiberglass: Fiberglass is a lightweight and corrosion-resistant material that is commonly used for tank construction in RAS. Fiberglass tanks (Figure 3.3) are easy to clean and maintain and are less likely to crack or leak compared to concrete tanks.



Figure 3.3. Fiberglass tanks used in RAS, Türkiye (Original photo, Deniz D. Tosun)

3. Polyethylene: Polyethylene is a flexible and durable material that is commonly used for tank construction in RAS. Polyethylene tanks are easy to clean and maintain, are resistant to chemicals and UV light, and are less likely to crack or leak compared to concrete or fiberglass tanks.

It is important to consider the specific requirements of each species being cultivated and the desired water quality when selecting the material for tank construction in RAS. The water chemistry and water quality can have an impact on the durability and performance of the tank, and the material selected should be able to withstand the conditions of the system.

3.2. Water treatment systems

Recirculating aquaculture systems (RAS) require efficient water treatment systems to maintain water quality and support healthy fish growth. The filtration system is an important component of RAS and is responsible for removing waste products, excess food, and other organic matter from the water RAS systems typically use a combination of physical, chemical, and biological processes to treat the water and maintain water quality. This may include mechanical filtration, chemical addition, and biological filtration using bacteria and other microorganisms (Masser, Rakocy and Losordo, 1999; Leonard, Blancheton, and Guiraud, 2000; Pfeiffer et al., 2008; Malone, 2013; Bregnballe, 2022).

RAS water treatment typically involves multiple steps, including:

3.2.1. Mechanical filtration

Mechanical filtration in RAS refers to the physical removal of solid particles from the water in the system utilizing a filtration device. In RASs, wastewater is typically rich in residual feed, excrement, and other solid particulate matter, which needs to be removed to the greatest

extent possible in the initial stage of water treatment. This is typically achieved through physical filtration to remove impurities and reduce the organic load before further water treatment steps. This type of filtration typically involves a filter screen or mesh that traps suspended solid particles, such as uneaten feed, excrement, and other debris, and prevents them from recirculating in the water. Mechanical filtration helps maintain water quality and clarity and is an essential component of a successful RAS operation. It reduces the solids in the water that can clog other components in the RAS, such as pumps, heat exchangers, and biofilters. These clogs can reduce the efficiency of the system and increase the risk of system failures, so removing solids through mechanical filtration is critical to ensure the long-term stability and performance of the RAS.The filtration device can be as simple as a mesh screen or as complex as a multi-stage filtration system that incorporates various types of filters (Figure 3.4), such as drum filters, sand filters, belt filters, or micro-screen filters (Ni and Zhang, 2007; Xiao et al., 2019).

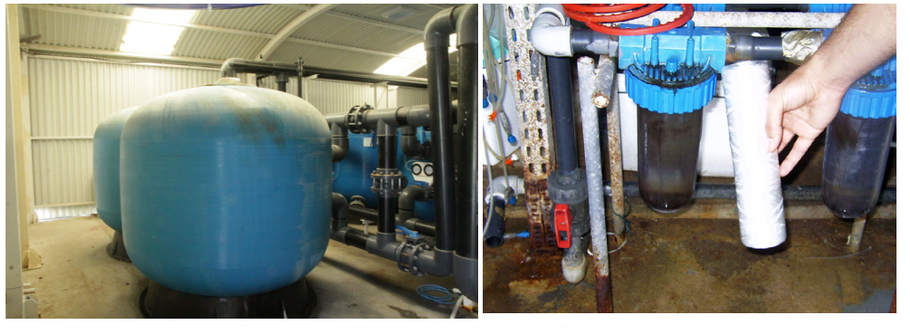

Figure 3.4. Types of mechanical filters used in Türkiye (Original photo, Deniz D. Tosun)

The design of a mechanical filtration system depends on the type of species being cultured, the water flow rate, and the total water volume of the RAS. For example, a RAS that cultivates finfish species would require a different type of mechanical filtration system than a RAS that cultivates shellfish. Similarly, the water flow rate and total water volume will dictate the size and configuration of the filtration system. There are various factors to consider when designing a mechanical filtration system, including the removal efficiency, the pressure drop across the filter, the backwash frequency and the maintenance requirements. A well-designed mechanical filtration system will effectively discard suspended solids and maintain water clarity while minimizing the pressure drop and the frequency of backwashing. Additionally, an easy system to maintain will reduce downtime and minimize the risk of system failures(-Losordo, Masser and Rakocy, 2000).

There are economic benefits to incorporating mechanical filtration into a RAS. By removing solid waste from the water, it is possible to reduce the amount of water that needs to be replaced and recycled, which can result in significant cost savings over time. Additionally, by maintaining high water quality and reducing the risk of disease outbreaks, it is possible to improve the health of the cultured species, which can result in increased production and profitability (Dolan et al., 2013; Andrei et al., 2016).

3.2.2. Biological filtration

Biological filtration is a critical aspect of Recirculating Aquaculture Systems (RAS) for ensuring water quality and the health of aquatic organisms. In RAS, biological filters are used to remove harmful waste products produced by fish or other aquatic organisms, namely ammonia and nitrite, by converting them into less toxic form such as nitrate. This process helps to maintain a stable and healthy environment for the aquatic organisms, which is essential for their survival and growth (Guerdat et al., 2010; Schreier et al., 2010).

The process of biological filtration in RAS involves the growth of beneficial bacteria on a substrate within the filter. The bacteria convert harmful waste products into less toxic form through a process known as the nitrogen cycle. The nitrogen cycle consists of several stages, including ammonia conversion to nitrite, nitrite conversion to nitrate, and nitrate conversion to nitrogen gas through denitrification. Biological filters are commonly categorized based on the type of bacteria used in the process. Nitrifying bacteria, such as *Nitrosomonas* and *Nitrobacter*, are responsible for converting ammonia to nitrite and then to nitrate. Denitrifying bacteria, such as *Pseudomonas*, are responsible for converting nitrate to nitrogen gas. The efficiency of biological filtration in RAS is impacted by several factors, including flow rate, water temperature, and pH levels. Maintaining optimal conditions for the bacteria is important to ensure the proper functioning of the biological filter. For example, nitrifying bacteria require a pH range of 7.0 to 8.0, while denitrifying bacteria prefer a lower pH range of 6.5 to 7.5. Water temperature also plays a critical role in the efficiency of biological filtration, as the optimal temperature range for nitrifying bacteria is between 20-30°C, while denitrifying bacteria prefer a temperature range of 15-25°C (Losordo et al., 2000; Eding et al., 2006; Guerdat et al., 2011).

In addition to the aforementioned factors, the size and design of the biological filter also impact its efficiency. The size of the filter should be proportional to the size of the RAS system, ensure the adequate surface area and allow for efficient water flow for the growth of bacteria. The use of bio-media, such as ceramic or plastic balls (Figure 3.5), can help to increase the surface area for bacteria growth and improve the efficiency of the biological filter.

Biological filtration plays a vital role in maintaining water quality in RAS, ensuring the health and survival of aquatic organisms. Proper design and maintenance of the biological filter are critical for the efficient removal of harmful waste products, and the maintenance of a stable and healthy environment for aquatic organisms. By understanding the factors that impact the efficiency of biological filtration and the role of bacteria in the nitrogen cycle, RAS operators can ensure the long-term success and sustainability of their systems. (Losordo et al., 2000; Eding et al., 2006; Malone and Pfeiffer, 2006).



Figure 3.5. Plastic balls that are used to increase surface area for nitrification (Original photo, Deniz D. Tosun)

It's important to note that regular maintenance of the biological filter is also crucial for its proper functioning. This includes regular cleaning or replacement of the bio-media, as well as monitoring and adjusting water parameters such as pH, temperature, and flow rate. Additionally, it's essential to avoid overstocking the RAS system, as this can lead to a buildup of waste products, which can harm aquatic organisms and potentially disrupt the nitrogen cycle in the biological filter (Steicke et al., 2009; Sharrer et al., 2007).

Another aspect of biological filtration in RAS is the use of bio-augmentation, which involves adding specific beneficial bacteria to the system to enhance the performance of the nitrogen cycle. This can help to reduce the time it takes for the biological filter to mature and can also improve its efficiency in removing harmful waste products (Neissi et al., 2022).

3.2.3. Chemical filtration

Chemical filtration is an important component of water treatment in recirculating aquaculture systems (RAS). The primary purpose of chemical filtration is to remove dissolved substances from the water, such as excess nutrients, heavy metals, or organic compounds.

There are several methods used in chemical filtration in RAS, including:

- Adsorption: uses materials, such as activated carbon or zeolites, to physically adsorb dissolved substances.
- Chemical precipitation: addition of chemicals, such as aluminum or iron salts, to the water to cause dissolved substances to precipitate out as solids.
- Ion exchange: uses resin beds to exchange ions in the water to remove dissolved substances.
- Reverse osmosis: uses pressure to force water through a semi-permeable membrane, removing dissolved substances in the process.
- Foam fractionation: Foam fractionation is a separation process that utilizes the differences in surface activity of components in a liquid mixture to separate them. In this process, a gas (usually air) is bubbled through the liquid, generating foam. As the bubbles rise, they carry with them the components that are more surface-active, such as proteins or surfactants, which adsorb onto the bubble surfaces. The resulting foam layer can be removed and the separated components recovered (Figure 3.6).



Figure 3.6. Foam fractionaters used in RAS systems in Türkiye

It is important to carefully manage the chemical filtration process, as the addition of certain chemicals can affect water chemistry and harm aquatic animals in the RAS. Regular monitoring of water parameters and adjustment of chemical filtration processes as needed is crucial for maintaining optimal water quality (Wheaton, 2002; Rurangwa and Verdegem, 2015).

Chemical filtration methods used in a RAS system can depend on a variety of factors, such as the specific water quality concerns, the species of fish being cultured, and the overall goals of the system. For example, ion exchange may be used to remove excess nitrates, while reverse osmosis may be used to remove high levels of dissolved salts. In addition to removing harmful substances, chemical filtration can also be used to add beneficial substances to the water. Addition of calcium and magnesium ions can help to maintain optimal water hardness, which can be important for the health and growth of certain species of fish (Helfrich and Libey, 1991; Sitek, 2020).

Another important aspect of chemical filtration in RAS is the management of the discharge of filtration by-products. In some cases, chemical filtration processes can result in the generation of residual chemicals or waste products that must be properly managed to avoid

environmental impact. Chemical filtration is an essential component of water treatment in RAS, but it must be carefully managed to ensure optimal water quality and avoid negative impacts on the environment. Regular monitoring of water parameters, such as pH, total dissolved solids, and dissolved oxygen, is critical in ensuring the effectiveness of chemical filtration in RAS. This information can be used to make informed decisions about the use of chemical treatments, such as the type and frequency of treatments, and can also provide early warning of any potential problems (Helfrich and Libey, 1991; Sitek, 2020).

3.2.4. Disease management systems

Ultraviolet (UV) and ozone systems are important components that are often used in recirculating aquaculture systems (RAS). UV sterilization systems efficiently utilize ultraviolet light to eliminate harmful bacteria, viruses, and other microorganisms in the water. UV sterilization remains an effective and efficient way to adequately maintain water quality and prevent infectious disease in RAS (Figure 3.7). Ozone treatment systems use ozone gas to disinfect water and remove organic compounds, such as dissolved organic matter, from the water. Ozone is an extremely reactive substance and is often used in combination with other water treatment methods to improve water quality in RAS. (Malone, 2013; Rurangwa and Verdegem, 2014; Huyben et al., 2018).



Figure 3.7. Disease management systems used in RAS in Türkiye (Original photo, Deniz D. Tosun)

3.2.5. Oxygenation

Fish require oxygen to carry out metabolic processes and maintain healthy growth. In RAS, the water is reused multiple times, which can deplete oxygen levels due to the high stocking density and high feeding rates. Therefore, maintaining adequate oxygen levels is crucial to prevent fish stress, disease, and mortality.The objective of controlling dissolved oxygen in RAS is to improve the efficiency of oxygen transfer while reducing the energy consumption. Several methods are available to increase oxygen levels in RAS, including mechanical aeration, oxygen injection, and oxygenation through algae photosynthesis. Mechanical aeration is the most commonly used method in RAS, where air is pumped into the water to increase oxygen levels. Oxygen injection involves injecting pure oxygen into the water, which can be more efficient in increasing oxygen levels but requires careful monitoring to prevent over-saturation (Figure 3.8) (Ebeling and Timmons, 2012; Malone, 2013; Espina and Matulic, 2019).



Figure 3.8. Oxygenation systems used in RAS in Türkiye (Original photo, Deniz D. Tosun)

3.2.6. Degassing

Degassing is an important step in water treatment in recirculating aquaculture systems. It involves removing dissolved gases, such as carbon dioxide, from the water to maintain optimal pH levels. Degassing can be accomplished through physical methods, like bubbling air through the water, or through chemical methods, such as the addition of alkalinity-adjusting agents. Incorporating degassing into the water treatment process helps to ensure stable water conditions and reduces the likelihood of pH fluctuations, which can be harmful to aquatic animals in the RAS (Ebeling and Timmons, 2012; Ebeling, 2000).

The specific treatment process and components used can vary based on the type of RAS, species of fish, and water quality requirements. It is important to regularly monitor water

parameters and adjust treatment processes as needed to ensure the health and growth of the aquatic animals in the system.

3.2.7. Pumping system

A pumping system is used to circulate water through the various components of the RAS system, including the mechanical filtration system, water treatment system, and tanks. The pumping system must be designed to provide adequate flow rates to maintain water quality and to prevent stagnation (Loyless and Malone, 1998; Mcmillian, Wheaton and Hochheimer, 2003; Malone, 2013; Badiola et al., 2018).

3.2.8. Monitoring and control system

A monitoring system is crucial for the proper functioning of a RAS as it plays a vital role in maintaining a healthy environment for the fish. It is used to track key parameters, such as water temperature, pH, dissolved oxygen, and total ammonia nitrogen, and to control the various components of the RAS system, such as pumps, heaters, and oxygenation systems. The monitoring system is responsible for ensuring the quality of the water in the indoor tanks, which directly impacts the growth and well-being of the fish living in the system. The use of computerized control systems can lower the need for manual labor and enhance the reaction to conditions that fall outside of acceptable parameters. The necessary monitoring systems will be determined by the design criteria, water quality goals, and a thorough evaluation of potential failure points in the system (Fowler et al., 1994; Al-Hussani et al., 2018; Aich et al., 2020)

3.2.9. Feed management strategies

Feed management is essential in RAS to ensure that fish receive the proper amount of food to support their growth and health. Overfeeding can lead to waste accumulation, which can cause poor water quality, disease, and stress to the fish. Underfeeding, on the other hand, can inevitably result in slow growth, poor feed conversion, and reduced profitability. Therefore, maintaining optimal feeding rates and avoiding wastage is crucial in RAS.

Several methods are applicable to manage feed in RAS, including manual feeding, automatic feeding, and demand feeding. Manual feeding involves the operator manually distributing the feed at specific intervals throughout the day. Automated feeding systems use a timer or controller to dispense feed at pre-set intervals, which can be programmed to adjust the feeding rate according to the fish biomass and feeding behavior. Demand feeding systems use sensors or software to detect fish feeding behavior, which triggers the automatic dispensing of feed. Additionally, monitoring feed intake and waste can help identify and address feeding issues (Badiola et al., 2012; Zhou et al., 2018).

Several challenges are associated with feed management in RAS, including feed quality, feed conversion, and the potential for overfeeding. The feed utilization by fish cultured in RAS often shows favorable comparison to that of fish raised in other types of culture systems. Feed conversion ratios (FCR) should be monitored to ensure that the amount of feed provided is efficiently converted into fish biomass. Overfeeding can lead to feed waste, which can cause poor water quality, disease, and stress to the fish. The quality of feed must be consistent and appropriate for the species being farmed to ensure good health and growth. RAS are susceptible to organic matter accumulation, particularly the dissolved organic matter (DOM), which is a significant drawback of this modern technology. Endogenous production of DOM in RAS primarily originates from fecal waste and feed spill. The contribution of exogenous DOM depends on the quality and quantity of makeup water, and the rate of water exchange, as well as the type of treatment system used. The excessive accumulation of DOM can inadvertently promote the growth of opportunistic bacteria, severely impair the bio-filtering performance, and hinder oxidative disinfection processes. Other challenges associated with DOM accumulation in RAS include contamination of the fish environment, jeopardizing the welfare of the fish, and deteriorating water quality, which increases the need for further water treatment and ultimately increases production costs (Masser et al., 1999; Pedersen et al., 2012; Van Rijn, 2013; Martins et al., 2010; Sun et al., 2014).

4. Benefits and Challanges of RAS

4.1. Benefits of RAS

Recirculating Aquaculture Systems offer several advantages:

- Tanks and equipment in RASs have a longer average lifespan compared to nets and boats, which allows for longer amortization periods.
- A decreased reliance on antibiotics and therapeutants can provide a marketing advantage for RAS-produced seafood, as it can be marketed as high-quality and safe seafood
- RASs can lead to a reduction in direct operational costs related to feed, predator control, and parasites.
- RASs have the potential to eliminate the release of parasites into recipient waters.
- Rearing species, such as salmon, in RASs before transferring them to cages can be beneficial, as it allows for a longer period of time for the young salmon to grow and reduces the amount of time they are exposed to the risks associated with off-shore environment. This potentially reduces the production time by optimized conditions.

- RASs can enable the production of a wide variety of species, regardless of their temperature requirements, as long as the costs of temperature control beyond ambient temperatures are energy-efficient.
- With RAS technology, it is possible to safely produce non-endemic species.
- RASs have the potential to greatly enhance feed management, as feeding can be closely monitored over 24-hour periods. Optimal environmental conditions in RASs can promote excellent Feed Conversion Ratios (FCRs), and some high-value marine species can reach market size in 50% less time compared to sea cages.
- RAS use less water compared to other aquaculture techniques.
- RAS farms can reduce the exposure of stock to stressful factors such as adverse weather, unfavorable temperature conditions, pollution incidents, and predation.
- RASs have improved opportunities for waste management and nutrient recycling.
- RASs have better hygiene and disease management
- RASs don't rely on surface water to produce aquatic organisms.
- Using RAS technology enables the production of a diverse range of seafood products in close proximity to markets.
- Producing seafood in RASs can reduce carbon dioxide (CO2) emissions associated with food transport.

RAS can offer several advantages over traditional aquaculture. It is important to evaluate these benefits in comparison to other production options and choose an economic and sustainable means of culture system that benefits both the producer, consumer and the environment. (Ebeling, 2000; Martins et al., 2010; Badiola et al., 2012, Murray et al., 2014, Ebeling and Timmons, 2010; Ebeling and Timmons 2012; Suantika et al., 2018).

4.2. Challanges of RAS

4.2.1. Challanges of mechanical filtration systems in RAS

Mechanical filtration in Recirculating Aquaculture Systems (RAS) has some limitations, including:

- Clogging: Mechanical filters can become clogged with debris, reducing their effectiveness and requiring frequent cleaning.

- High maintenance: Mechanical filters require regular maintenance and cleaning to function effectively, which can be time-consuming and labor-intensive.
- Cost: Mechanical filters can be expensive, especially for larger RAS systems, and the cost of replacement parts and maintenance can add up over time.
- Vulnerability to damage: Mechanical filters are vulnerable to damage from sharp objects or other debris in the water, which can reduce their effectiveness or cause them to fail completely.
- Limited capacity: Mechanical filters have limited capacity and may need to be replaced more frequently in high-volume systems.
- Power outages: Power outages can disrupt the mechanical filter, leading to a breakdown in water quality and potentially harming fish and other aquatic organisms.
- Scalability: Scaling up the mechanical filtration system to accommodate larger RAS systems can be challenging and require significant modifications to the filter design and operation.
- Noise: Mechanical filters can generate noise during operation, which may be distracting or disruptive in some RAS systems.
- Space requirements: Mechanical filters may require a significant amount of space to accommodate their size and operation, which can be challenging in smaller RAS systems.
- Complexity: Mechanical filters can be complex to operate and maintain, requiring specialized knowledge and skills to ensure effective and efficient performance.
- Energy consumption: Mechanical filters consume energy to operate, which can contribute to the overall operating costs of the RAS system.
- Inefficient removal of smaller particles: Mechanical filters may not be effective in removing smaller particles

Mechanical filtration plays an important role in maintaining water quality in RAS systems, it is important to carefully consider its limitations and design the system accordingly to ensure optimal performance (Blancheton, 2000; Dolan et al., 2013; Gichana et al., 2018).

4.2.2. Challanges of biological filtration in RAS

Biological filtration in Recirculating Aquaculture Systems (RAS) has some limitations, including:

- High sensitivity to water quality: Biological filters are very sensitive to water quality and can become easily overloaded or clogged if the water is not properly maintained.
- Need for continuous monitoring: The biological filter requires constant monitoring and maintenance to ensure that it is functioning properly and effectively removing waste.
- Limited capacity: The capacity of the biological filter is limited and can become overwhelmed if the RAS system is overstocked or if water quality deteriorates. Ammonia buildup is a common issue in RAS systems, which can harm fish and other aquatic organisms.
- Fish welfare can be negatively affected, and exposure to stressful situations can increase in RASs due to factors such as high stocking density, chronic exposure to poor water quality and metabolic by-products, which can result from inadequate water treatment technology or inexperienced management.
- Cost: The cost of setting up and maintaining a biological filtration system can be high, especially for larger RAS systems.
- Vulnerability to disease: The biological filter can become contaminated with harmful bacteria or parasites, which can harm fish and other aquatic organisms. The filter must be carefully monitored and treated to prevent disease outbreaks.
- Maintenance and cleaning: The biological filter requires regular maintenance and cleaning to keep it functioning effectively, which can be time-consuming and labor-intensive.
- Power outages: Power outages can disrupt the biological filter, causing a breakdown in the nitrogen cycle and leading to ammonia spikes and other water quality issues.
- pH fluctuations: Sudden changes in pH levels can disrupt the biological filtration process and harm the filter's microbial population.
- Scalability: Scaling up the biological filtration system to accommodate larger RAS systems can be challenging and require significant modifications to the filter design and operation.

It is necessary to effectively monitor and maintain the biological filter for a successful production in RAS. The limitations of the system must be considered by the farmer, and the system should not be overwhelmed (Blancheton, 2000; Dolan et al., 2013; Gichana et al., 2018).

4.2.3. Challanges of chemical filtration in RAS

There are several limitations to the use of chemical filtration in recirculating aquaculture systems (RAS):

- Cost: Chemical filtration methods, such as reverse osmosis and ion exchange, can be expensive to implement and maintain.
- Chemical residuals: Some chemical filtration processes can result in the production of residual chemicals that must be managed and disposed of properly to avoid environmental impact.
- Water chemistry changes: The addition of certain chemicals during the filtration process can alter water chemistry, potentially affecting the health and growth of aquatic animals in the RAS.
- Maintenance requirements: Chemical filtration systems, particularly those using resin beds, can require frequent cleaning and maintenance to maintain their effectiveness.
- Limited removal effectiveness: Chemical filtration may not be effective in removing all dissolved substances, and in some cases, may only partially remove the target substance.
- Complexity: Chemical filtration processes can be complex and require specialized knowledge and expertise to implement and manage effectively.

It is important to consider chemical filtration as one component of a holistic water treatment approach in RAS, rather than relying solely on chemical treatments. It is also important to regularly monitor water quality and adjust treatment processes as needed to ensure optimal water conditions and support the health and growth of aquatic animals in the RAS (Wheaton, 2002; Rurangwa and Verdegem, 2015; Helfrich and Libey, 1991; Sitek, 2020).

4.2.4. Challanges of disease management systems in RAS

There are several limitations to the use of disease management systems in RAS;

- Limited effectiveness against some pathogens: While U.V. systems can be effective in controlling or eliminating some pathogens, they may not be effective against all types of pathogens, particularly those that are resistant to U.V. radiation.
- High power consumption: U.V. systems require a significant amount of energy to operate, which can contribute to higher energy costs for the recirculating aquaculture system.

- Limited penetration of U.V. radiation: The effectiveness of a U.V. system depends on the intensity and duration of the U.V. radiation exposure, which can be reduced by factors such as water quality, the thickness of biofilms or suspended solids in the water.
- Reduced effectiveness over time: The U.V. lamps used in U.V. systems lose their effectiveness over time and need to be replaced periodically to maintain the desired level of U.V. radiation exposure.
- U.V. systems require regular maintenance, including cleaning of the quartz sleeve and replacement of the U.V. lamps. Failure to perform maintenance can result in reduced effectiveness of the U.V. system.
- Initial cost: The initial cost of purchasing and installing a U.V. system can be high, particularly for larger recirculating aquaculture systems.
- Dependency on chemical treatments: Some recirculating aquaculture systems rely heavily on chemical treatments, such as hydrogen peroxide, to manage diseases. However, this can lead to the buildup of residual chemicals in the system, which can have negative impacts on fish health and water quality.
- Lack of skilled personnel: Effective disease management in recirculating aquaculture systems requires specialized knowledge and skills, which may not be readily available in some regions or for all operators.
- Risk of over-treatment: Ozone is a powerful oxidant that can quickly and effectively destroy pathogens, but it can also harm fish if over-applied. Ozone systems require careful monitoring to prevent over-treatment.
- Ozone systems can be expensive to install and maintain, particularly for larger recirculating aquaculture systems.
- Ozone can be less effective in poor water quality conditions, such as low oxygen levels or high levels of organic matter, which can reduce the effectiveness of the ozone treatment.
- Ozone systems require regular maintenance, including cleaning and replacement of ozone generators, to ensure effective operation.
- Formation of harmful byproducts: Ozone can react with organic matter in the water to form harmful byproducts, such as bromate or aldehydes, which can be toxic to fish or other aquatic organisms.

- Safety concerns: Ozone is a highly reactive gas that can be hazardous to human health if not properly managed. Ozone systems require appropriate safety measures to prevent exposure to ozone gas.

A RAS operator should keep these limitations in mind and monitor the system throughout the rearing period for a successful production (Gullian et al., 2011; Sharrer et al., 2005; Sharrer and Summerfelt, 2007; Summerfelt et al., 2004; Gonçalves and Gagnon, 2011)

4.2.5. Challanges of oxygenation in RAS

Maintaining optimal oxygen levels can be challenging in RAS due to several factors, such as changes in water temperature, feeding rate, stocking density, and filtration efficiency. Overfeeding and under-filtering can cause oxygen depletion in the system, and sudden changes in water quality can also affect oxygen levels. Monitoring and controlling oxygen levels in RAS require constant attention and adjustments to maintain optimal conditions for the fish.

5. RAS in Türkiye

In our country, as of the data for the year 2022 of the Ministry of Agriculture and Forestry, there are 2239 licensed aquaculture production facilities. Of these facilities, 997 operate with open-system concrete pools, 914 operate with net cage systems in seas and reservoir lakes, 193 operate with open-system earthen ponds, 54 operate in pond areas, 24 are open/semi-closed tank systems, 20 operate with rope/raft systems, and 9 operate with recirculating aquaculture systems. As can be seen from the data, the number of enterprises operating with RAS represents only 0.4% of the existing production facilities. Shrimp species, trout, tilapia, sparidae species, sea bream, sea bass, Sargoz, Sivriburun karagöz, Karagöz, Minekop, Trança, Granyöz, Eşkina, Sinagrit, Lahoz, ornamental fish and plants are produced in licensed RAS facilities. It is known that there are currently new license applications related to RAS in our country. There are various initiatives for the use of closed circuit systems in the production of new potential species. The most recent example that can be given is the blue crab production facility licensed in 2023 and established in Muğla/Milas. It is also known that studies are being carried out for mussel production in closed circuit systems.

Apart from commercial RAS applications, there are also government and educational RAS units that are used for research and wildlife stocking purposes. These RAS units are primarily used for studying and understanding the biology and ecology of different aquatic species, and for developing sustainable practices for aquaculture (Figure 5.1). The government and educational RAS units are often used for breeding and rearing rare and endangered aquatic species for restocking purposes in their natural habitats.



Figure 5.1. Istanbul University, Faculty of Aquatic Sciences, RAS Research Laboratory and Sapanca Inland Fisheries Production Research and Application Unit, trout hatchery recirculating aquaculture system

6. Conclusion

RAS technology has become an essential tool in aquaculture and has been increasingly used in Türkiye in recent years. Our country's geographic location, diverse aquatic species, government support and significant investment in technology make it an ideal candidate for RAS applications. The use of RAS in Türkiye is expected to continue to grow, and the country's government and industry leaders are taking steps to ensure that Türkiye remains at the forefront of RAS development and implementation.

One of the major inhibiting factors for the growth of RAS in Türkiye is the initial investment required to set up the necessary infrastructure. The high cost of purchasing, installing, and maintaining RAS equipment can be a significant barrier for small and medium-sized aquaculture businesses in Türkiye. In addition, the lack of expertise and skilled labor in the field of RAS can further increase the cost of implementing these technologies. To address these issues, the Turkish government has launched various initiatives to provide financial support and incentives for businesses to invest in RAS technology. However, more needs to be done to ensure that RAS technology is accessible to all aquaculture investors, regardless of their size.

Another inhibiting factor for the growth of RAS in Türkiye is the cost of electricity. Electricity prices in Türkiye have increased significantly in recent years, which can make operating RAS systems expensive. Given that RAS systems typically require large amounts of electricity to operate, the cost of electricity can be a significant factor in determining the viability of implementing RAS technologies. Addressing the cost of electricity will require collaboration between the government and the energy sector to develop sustainable and affordable energy solutions for RAS systems. Additionally, the development of more energy-efficient RAS systems and the use of renewable energy sources can also help to reduce the cost of electricity and make RAS technology more accessible and affordable for aquaculture businesses in Türkiye. With the right approach, RAS technology has the potential to revolutionize aquaculture in Türkiye, improve efficiency, and create new opportunities for economic growth.

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CHAPTER 14

APPLICATION OF IMTA SYSTEMS IN AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.014

1. Introduction

Many technological developments in our world are inspired by nature through bio imitation. Likewise, the development of breeding techniques for living things under controlled conditions began with the imitation of nature. The integrated multi trophic aquaculture approach is also a production plan inspired by the food chain model designed to eliminate energy loss in the environment (Ridler et al., 2007; Chopin, 2013; Zhang et al., 2019). Two or more species (one as primary species and others as extractive species) from different levels in the food chain are farmed together without requirements of additional feed, using the waste products of one species at the other level in an integrated multi-trophic aquaculture (IMTA) system. High income and waste bioremediation are achieved since more than one product is produced in the same system in this way (Barrington et al., 2009; Chary et al., 2020; Mohsen and Yang, 2021; Hossain et al., 2022). Necessary information about the purpose of use of IMTA systems, system design and selection of species to be grown in this system, advantages and disadvantages of the system are presented below. In addition, information is given about the studies, theses and research about the use of IMTA systems in Turkish aquaculture.

2. History and Application of IMTA Systems

IMTA has progressed from fields where rice and fish were farmed together 2000 years ago to the concept of integrated aquaculture introduced in the 1970s. The first term "integrated multi-trophic aquaculture" was used by Thierry Chopin and Shawn Robinson in 2004 (Chopin and Robinson, 2004). although there were references to the use of different trophic levels to reduce nutrient loadings from aquaculture or to increase production in the 1970s. IMTA was practiced in China in the 1980s, particularly in Sanggou Bay without saying IMTA (Fang et al., 2016). In the 1980s, a local salmon producer asked help from the Canadian Department of Fisheries and Oceans to farm mussels in the same aquaculture system, and a study began in the Bay of Fundy on the Canadian east coast. The scientific literature of that day reported that salmon used only 30% of their feed on a dry weight basis, leading to a debate over how to reach the other 70%. Recovering these lost nutrients has been a driving force for IMTA overall (Robinson, 2020). Activities in the Bay of Fundy have provided one of the most studied model systems for the finfish-focused IMTA since 2001, and when combined with sites on the Pacific coast, the benthic IMTA has produced many of the first positive data for the finfish-mussel and seaweed IMTA. Salmon, mussel, macroalgae, sea urchin and sea cucumber were used in these projects (EUMOFA, 2020).

In addition, studies that salmon, mussel and kelp were farmed together were conducted in Norway which is one of the largest salmon producers. As a result, it was observed that the nutritional value of mussel and kelp grew better by removing some of the nitrogen produced by the fish (Bellona Report, 2013). In another IMTA study conducted in Denmark, it was reported that all of the nutrients (88 tons of nitrogen and 9.6 tons of phosphorus) given to the aquatic environment from a farm producing 2105 tons of rainbow trout per year were recovered with 7-9 thousand tons of mussel production (Dolmer and Minnhagen, 2014). In a study conducted in Portugal, *Gracilaria vermiculophylla*, a macroalgae species integrated into the wastewater of a RAS system where sea bass, turbot and sole fish were farmed was reached up to 156 kg dry weight and it was stated that 8.8 kg of nitrogen in the aquatic environment was removed as a result of this production (Abreu et al., 2011). In the same study, it was reported that all of the nitrogen in the environment could be removed by growing the specified macroalgae species in a larger area. In a similar study, it was stated that 20-25 tons of nitrogen in the water was eliminated with 2500 tons of mussel production (Møhlenberg, 2010). In another study in Canada, it was reported that organic and inorganic substances released into the water through salmon feed waste and feces meet the nutritional needs of mussels and sea cucumbers (Wang et al., 2013). In addition, in a study conducted with salmon, it was stated that there is a similarity between the amounts of EPA and DHA contained in fish feces and the amounts obtained from some phytoplankton species (George and Parish, 2013). After 2004, IMTA-related projects started in Norway, either through direct financing or as part of EU-financed projects with Norwegian partners. The main ones are POLYCULT 2004–2006, INTEGRATE 2006–2011, MACROBIOMASS 2010–2012 and MAXIMTA, EXPLOIT and IDREEM, 2012–2016, IMPAQT 2018-2021 (EUMOFA, 2020). Currently, AquaVitae (2019–2023), ASTRAL (2020–2024) projects are being worked on related to this topic (EUMOFA, 2020).

The requirements for IMTA are as follows according to Barrington et al. (2009):

- To determine the economic and environmental value of IMTA systems and their by-products;
- Choosing the right species and available technologies suitable for habitat, environmental and oceanographic conditions;
- Ensuring that species are complementary in terms of ecosystem functions;
- Matching growth rates and achievable biomass for bio mitigation need;
- To promote effective government legislation/regulations and support to facilitate the development of IMTA practices and the commercialization of IMTA products;
- Recognizing the benefits of IMTA and training stakeholders about this practice;
- Supporting research and development (R&D) and business persistence for IMTA.

3. Purpose of Integrated Multi Trophic Systems

Aquaculture has been the fastest growing food production sector in the world with a rate of approximately 4.46% between 2018-2022. It is stated that aquaculture plays a critical role in meeting the protein needs of the increasing world population (FAO, 2022). The total aquaculture and fisheries production of the world was 177.8 million tons in 2020. Aquaculture production is 87.5 million tons including 57.5 million tons of fish, 17.7 million tons of molluscs and 36 million tons of macroalgae are produced. Total sales value is 281.5 billion USD (FAO, 2022). The total aquaculture production is 471.686 tons, 467.048 of which belong to fish and 4585 tons of mollusc production in Türkiye. However, there is no macroalgae culture production in Türkiye yet (TUIK, 2023).

Fish feeds used in aquaculture production contain high levels of nitrogen and phosphorus (Chatvijitkul et al., 2018). Therefore, nitrogen and phosphorus originating from the feeds used in fish farming and deriving from fish feces are released to the environment with the relative subsequent effects. Today, extruded feeds that can be highly metabolized by fish are produced

with the developing feed technology and the loss of feed used in aquaculture is considerably reduced, but it was determined that 41.6 kg of nitrogen and 5.3 kg of phosphorus waste are generated from 1 ton of commercial trout feed in a cage rearing system (Farabi et al., 2020). The feeds that are discarded by excretion or mixed with the water without being consumed create a nutrient load in the water and may cause eutrophication in the long term. At this point, IMTA systems, whose basic principle is to ensure the natural elimination of these substances, become strategic for further development of aquaculture. In fact, IMTA concept represents an alternative approach to achieve sustainable aquaculture (Soto et al., 2007).

Many species including fishes, crustaceans, molluscs, echinoderms and macroalgae are being largely produced all around the world (FAO, 2022). A large part of the fish production is carried out in net cages and the feed that cannot be consumed by the fish during this production is given to the water body together with the fish excrement. Deposit feeders (e.g. sea cucumbers) and filter feeders (e.g. mussels and oysters) which are placed under or next to the fish cages, use the waste as feed by contributing to the purification and bioremediation of the water and the sediment. As well as, macroalgae provide oxygen and remove nutrients from wastes, especially dissolved nitrogen, phosphorus and carbon (Chopin and Robinson, 2004). These productive systems fully fill the requirements of the Blue Growth.

Blue Growth strategy plays a major role in the implementation of the European Green Deal for marine environments (Interreg Europe, 2023). The Green Deal, which entered into force in 2019 by the European Union, offers various action plans to fight the climate crisis. These are mainly targets such as clean energy, sustainable food production, industry, transportation and biodiversity. The ultimate goal is reaching zero carbon emissions by 2050, economic growth apart from resource use, and that no person or region is left out of these developments (European Commission, 2021). IMTA systems are generally recognized as a way to ensure circularity and sustainable practices in aquaculture while maintaining and increasing productivity and the supply of high quality products (Wartenberg et al., 2017; Papageorgiou et al., 2023). It also harmonizes with the European Green Deal, as it eliminates the waste released by fish farming into the environment. On the other hand, Paris Agreement aims to limit the global temperature increase caused by anthropogenic greenhouse gas emissions to below 2 degrees celsius in the long term compared to the pre-industrial period, and for this, national contributions, mitigation, adaptation, loss/damage, financing, technology development and transfer, capacity building, transparency focuses on due diligence issues (United Nations, 2023). Although aquaculture has a very low carbon footprint compared to terrestrial animal production (Tsakiridis et al., 2020), waste feed and feces can cause carbon, nitrogen

and phosphorus to accumulate in water bodies. IMTA systems destroy this accumulation and improve food insecurity, in other words, it is the circular economy model of seafood products (Figure 1).



Figure 1. Green Deal (Loeffler, 2023) and Paris Agreement (United Nations, 2023)

It is believed that invertebrates and algae cultivation can be improved, as well as helping the aquaculture feed industry to seek alternative raw materials thanks to IMTA (Lama et al., 2022). There is potential to include macroalgae, invertebrates or part of multiple chains as alternative raw materials or feed additives to components in fish feed (Hua et al., 2019). Macroalgae to be produced with IMTA systems will help meet the protein needs of the world, and naturally occurring macroalgae can be used in a beneficial way by participating in this production system. It is also believed that IMTA can help increase productivity and employment and provide a more sustainable, circular product that is desirable and at a higher price for consumers (Barrington et al., 2009; Chopin, 2013; Piper et al., 2021). Farming more than one species at the same time increases profitability, and diversification of the products increases the flexibility of enterprises. Integrating low trophic level species in the food chain with other seafood is advantageous not only for nutritional needs, but also for sharing infrastructure and distribution channels and saving space (Barrington et al., 2009; Buck et al., 2018). Conservation up to 90% of the energy loss at each step of the food chain is also possible by IMTA systems (Golley, 1960; Ren et al., 2012).

4. Design of the System and Species Cultivated

Polyculture of rice and fish farming is probably one of the oldest methods, where rice fields provide a habitat for fish and other aquatic life, while fish contribute to the nutrient cycle by feeding on invertebrates and other organic particles produced in nature (Chopin and Sawhney, 2009). However, IMTA is a new system for the western world. Studies on this subject started in the 2000s (EUMOFA, 2020). Net cages are widely used in aquaculture to produce fish in marine and inland systems. The cages have floating pipes structures and nets stretched to

these pipes (Figure 2). Fish held in this net are fed, controlled and harvested when they are large enough. As the fish are kept inside, water changes constantly thus maintaining the high water quality without pumping systems and avoiding waste accumulation (Beveridge, 2004).

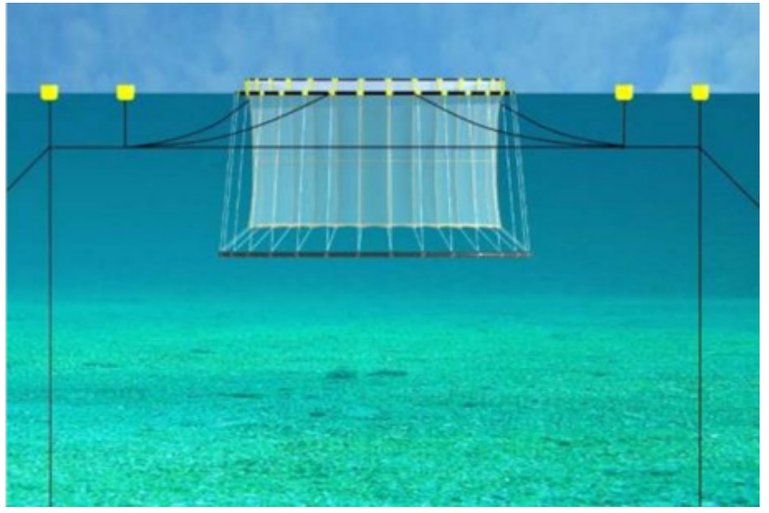

Figure 2. Fish cage (Badinotti, 2023)

Aquatic invertebrates feed on a variety of food including algae, detritus, other invertebrates and even some small vertebrates (Bouchard, 2004). Filter feeders such as mussel, clams etc. are fed by filtering the water they are in (Gosling, 2008), plankton and other microscopic creatures from the water are consumed as food by them (Newell, 2004; Setälä et al., 2014). Deposit feeders (e.g. sea cucumbers) ingest the particles from sediment (Lopez et al., 2022). They can be used as a food product and to prevent water pollution (Lindahl et al., 2005; Oliveira et al., 2011; Li et al., 2019). Macroalgae is another name for seaweed which is divided into groups as green, red or brown (Hurd et al., 2014). These plants can perform photosynthesis, which means they can convert carbon dioxide in water into oxygen. They consume nitrogen and phosphorus found in water as nutrients (Moreira and Pires, 2016). Macroalgae are consumed as food, especially in Far East cuisine, and are also used as feed raw materials, feed additives, fertilizers and biofuels in health and cosmetics fields (Ktari, 2017; Buschmann et al., 2017; Øverland et al., 2019; Biris-Dorhoi et al., 2020; Lourenço-Lopes et al., 2020; Deepika et al., 2022).

In IMTA systems, primarily mussels or oysters are placed under the fish cages or on the downstream side, and macroalgae are placed in the next stage, as well as sea cucumbers, sea

urchins and lobster can be disposed to the bottom (Figure 3). There are different systems for secondary and tertiary species as well as they are usually fixed with ropes (Table 1).

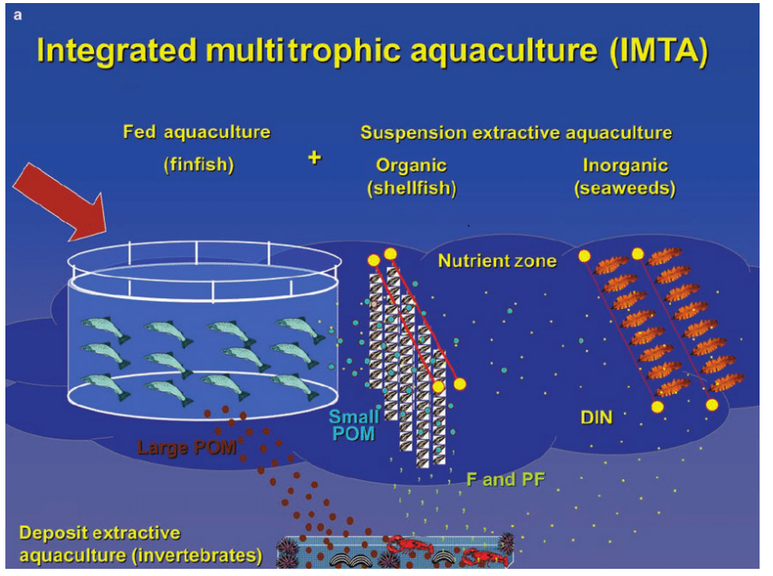

Figure 3. IMTA system (FAO, 2009)

Species of particular interest and high growth potential in IMTA systems in temperate marine waters by Barrington et al. (2009):

Fish: Salmon and trout (Salmonidae), turbot (Scophthalmidae), sea bass (Serranidae, Moronidae), mullet (Mugilidae), cod and haddock (Gadidae), halibut (Hippoglossus), flounder (Pleuronectidae)

Macroalgae: Green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta) macroalgae species

Molluscs: Mussel (Mytilidae), abalone (Haliotidae), pectin and scallop **(**Pectinidae), oyster (Ostreidae)

Echinoderms: Sea urchin (Echinidae), sea cucumber (Phyllophoridae)

Marine worms: Ringworm and bloodworm (Glyceridae)

Crustaceans: Lobster (Homaridae)

Table 1. Orders of alternatice species for IMTA (Barrington et al., 2009)			
1. Stage	**2. Stage**	**3. Stage**	**4. Stage**
Fish	Mussel	Macroalgae	Sea cucumber
Shrimp	Macroalgae	Sea urchin	
Fish	Macroalgae	Sea cucumber	Sea worm
Fish	Lobster		

IMTA systems will be used in at least 2 stages and this number can be increased according to the variety of species required (Figure 4). These species were chosen for their established aquaculture practices, habitat suitability, bio mitigation capability and economic value (Barrington et al., 2009). However, for IMTA systems to be successful, the species to be produced must be local and regulated according to the region.

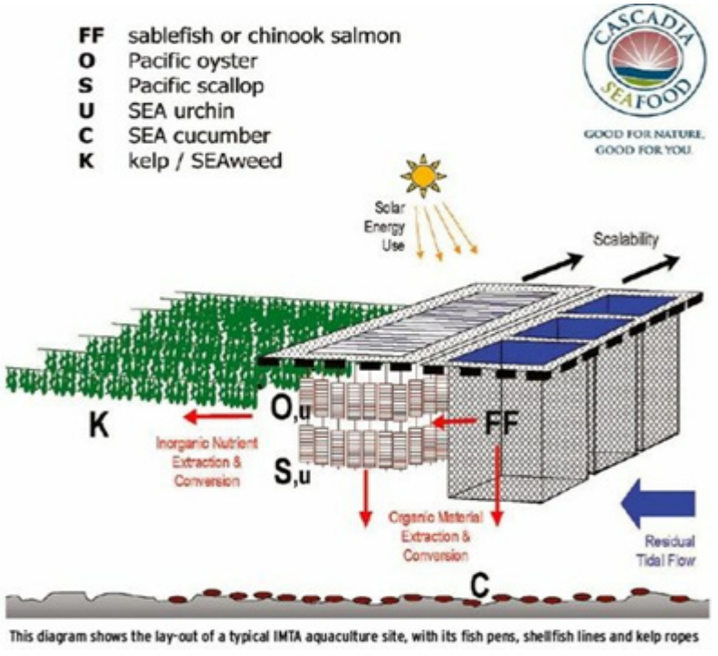

Figure 4. IMTA design (Fishfarmingexpert, 2018)

5. SWOT Analysis for IMTA

SWOT (strengths, weaknesses, opportunities, and threats) analysis can be an effective tool for evaluating the strengths, weaknesses, opportunities, and threats associated with implementing IMTA. One of the strengths of IMTA is its sustainability, which is becoming increasingly important as consumers seek eco-friendly products (Chopin, 2013; Hossain et al., 2022), additionally, IMTA can help to reduce costs by maximizing the use of resources and minimizing waste (Barrington et al., 2009; Chopin, 2013; Qiu et al., 2022). However, one of the weaknesses of IMTA is the technical knowledge and expertise required to successfully implement the system (Buck et al., 2018). There may also be challenges in managing and controlling diseases due to the diversity of species involved (Barrington et al., 2009; Kleitou et al., 2018). In terms of opportunities, the growing demand for sustainable seafood products provides a significant market for IMTA products (Buck et al., 2018; Park et al., 2018). However, there may also be threats such as limited market demand for certain species and competition from other aquaculture methods (Rosa et al., 2020). A SWOT analysis can help to identify these factors and inform decision-making to maximize the benefits and minimize the risks of implementing IMTA (Teoli et al., 2022).

Table 2. SWOT analysis for IMTA (EUMOFA, 2020)	
Strengths • Feed saving • Environment • Public opinion • Economic • More production in limited space • High income • Environmental improvement • Easy to apply to small businesses • In line with international agreements • Fight with food insecurity • Resistance to climate change • Carbon footprint reduction	**Weaknesses** • The need for simultaneous farming of more than one species • To correct the lack of information on different farmed species. • Transforming more durable food webs into more fragile food chains • The need for research to select and scale suitable species • The need to create new legislation and regulations
Opportunities • Bioremediation • Sustainability • Market: pricing, high-value products, packaging, niche opportunities	**Threats** • Low profitability compared to initially established systems • Lack of funds • Lack of regulations • Disease risk • Biosecurity problems

6. Best Practices of IMTA

The most commercially developed country in the use of IMTA systems is China (Fang et al., 2016; Chopin, 2018). On the other hand, Chopin et al. brought this term and issue to the agenda and pioneered the research (Chopin, 2011). However, related projects have been carried out in many European countries such as Norway, United Kingdom, Spain, Italy and Türkiye (Kleitou et al., 2018; EUMOFA, 2020, IMPAQT, 2023).

Saccharina japonica farming in winter and spring, followed by *Gracilaria* sp. farming in summer and autumn is widespread in Sanggou Bay-China (Figure 5). This provides a very

good income for local farmers and helps to improve the environmental conditions. Farmers produce 1.500 tons (dry weight) of seaweed per km², which is estimated at around 40 tons of nitrogen, 5 tons of phosphorus and 500 tons of carbon in this system. Additionally, seaweed and oysters are farmed together in another part of the bay. The news of this "eco-farming" model has spread rapidly over the past decade, thanks to educational programs in northern China (Zhang et al., 2016).



Figure 5. Sanggou Bay macroalgae production, China (Fishsite.com, 2021)

Another example of IMTA in Sanggou Bay consists of small cages containing Japanese sea bass, Gracilaria in longlines, and Pacific oysters in angler nets. According to the results of studies on this combination, fish feces provides 30 percent of the nutrients needed by oysters, while uneaten feed provides an additional 5.6 percent. The oyster production in areas where these nutrients are available was estimated to be 30% larger in meat production than those grown in a monoculture system (Zhang et al., 2018). Abalone, seaweed, and sea cucumbers are also commonly integrated species combinations used in Sanggou Bay, and the last two species are grown in angler nets outside of seaweed longlines. Sea cucumbers remove sediment from abalone nets, improve farming conditions and reduce the farm's environmental impact. Macroalgae which are very low in price will be farmed together with sea cucumber and abalone to increase the farmer's income (Zhang et al., 2018). Finally, with the studies carried out in this bay, it has been determined that the benthic environment is still healthy after 60 years of intensive aquaculture, and the reason for this is IMTA development in the area (Fang et al., 2016).

7. Current State of Türkiye and Suggestions for the Future

The prevalence of cage fish farming in Türkiye lays the groundwork for the development of IMTA. Although mussel farms have become widespread in our country in recent years, macroalgae have not yet started commercial production. However, there are commercial examples where these species are diversified and produced together in IMTA systems around

the world. Intensive fish or shrimp culture is now practiced in many places as integrated units with seaweed and mollusc culture (Samocha et al., 2015; Neori et al., 2017, Chang et al., 2020; Gao et al., 2022). In IMTA systems, components such as algae and molluscs extract nutrients from the waste of commonly farmed species (fish or shrimp). This approach, in addition to being a balanced form of ecosystem management, also prevents environmental impacts from aquaculture. It also opens up opportunities for new valuable seaweed products. Also among the promising candidates of these systems are lobster and sea cucumber (Baltadakis et al., 2020; Grosso et al., 2023).

Studies have been conducted with fish and mussels used together, setting an example for IMTA systems in Türkiye (Ercan, 2009; Şirin, 2012; Yıldız et al., 2013; Kurtay, 2020; Hanif, 2023). This system is not yet widely used in practice in our country. Today, trial studies in which fish, mussels and macroalgae are reared together are being evaluated on a pilot scale PhD project in Istanbul University by Esin Batır and her supervisor Mustafa Yıldız. The growth and nutrient composition of the species employed in this project together and the water quality are being measured. Although these studies are not yet accessible as literature, it is thought that they will be reflected in the literature in a short time.

Although we are still at the beginning when we compare it with the world, this issue is indispensable because it is very important for aquaculture to be sustainable and environmentally friendly. It has been seen that these systems provide benefits everywhere such as RAS, offshore and flow-through systems.

There is also still little information available in Europe compared to China about the relevant interactions, risks and impacts of IMTA to inform the decision-making process. This can cause significant delays and hinder the development of commercial scale systems. Impact of IMTA, commercial-level development on biosecurity and disease management and their potential to improve sustainability in an ecosystem setting are also not fully developed.

Some suggestions are made to ensure the spread of IMTA (Barrington et al., 2009):

- Determination of the economic and environmental value of IMTA systems and by-products.
- Selecting the right species suitable for habitat, current technologies, environmental and oceanographic conditions, complementary to ecosystem functions, capable of achieving a substantial biomass for efficient bioremediation, and which commercialization will not create major barriers.

- Developing effective government legislation/regulations and incentives to facilitate the development of IMTA practices and the commercialization of IMTA products.
- Recognizing the benefits of IMTA and educating stakeholders about this practice.
- Establishing R&D and development continuity for IMTA.

Sharing knowledge and techniques between practitioners and researchers will enable Europe to develop appropriate IMTA systems, while governments will be key to promoting the concept by creating policies and organizing technical training with research institutions.

Since a fish farmer will not have the skills to farm shellfish or seaweed, or vice versa, knowledge transfer or creative co-farming arrangements must be established and negotiations must be made on the selection of the appropriate species to be used to achieve this (Barrington et al., 2009; Zhang et al., 2009), extra challenges associated with the production and processing of new or multiple species, such as drying seaweed or depurating filter feeders should also be considered (Troell, 2009; Chopin, 2013). The value of IMTA systems must be defined and measured to inform stakeholders and consumers about the social, marketing, environmental and economic benefits and to attract investors. The overall costs of IMTA compared to monoculture should also be considered at a level other than simple economic value, while benefits at an ecosystem level should be emphasized in regulation (Knowler et al., 2020). Prioritizing the production of native species and keeping production systems diverse protect aquaculture operators from risks such as disease outbreaks and climate change also allows them to take a more resilient and nature friendly approach as highlighted recently by FAO. Moving away from monoculture and adapting more natural multi-trophic farming systems is a core part of IMTA. IMTA systems are initially suitable for small and medium-sized companies that work closely with local communities and have more autonomy in deciding their own operational practices. Also, traceability and actual pricing will play a valuable role in the coming years because younger generations are more open to sustainability and transparency in their supply chains (Buck et al., 2018).

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PART IV

INNOVATIVE DISEASE CONTROL AND DIAGNOSIS

CHAPTER 15

RESEARCH AND INNOVATIVE APPLICATIONS FOR INFECTIOUS DISEASE CONTROL

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DOI: 10.26650/B/LS32LS24.2024.005.015

1. Introduction

Research and innovative strategies/applications for infectious disease control are explored in this chapter. The first of four sections focus on how “functional feeds” can improve infectious disease resilience in farmed fish. Section two take a closer look at bacteriophages as biological control agents of notable bacterial pathogens. In section three, the topic is experimental biotechnology-based applications for finfish aquaculture based on natural or synthetic antimicrobial peptides (AMPs). The fourth and final section gives an introduction to the important role “omics”-studies now play in bacterial fish pathogen research. For simplicity, the chapter focuses on for Türkiye economically important bacterial fish pathogens and research related to European seabass, gilthead seabream and rainbow trout as key species of Mediterranean and European aquaculture.

2. Functional Feeds for Improved Disease Resilience

Fish feed formulation has been improved by the inclusion of supplements to boost not only feed utilization and growth performance but also infectious disease resilience. Such “functional feeds” include either pro/parabiotics (live/dead bacteria or cell components), various immune-stimulating compounds, inorganic feed supplements or microbial enzymes. The principle is that these supplements modify the gut microbiota of the host to strengthen the

natural defenses against pathogen colonization or boost the immune system response towards targeted infectious agents.

Numerous bacteria and some yeasts have been tested and proposed as suitable probiotics for finfish aquaculture. Probiotics may improve fish growth performance, increase infectious disease resilience, strengthen immune/health status and/or the intestinal epithelial barrier integrity besides modifying the gut microbiota. Suggested mechanisms of action are competitive exclusion through antimicrobial peptide (AMP) production, nutrient/energy competition, adhesion site competition, aiding host nutrition through breakdown of otherwise inaccessible fibres, contribution to de novo synthesis of macro/micronutrients, host immune response stimulation and virulence reduction through for example quorum sensing (QS) inhibition (Zorriehzahra *et al*., 2016). Well-established probiotics include bacteria from genus *Bacillus*. The immune-stimulating potential of two *Bacillus subtilis* spore-forming strains was recently investigated *in vitro* using rainbow trout cell lines (Docando *et al*., 2022). The strain with the best binding capacity to intestinal epithelial cells, *B. subtilus* ABP1, was able to modulate cell proliferation, secretion of IgM and surface expression of MHC II on splenic B-cells. It also induced transcription of inflammation related genes, antimicrobial genes and genes involved in T-cell responses after only a single oral administration. More specifically, an increase in pro-inflammatory cytokines transcription were seen in the gut explants as well as the RTgutGC cell line. An upregulation of antimicrobial peptides cathelicidin and hepcidin were also observed; although only in the RTgutGC cell line. The probiotic ABP1 strain modified transcription levels of genes taking part in the adaptive response. In spleen and kidney, several genes involved in the T-cell response were up-regulated. The up-regulation of il-l4/il-13 transcription in intestinal tissue suggests that these cytokines like others may promote Th2-mediated responses to vaccines (Ranasinghe *et al*., 2014). A single oral administration of *B. subtilis* does not elicit a strong inflammatory response but, rather modulates expression of genes involved in the adaptive response, which seems like a positive trait for an oral adjuvant. Another positive trait is that robust *B. subtilis* spores can be incorporated into fish feed without further protection like encapsulation or refrigeration during storage. The authors concluded that the ABP1 strain has a clear potential for the future design of novel oral vaccination strategies for fish. Another study from the same year, investigated if *B. subtilis* spores can be used as efficient antigen-carriers for oral vaccine delivery (Gonçalves *et al*., 2022). To this end, genetic manipulation was used to display the Vibrio antigenic protein OmpK at the spore surface. Anti-*V. anguillarum* antibodies could be detected in the serum of European seabass juveniles fed diets containing OmpK-carrying spores. The chosen carrier

for spore surface display of OmpK proteins was the crust protein CotY. It was fused both C- and N-terminally to CotY to maximize chances of success in protein display on *B. subtilis* spores. Both oral-vaccination and *V. angullarium* challenge was performed in an experimental recirculating water system (RAS). Experimental fish were first fed a dry pelleted commercial feed, with incorporated CotY-OmpK carrying lyophilized spores, for 1 month. Non-vaccinated and vaccinated fish were thereafter challenged by intraperitoneal injection of *V. anguillarum* DSMZ 21597. Over 7 days, the cumulative mortality of orally vaccinated European seabass was reduced significantly from 40% to 13% ($p < 0.05$). The authors concluded that *B. subtilis* spores can be used effectively as antigen-carriers for oral vaccine delivery in fish.

An earlier study from 2019 investigated how dietary administration of probiotics *Lactobacillus delbrukei* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *Citrobacter farmer* affects rainbow trout (Mohammadian *et al*., 2019). In these probiotic-fed fish, serum lysozyme and complement activities were higher than in controls. Probiotic feeding led to, in some cases, highly significant upregulation of cytokine expression in fish intestines. The higher *in vitro* antagonist activity of the former two probiotics were confirmed through challenge *in vivo*. The relative survival of fish fed probiotics *L. bulgaricus* and *L. acidophilus* was 63.7% and 51.6%, respectively. In contrast, the survival of fish fed *C. farmeri* was similar to the control group fish and only 9.1%. The authors concluded that improved resistance to *L. garvieae* could be explained mainly by increased non-specific immune responses such as serum lysozyme and complement activities. Another functional feed trial evaluated the acute phase response in rainbow trout fed a combination of probiotic *Saccharomyces cerevisiae* and prebiotic mannan-oligosaccharides (Castro-Osses *et al*., 2017). A differentially modulated acute phase protein response was observed with these dietary supplements. One day after bacterial challenge, an increase in HAPT, IL-1β, IL8 and LECT2 gene expression was detected whereas an overexpression of HAPT, SAA, LECT2 and IL-1β was evident under chronic stress caused by high stocking density. The authors concluded that rainbow trout fed the functional feed diet had a faster and acute phase response to bacterial challenge with *V. anguillarum*.

The aim of the next study was to evaluate endemic probiotic bacteria in the nurturing of seabass larvae (Makridis *et al*., 2021). An unidentified *Phaeobacter* sp. isolated from bonito yolk-sac larvae (*Sarda sarda*) inhibited *Vibrio anguillarum in vitro*. The probiotic strain was bioencapsulated in live food and tested in a large-scale trial with seabass larvae (*Dicentrarchus labrax* L.). After 60 days, both the survival and the specific growth rate were significantly higher for fish receiving probiotics ($p < 0.05$). The gut communities of fish fed probiotics were more similar with fewer bacterial phylotypes identified by DGGE analysis. It

was thus speculated that the probiotic *Phaeobacter* inhibited gut colonization of other bacteria. Seabass larvae fed the probiotic showed increased survival ($p < 0.05$) after experimental infection with *Vibrio harveyi*. The authors concluded that the native probiotic strain had an advantageous effect on larval rearing under industry-like conditions. The purpose of another study was to investigate the probiotic potential of *Bacillus velezensis* D-18 isolated from fish farm wastewater (Monzón-Atienza *et al*., 2021). This strain was evaluated *in vitro* through various tests. It was found that the D-18 strain was able to survive a 90-min long exposure to 10% seabass bile but, not a pH below 4. *V. anguillarum* growth was inhibited significantly by 30% ($p < 0.05$) after 24 hours in co-culture with *B. velezensis D-18.* Also, the bacterium's adhesion capacity to mucus was significantly reduced by 60% in the presence of this probiotic D-18 strain. In a *V. anguillarum* challenge trial, the observed survival rate of fish fed the probiotic D-18 strain increased by 43%. The authors therefore considered isolate *B. velezensis* D-18 as a good candidate probiotic for the prevention of *V. anguillarum* infection in European seabass. Besides bacteria, yeast can be used as probiotics in aquaculture. The aim of the next feed trial with gilthead seabream (*Sparus aurata*) was to investigate the probiotic potential and immune-stimulating activity of marine extremophile yeast *Kluyveromyces lactis* M3 (Guluarte *et al*., 2019). Bactericidal activity in skin mucus against the three tested pathogenic bacteria, *Vibrio parahaemolyticus*, *V. harveyi*, and *V. anguillarum*, was significantly higher ($p < 0.05$) in fish fed dietary *K. lactis* M3 (0.55% or 1.1%) for 15 days. The nitric oxide production, peroxidase activity and Concanavalin A (ConA) lectin union levels increased strongly in fish skin mucus as well. But, for peroxidase activity and lectin union, the increase was significant only in fish fed the lowest concentration of the putative probiotic yeast. Taken together, the results suggested that *K. lactis* M3 had an immunostimulating activity and resulted in high antioxidant capability in the fish host and the authors thus considered it suitable probiotic candidate for finfish aquaculture.

Already two decades ago, a pioneering Turkish study was undertaken to investigate the effects of dietary plant extract intake on the immune status of rainbow trout (Karatas Dügenci *et al*., 2003). After three weeks of feeding, it was found that extracts from mistletoe (*Viscum album*), nettle (*Urtica dioica*) and ginger (*Zingiber officinale*) caused an enhanced extracellular respiratory burst activity ($p < 0.001$) in experimental fish. Particularly rainbow trout fed the diet containing 1% ginger root extract exhibited a non-specific but significant immune response. Both phagocytosis and the extracellular burst activity of blood leukocytes were significantly higher than in control group fish. All dietary plant extracts increased the total plasma protein level with the exception of 0.1% dietary ginger extract. Nonetheless, the

highest level of plasma proteins was observed in fish fed 1% dietary ginger. Since then, a wide spectrum of other phytogenic compounds/extracts has been studied experimentally. However, only a few recent examples will be provided herein as a comprehensive review of this research area is outside the scope of this book chapter. One study demonstrated that dietary administration of dihydroquercetin (0.1%) from the medical plant deodar (*Cedrus deodara*) significantly increased phagocytosis, respiratory burst, IgM level, complement, antiprotease and bactericidal activities in gilthead seabream (Awad *et al*., 2015). Another study reported a significant increase in immune parameters, principally in gilthead seabream fed only fenugreek seeds or combined with probiotic *Bacillus subtilis* (Bahi *et al*., 2017). Moreover, qPCR showed that dietary supplementation boosts significantly the head-kidney expression of immune-associated genes, especially IgM gene expression. Gilthead seabream fed lemon peel (1.5% and 3%) enriched diets for 15 days exhibited better growth, humoral (serum IgM) and cellular (leucocyte peroxidase activity/phagocytosis in head kidney) immunity (Garcia Beltrán *et al*., 2017). This supplemented diet also modified the expression of some immune-related genes. However, no notable changes to the antioxidant status of fish was noted and decreased growth promotion was observed after thirty days. Gilthead seabream fed diets enriched with oregano (0.5% and 1%) had enhanced humoral (serum protease activity and skin mucus IgM/bactericidal activity) and cellular (leucocyte phagocytosis in head kidney) immunity after two weeks but also one month (Garcia Beltrán *et al*., 2020). In contrast, supplemented oregano did not trigger any growth promoting effect or rise in total skin mucus/serum antioxidant activity. It was demonstrated that dietary ribwort plantain (*Plantago lanceolata*) is immunostimulatory as myeloperoxidase activities and the oxidative radical production were enhanced in rainbow trout after 3 months (Elbesthi *et al*., 2020). The authors concluded that ribwort plantain can be used as a growth promoter and immunostimulant in rainbow trout farming. The next study examined the dietary intake of *Jasonia glutinosa* (rock-tea) and its effect on the oxidative and immune status of gilthead seabream (Espinosa *et al*., 2020). It was found that the immunostimulant effect of dietary rock-tea is evident after 15 days of treatment but, not after 30 days. However, the antioxidant protection was maintained after 30 days. The effect of an aqueous methanolic extract of thin-skinned plum (*Prunus domestica*) on growth, immune responses and *Yersinia ruckeri* resistance was recently studied in rainbow trout (Terzi *et al*., 2021). Elevated respiratory burst and potential bacterial killing activity was observed on the 7th day in fish fed 1% plum extract. Simultaneously, expression of interleukin and COX-2 genes was elevated in the kidney and intestine of experimental fish. After two weeks, increased myeloperoxidase activity was recorded. However, no difference in lysozyme activity or histological changes in organs were observed in fish fed the plum extract containing diet. A significantly

increased survival rate ($p < 0.05$) of fish from all plum extract-fed groups was observed after challenge with *Y. ruckeri*. The dietary supplement could thus, according to the authors, strengthen innate immunity and improve resilience against *Y. ruckeri* infection. A limitation of several mentioned studies is that they fail to identify/isolate the bioactive compound of the studied plant extracts or to reveal the underlying "mechanism of action" for such phytogenic compounds. But, one review suggests that the mucosal immune-related responses of bioactive compounds from plants are mediated by activation of TRP cation channels in both immune and epithelial cells of mucosal tissues (Firmino *et al*., 2021). These compounds may activate such channels leading to increased intracellular Ca^{2+}, and therefore to the modulation of pro/anti-inflammatory cytokines expression as well as the activation of signalling pathways and antioxidative enzymes.

Other biological immunostimulants have been considered for aquaculture use. For example, it was tested whether IgM in blood from slaughtered rainbow trout could be a functional feed supplement able to confer protection against infectious disease (Chettri *et al*., 2019). For this purpose, purified serum IgM from *Yersinia ruckeri* vaccinated rainbow trout was encapsulated in alginate microparticles and top-coated on fish feed. But, the IgM containing diet did not significantly enhanced survival of fish challenged with *Y. ruckeri.* It should however be mentioned that some protection (10%) was observed for the fish group fed the highest IgM dose (400 μg/fish/day). The authors thus speculated that elevated IgM levels at bacterial entry sites (stomach, intestine and anal opening) could prevent invasion or inactivate pathogens at the intestinal wall or in the lumen. Spray-dried plasma (SDP) from porcine blood has also been evaluated in the growing diets for gilthead sea bream fingerlings (Gisbert *et al*., 2015). Results indicated that dietary SDP promoted fish growth as fish fed 3% SDP on average were 10.5% heavier ($p < 0.05$) than control diet fish. It was also found that SDP improved the antioxidative defences in the fish intestine ($p < 0.05$) and increased the density of goblet cells ($p < 0.05$). Furthermore, the nonspecific serum immune response was greater ($p < 0.05$) in fish fed a SDP containing diet. It was thus concluded that inclusion of SDP in feed likely would be beneficial for farmed fish. European seabass fed AviPlus® Aqua, containing a blend of organic acids and nature identical compounds thymol and vanillin, experienced an upregulation in pro/anti-inflammatory cytokines (Busti *et al*., 2020). While the said product lead to an increase in gut microbiota diversity and increased abundance of beneficial lactic acid bacteria in the mentioned study, it did neither affect gene-regulation nor gut microbiota diversity/composition in rainbow trout (Pelusio *et al*., 2020). Methionine can be a limiting amino acid when fish meal has been replaced with plant protein in fish diets. For this reason, the effect of methionine-supplemented

diets on immune responses has been studied in European seabass (Machado *et al*., 2020). After two weeks, diets supplemented with methionine led to changes in the expression of among others leucocyte-related genes in examined fish. But the presumed immune-enhancing role of methionine became more evident after 12 weeks with an increased neutrophil density and decreased expression of apoptotic genes. Although the plant-protein diet supplemented with 0.22% L-methionine had a similar content of this amino acid as the fish-meal diet, the former diet reduced expression of several immune-related genes. According to the authors, this indicates that the methionine requirement for an optimal immune status could be higher when farmed seabass are fed a plant protein-based diet. Another study evaluated the administration of two exopolysaccharides (EPS), derived from putative probiotic strains, and their possible effect on the immune/antioxidant status of European sea bass larvae (Mahdhi *et al*., 2020). Expression levels of scavenging enzymes (Cat, Sod, Gr) genes in the liver and immune-relevant genes (infg, il1b, il8, il6 and tcr-β) in the head kidney/gut were modulated. The fatty acid profiles of European sea bass larvae were not affected by EPS administration. The authors concluded that the tested EPS can enhance immune responses without negative effects on the biochemical composition of fish and thus considered them as natural functional aquafeed ingredients for European sea bass.

The last examples in this section involve commercially available feed supplements. Supplements based on a coconut fatty acid distillate (DICOSAN®) and a probiotic *Bacillus amyloliquefaciens* strain were evaluated for use in the farming of gilthead sea bream (Simó-Mirabet *et al*., 2017). MCFAs in the form of a coconut oil increased the nutrient absorption surface and feed intake promoting anabolic action on the somatotropic axis and thus growth rates. The *Bacillus* probiotic, on the other hand, induced anti-oxidant/anti-inflammatory effects with changes to circulating cortisol and IgM levels, leukocyte respiratory burst, mucosal expression levels of cytokines, lymphocyte markers and membrane IgT in posterior intestine. The immune-modifying property of the commercial aquafeed Protec™, containing glucans, zinc, vitamin C and E, was tested in a recent rainbow trout trial (Bulfon *et al*., 2019). This functional feed increased notably the synthesis of specific IgM against *Lactococcus garvieae* and the respiratory burst activity of leukocytes after intraperitoneal vaccination. On the other hand, the serum lysozyme activity remained unaffected. Therefore, these authors concluded that Protec™ is able to improve both the rainbow trout innate and adaptive immune response. The stress- and immuno-modulating abillity of dietary β-glucans (commersial product Macrogard®) in lymphoid organs from healthy and *Aeromonas hydrophila*-infected rainbow trout was evaluated in the next study (Douxfils *et al*., 2017). It was found that the spleen is highly

responsive to dietary β-glucans both in infected and healthy fish and thus notably contribute to the reinforced immunity. However, to high doses of β-glucans as well as prolonged use could lead to a non-responsive physiological status and, as a result, a poor immune response. A more recent study comparing β-glucans from wild type and null-mutant (Gas1) baker's yeast (*Saccharomyces cerevisiae*) against the commercial product MacroGard® suggest that their efficiency may vary according to their origin and structure (Cornet *et al*., 2021). The commercial product MacroGard® performed well after two weeks. On day 36 however, Gas1 β-glucan increased the Ig proportion, lysozyme activity and expression of some immune genes (hepcidin and mcsfra) compared to MacroGard®. Thus the resistance of rainbow trout juveniles to *A. salmonicida achromogenes* infection was increased as highlighted by better survival rate two weeks' post-challenge. These authors concluded that Gas1 β-glucan could prevent diseases in aquaculture more efficiently than other β -glucans in current use.

In conclusion, several putative beneficial bacteria, some yeasts, numerous phytochemicals and secondary metabolites/bioactive compounds from various plants/organisms have been studied for their beneficial effects on infectious disease resilience and thus fish health. But, relatively few of them have made it into commercial products and daily use in finfish aquaculture. Also, despite intensive research, several feed trials fail to isolate the bioactive compound or to identify the exact underlying "mechanism of action" for the studied feed supplement.

3. Bacteriophages as Biological Control Agents

Despite vaccination and/or pharmaceutical/antibiotic treatment, infectious diseases and parasite infestations still cause notable economic losses in global aquaculture. Alternatives are warranted due to concerns related to widespread chemical drug and antibiotic resistance, but also lack of effective vaccines against local strains of important bacterial pathogens. Pharmaceuticals have for example been used against lice infection in aquaculture, but they pollute surrounding areas, harm wild crustaceans and have led to the widespread presence of multi-resistant salmon lice (Fjørtoft *et al*., 2021). Since 2010, a popular alternative strategy for biological control have been cleaner fish, lumpfish (*Cyclopterus lumpus*) and species of wrasse (family Labridae), used to deal with sea lice infestations in farmed Atlantic salmon (Brooker *et al*., 2018). Despite their success, concerns have been raised related to cleaner fish welfare and their likely role as carriers/reservoir of salmon pathogens in aquaculture (Gulla *et al*., 2016; Scholz *et al*., 2018). This section however focuses on the use of bacteriophages as biological agents to prevent/control bacterial fish disease. Currently, there are only a couple of registered phage products for use in fish farming. ACD Pharmaceuticals is a Norwegian company that has specialized in such applications and launched the world's first licensed phage

product CUSTUS™ for aquaculture use in 2018. A second example is the "phage cocktail" product BAFADOR® (Schulz *et al*., 2019).

Bacteriophages (or simply phages) represent a sustainable alternative to antibiotics in that they specifically target disease-causing bacteria and are harmless to farmed fish, the environment and consumers of fish products. They are, for example, being isolated for potential use in shrimp and Pacific oyster farming against several problematic bacteria (Kim *et al*., 2019; Richards *et al*., 2021; Stalin and Srinivasan, 2017). Tailed phages (order Caudovirales) attach better to bacterial receptors, so they are preferable for this type of application. Furthermore, the multiplicity of infection (MOI) is an important parameter critical for the efficacy of phage applications. Good phage candidates should be able to propagate efficiently both *in vivo* and *in vitro* as the latter is essential for large scale production. Propagation under *in vitro* conditions can be studied in growth curve experiments. Collected data allow calculation of valuable parameters including burst size and latent period duration, as well as the modeling of production variables for large-scale bacteriophage production. A large burst size and a very short latent period is crucial in order to develop an efficient application. Good phage candidates have a narrow but, not to narrow bacterial host range. This would ensure that the microbiomes of aquaculture environments and the farmed fish are left as undisturbed as possible. In turn, this could reduce the risk of secondary infections and likely lead to faster host recovery. If the candidate passes the mentioned criteria, later characterization would include determination of phage particle stability. Produced viral particles must be durable to withstand storage as well as administration to reach their intended target bacteria in efficient titres.

The genomes of phage candidates must be sequenced to make sure that they are safe and harmless. In particular, only strictly lytic phages are applicable, as they always lead to lysis of bacteria. Genetic characterization should identify presence/absence of important determinants including lysogenic cycle genes, virulence genes, toxin genes, modified nucleotide biosynthesis genes, DNA-dependent RNA polymerases, endonuclease genes and antibiotic resistance genes (Fernandez *et al*., 2019). Phage genome analysis can verify the lack of genes involved in lysogeny, such as integrases, the lytic cycle repressor and site-specific recombinases. Endonuclease genes in a phage genome would suggest that the isolate is not a "super spreader" (Keen *et al*., 2017). Such phages facilitate the release of intact bacterial gDNA to the environment that can be taken up by other naturally competent bacteria. Therefore, it is important to identify if phages degrade bacterial chromosomes prior to lysis. In addition, genomic analysis is a reliable way to determine whether the phage genome encodes undesirable toxins or antimicrobial resistance determinants. In that case, the

phage should never be considered for this application. The identification of genes involved in the biosynthesis of modified nucleotides in a phage genome would suggest that the viral DNA is resistant to the activity of certain bacterial restriction endonucleases. Selection of phages harbouring modified nucleotides in their genomes may limit the chance of bacterial pathogens being or becoming resistant to the applied phage. A major concern related to such applications for aquaculture is development of phage resistance in bacteria through different mechanisms including prevention of phage adsorption, blocking DNA entry, abortive infection and degradation of viral DNA by CRISPR/Cas and restriction-modification systems (Labrie *et al*., 2010). However, research has indicated that phage resistance may come at a high fitness cost leading to reduced virulence for example in the fish pathogen *Flavobacterium columnare* (Laanto *et al*., 2012). Mutations in bacterial genes for receptors may prevent phage absorption but, simultaneously affect bacterial virulence as such surface proteins are involved in adherence of pathogenic bacteria to fish host cells. An easy option to reduce the risk of acquired resistance is "phage cocktails" like the BAFADOR®-product which contains a mix of 7 different phages that was found to limit mortality of rainbow trout after mixed experimental infection with *A. hydrophila* and *Pseudomonas flourescens* (Schulz *et al*., 2019).

In theory, recirculating aquaculture systems (RASs) would be an optimal environment for a bacteriophage application as the majority of water is recycled. A recent paper investigated the fate of a *F. columnare*-infecting phage FCL-2 in a RAS farm with rainbow trout (Almeida *et al*., 2019). The phage titer dropped fast within the first few days but, persisted in the aeration unit for two weeks. In rearing water, rainbow trout mucus and bioreactor carrier media from the fixed/moving bed biofilters, they were still detectable after three weeks. Higher phage titers in filter carrier media suggested that present bacterial biofilm may influence phage detainment. The authors speculated that unspecific interactions with biofilm exopolysaccharides or the presence of some bacterial host in the biofilter microbiome was the reason for this observation; although *F. columnare* were not detected during the experiment. Importantly, there were no indications that phage addition influenced water quality or fish health, demonstrating it is a safe and potent strategy for maintaining biosecurity in RAS systems. The same phage FCL-2 had previously been shown to protect rainbow trout (*Oncorhynchus mykiss*) fingerlings against *F. columnare* infection under controlled experimental conditions mimicking real conditions at a fish farm (Laanto *et al*., 2015). Only a single administration of phages into the water in a flow-through tank system rescued the rainbow trout population. The survival increased significantly from 8.3% in the untreated control group to 50% in the experimental group after phage administration (MOI 1) to the water. Promising results indicated that the

phage can persist and replicate despite water flow as long as host bacteria are present in this simulated farm environment as FCL-2 titers remained stable for at least 6 weeks.

Bacteriophages would also likely be efficient biocontrol agents in hatchery environments during the early stages of fish production. Another study established an infection bath challenge to investigate the potential of two bacteriophages to control *Flavobacterium psychrophilum* colonization of rainbow trout (*Oncorhynchus mykiss*) eyed eggs to prevent post-swim up fry mortality (Donati *et al*., 2021). It was found that these phages do not negatively affect the eyed eggs survival or do not strongly adhere to their surface either. Notably, they lead to a 12 to 15-fold reduction in eyed egg-associated bacteria for 24 hours. While FpV4 phages disappeared after 1 day, it was possible to detect FPSV-D22 phages for 68 hours post-infection. It should be noted that this experiment only explored short-term disinfection efficiency. It was thus concluded that further studies are needed to determine if bacteriophage control of contaminating bacteria can be maintained for a longer period. Phages could also be used to reduce the load of pathogenic bacteria in aquaculture live feeds like Artemia and rotifers. It has for example been shown that rotifers used in fish larviculture have a high bacterial load and that they can be a major source of bacteria introduced into rearing tanks at hatcheries (Turgay *et al*., 2020). A recent paper isolated and characterized two lytic phages against *Vibrio alginolyticus* and investigated if they had potential as biological control agents of this bacterium in aquaculture live feeds (Kalatzis *et al*., 2016). Both Myoviridae phages had the same host range including several *V. alginolyticus* strains, one strain of *V. harveyi* and *V. parahaemolyticus*, respectively. However, they were unable to infect tested *V. anguillarum*, *V. ordalii*, *V. owensii* and *V. splendidus* strains. In vivo administration of the two component phage cocktail (φSt2 and φGrn1) directly on live prey *Artemia salina* cultures at MOI = 100, led to a 93% reduction in viable vibros after 4 hours. Both φSt2 and φGrn1 have short latency times (about 30 min), but differ notably in their burst sizes (97 and 44 virions/cell, respectively). The authors thus considered them good candidates for a phage application and concluded that administration of φSt2 and φGrn1 to live feed Artemia-cultures could selectively reduce *Vibrio*-load in fish hatcheries and thus in theory minimalize the risk of disease outbreaks.

The method of delivery is important for a successful outcome of bacteriophage applications in aquaculture. A recent study tested three phage delivery methods. Bath submersion, phage-coated material and oral administration via feed were experimentally tested to prevent/treat *Flavobacterium columnare* infections in rainbow trout fry (Kunttu *et al*., 2021). In the flow-through experiment that resembled a real-world situation, phage bath submersion (MOI 10) significantly reduced fish mortality with the greatest effect observed when treatment

coincided with first symptoms of disease. While water administration of phages is conceptually simple, it could be impractical in certain commercial-scale aquaculture environments. Therefore, phage-coated plastic sheets and fish feeds were tested. Although less effective, phage-coated plastic sheets delayed fish mortality significantly in the flow-through system. This result suggests that immobilisation of phages on for example biofilters, could be an efficient way to reduce load of pathogens in inlet and tank water at flow-through fish farms. Prophylactic oral administration of phage-coated feed did not have a notable effect on fish mortality likely due to the external nature of columnaris disease. Viral particles could only be detected in skin mucus, kidney and intestine in some fish after 7-days and transmission of phages through the gut appeared inefficient. Finally, *F. columnare* phages retained their infectivity for at least four months in Shieh medium and autoclaved lake water.

In summary, few of the studied bacteriophages against fish pathogenic bacteria have so far made it into common use in aquaculture. This is mostly due to unresolved issues concerning their practical use in aquaculture environments. As such, phage applications are obviously most suitable for closed/semi-closed aquaculture systems. More research is required to identify the optimal phage dose and delivery method which will be critical for success of both preventive and treatment approaches. Also, correct timing is everything for phage applications and may require new or improved methodology for early detection or constant monitoring of bacterial pathogens and infection pressure in aquaculture environments.

4. Biotechnology-Based Applications for Finfish Aquaculture

Fish vaccines have been on the market since the early 1990s and represented a paradigm shift for the aquaculture industry that made producers less reliant on antibiotics to fight bacterial disease. However, despite the overwhelming success of vaccination, farmed fish remain susceptible to disease as they are often kept in semi-closed systems at high stocking density. Furthermore, commercially available fish vaccines may be inefficient against local strains/variants of fish pathogenic bacteria and the only viable option may thus be antibiotic treatment. The latest generation of fish single/multi-component and DNA/RNA vaccines is developed and provided to the aquaculture sector using biotechnology and large-scale production techniques. However, most recent DNA/RNA vaccines are against viral agents as bacterial pathogens usually are easy to culture. Well-proven effective vaccines can therefore be developed and produced at a fraction of the cost of more technologically advanced vaccines. Novel researched strategies to fight infectious disease in aquaculture includes among others RNA-interference against viral disease in shrimp farming (Gong and Zhang, 2021), application of aptamer technology in aquaculture biosecurity (Chong and Low, 2019)

and silver nanoparticles with antibacterial activities against fish pathogens like *Aeromonas salmonicida* (Shaalan *et al*., 2018). However, this section will consider another promising biotechnology-related research area; namely the use of natural or synthetic antimicrobial peptides to fight infectious disease in aquaculture.

Antimicrobial peptides (AMPs) are small natural host defence proteins produced by a wide range of multicellular organisms including fish and even some of their commensal bacteria. They are an integral part of the host innate/mucosal immunity system and participate in the chemical/physical barrier that represent the first line of defence against infection. AMPs execute their antimicrobial properties through many “modes of action” that roughly can be grouped into two categories; either destabilization of plasma membranes or action against specific intracellular targets. For instant, AMPs can be inhibitors of nucleic acid synthesis/metabolism, protein biosynthesis/metabolism, proteases, protein folding chaperones, cell wall biosynthesis and cell division (Le *et al*., 2017).

AMPs have several advantages over traditional antibiotics. They have a broader range of antimicrobial activity and may also be anti-fungal and anti-viral. Secondly, AMPs are generally more bio-degradable and thus less likely to persist in the environment. On the other hand, precautions should be taken as bacteria can evolve resistance to AMPs. Bacterial resistance mechanisms against AMPs include repulsion by cell surface and/or plasma membrane modifications, interception by competitive binding to high-affinity extracellular proteins, removal or secretion by efflux pumps or proteolytic inactivation/destruction by enzymes (Joo *et al*., 2016). Fortunately, the pharmacodynamics of AMPs are much more favourable than for conventional antibiotics (Lazzaro *et al*., 2020; Yu *et al*., 2018). AMPs kill much faster than antibiotics allowing less time for resistance to evolve. But even more important, the concentration range in which resistance can evolve is smaller for AMPs than for antibiotics. It implies that the mutant selection window where viable bacteria are able to evolve resistance is very narrow. It has also been suggested that synergism between different AMPs may be used to reduce the risk of resistance evolving (Yu *et al*., 2016). But, the use of AMPs in aquaculture still face some unsolved challenges. Despite intensive research into efficient production methods for AMPs, limitations including degradation, low yield and loss of activity persists (Deo *et al*., 2022). To date, no standardized method can reliably provide the high yields required for a practical low-cost aquaculture application. Another challenge is solving the intrinsic limitations of antimicrobial peptides (AMPs), especially their susceptibility to digestion by proteolytic enzymes in the host gastro-intestinal tract that affects their bioavailability. For this reason, more research is needed into strategies for enhanced proteolytic stability of AMPs (Lai *et al*., 2022)

as well as their robust oral delivery/administration (Gent *et al*., 2021; Wang *et al*., 2021) before they can be used efficiently in veterinary and human medicine.

A study from 2016 aimed to investigate the antimicrobial activity of four synthetic variants of hepcidin against *Vibrio anguillarum* as well as the ability of the most effective peptide to protect European Sea bass against infection with this bacterium (Álvarez *et al*., 2016). After intraperitoneal injection, this synthetic hepcidin reached internal organs such as intestine, head kidney, spleen and liver. No cytotoxic effects were observed. Administration of the most potent hepcidin lowered significantly the accumulated mortality (23.5%), compared to fish in the control group (72.5%), over a three-week period. Thus, the authors concluded that synthetic hepcidin have a clear potential as a therapeutic application in aquaculture. Another publication explored the effects of a hepcidin-like protein as a dietary supplement in grouper (*Epinephelus lanceolatus*) culture (Ting *et al*., 2019). This recombinant tilapia hepcidin rTH2-3 was thermostable and exhibited broad-spectrum antimicrobial activity. Examined fish showed enhanced superoxide dismutase (SOD) activity after rTH2-3 feeding. It was also found that the recombinant hepcidin rTH2-3 increased microbial gut diversity. In *Vibrio alginolyticus* challenged grouper, differential regulation of several immune-related genes was observed in the liver and spleen. These were genes associated with the major histocompatibility complex (Mhc), antimicrobial/pro-inflammatory functions and complement system factors. Taken together, overall immunity appeared to be improved. The authors concluded that long-term supplementation with rTH2-3 likely would be beneficial for farmed grouper through microbial gut community and immuno-modulation.

As mentioned, AMPs may have both anti-bacterial and anti-fungal activities. An example from literature is the synthetic short and compositionally simple peptide, KK16 (Bhat *et al*., 2022). KK16 has high affinity towards two virulence proteins, namely the outer membrane protein (omp) and a aerolysin of *Aeromonas sobria*, according to a molecular docking experiment. The antimicrobial activity of the peptide, which was synthesised using Fmoc-chemistry, was evaluated *in vitro* against amongst others *A. salmonicida*, *A. sobria*, *Edwardsiella tarda*, *A. hydrophila*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The antimicrobial KK16 showed potent activity against these pathogens with MICs and MBCs, ranging from 7.81 to 500 μM, and 15 to 900 μM, respectively. Moreover, the peptide retained its activity in presence of salt/serum and was also stable at higher temperatures. Even at higher concentrations, the synthetic peptide displayed little cytotoxic and haemolytic activity. KK16 may interfere with replication as a peptide-DNA binding assay revealed that it was capable of binding bacterial gDNA. Fluorescent microscopy observed uptake of propidium iodide by treated bacterial cells, indicated that the antimicrobial peptide likely has membrane disruptive activity. In an *in vitro* experiment with embryonated fish eggs, the KK16 peptide completely inhibited the growth of fungus *Saprolegnia parasitica* at peptide concentrations ≥ 30 μM. In summary, the stable KK16 peptide has potent antibacterial and antifungal activity with little cytotoxic effect on host cells. Hence, the authors concluded that it is a promising anti-infective agent for combating both common bacterial and fungal infections in aquaculture.

Epinecidin-1 is an AMP that originate from the orange-spotted grouper (Chee *et al*., 2019). Synthetic Epi-1 has several pharmacological activities counting immunomodulatory, anti-cancer/microbial not to mention wound healing properties. The synthetic 25-mer Epi-1 was highly active, with minimum bactericidal concentrations (MBCs) < 2μM, against several Gram-negative bacteria including *Vibrio alginolyticus, Pasturella multocida*, *Morganella morganii*, *Aeromonas sobrio*, *Aeromanas hydrophila* and *Flavobacterium meningosepticum*. The peptide was however less effective against *Vibrio vulnificus* and *Pseudomonas fluorescens* with MBC values of 4.19 μM and 67.04μM, respectively. A study from 2007 showed that Epi-1 peptide administration was effective in promoting a significant increase in fish survival after experimental *Vibrio vulnificus* infection in tilapia (*Oreochromis mossambicus*) and grouper (Pan *et al*., 2007). Very few AMPs from topmouth culter (*Erythroculter ilishaeformis*) have been characterized. In a recent study, an AMP belonging to the liver-expressed antimicrobial peptide 2 (LEAP-2) family, was isolated from the head kidney of topmouth culter (Chen *et al*., 2020). The topmouth culter derived LEAP-2 peptide inhibited growth of aquatic bacteria, including antibiotic-resistant strains, with MIC values ranging from 18.75 to 150 μg/ml. It was lethal for ampicillin-resistant *A. hydrophila* and took less than 60 min to kill this bacterium at a concentration of 5 × MIC. In a SYTOX green stain uptake assay, it was observed with scanning electron microscopy (SEM) that the AMP impaired the integrity of bacterial membrane by eliciting pore formation. Inducible drug resistance was not detected in test bacteria. But interestingly, the AMP efficiently delayed the occurrence of ampicillin drug resistance in a sensitive strain of *Vibrio parahaemolyticus* and sensitized ampicillin-resistant bacteria to ampicillin. A chequerboard assay revealed that topmouth culter LEAP-2 worked in synergy with ampicillin. In other words, the AMP showed great promise for use in combination with antibiotics. Topmouth culter LEAP-2 boosted as expected the therapeutic effect of ampicillin *in vivo* and thus lessened significantly ampicillin-resistant *A. hydrophila* infection. The authors concluded that the LEAP-2 family AMP is a promising alternative to antibiotics with a potential for application against antibiotic-resistant bacterial infection in aquaculture industry.

European sea bass (*Dicentrarchus labrax* L.) is a key species in marine aquaculture, but highly susceptible to nodavirus (NNV) infection that can lead to high mortality rates in fish larvae and juvenile stages. A present-day study thus aimed to assess if AMPs exert immunomodulatory preventive actions in sea bass against this virus (Cervera *et al*., 2023). Sea bass dicentracin (pDIC), betadefensin (pDB1), hepcidin (pHAMP2) or NK-lysin (pNKL) encoding plasmids were conctructed to this end and injected intramuscularly into sea bass juveniles to investigate the function of these AMPs. Neutrophilic granulocytes were recruited to the site of injection and an immune-related gene analysis revealed a strong activation of the inflammatory response. But, hepcidin (pHAMP2) plasmid-expression negatively affected NNV-infected fish and an increase in mortality was observed. It was concluded that although these plasmid-encoded AMPs apparently had immune-stimulatory effects on European sea bass, they failed to improve resistance to NNV.

A study from 2020, *in vitro* tested synthetic peptides including European sea bass Dicentracin (Dic), NK-lysin peptides (NKLPs) and frog Caerin1.1 as antimicrobial agents for aquaculture (León *et al*., 2020). Results showed that the highest bactericidal activity against fish and human pathogens was obtained with Caerin1.1 followed by sea bass Dic. NK-lysin peptides were the least potent as sea bass NKLP20.2 had negligible activity. *Aeromonas salmonicida* was unexpectedly unaffected by all tested fish peptides. The synthetic peptides were able to inhibit infection by some of the most devastating viruses in aquaculture such as nodavirus (NNV), viral septicaemia haemorrhagic virus (VHSV), infectious pancreatic necrosis virus (IPNV) and spring viremia carp virus (SVCV). But, their effectiveness was highly dependent on virus type. Overall, IPNV was the most resistant as Caeerin1.1 and sea bass NKLP20.2 were unable to reduce its titre whereas other peptides tested reduced the titre by 43 - 78%. It was concluded that synthetic peptides, at least *in vitro*, have great antibacterial and antiviral activity against important fish pathogens and thus are potential therapeutic agents in aquaculture.

Live feed rotifers often have a high load of bacteria that can be passed on to marine fish larvae and potentially lead to bacterial disease. For this reason, an adapted MIC-protocol was employed to study the potency of ten AMPs against bacterial pathogens under simulated rotifer culture conditions (Woods *et al*., 2022). All AMPs had potent antimicrobial activity against bacterial pathogens at very low salt concentration. However, in artificial seawater (25‰), the majority of the AMPs had an MIC value greater than 65 µg/mL. The only exception was two AMPs with MIC values about 32.5 µg/mL against *Vibrio rotiferianus* and *Tenacibaculum discolor*, respectively. The authors concluded that the ten tested synthetic AMPs were ineffective at reducing the bacterial load in brackish salt concentrations of a typical commercial rotifer culture.

As discussed, AMPs show great promise as alternatives to traditional antibiotics given their advantages including a broader range of activity and assumed lower risk of resistance development in bacterial pathogens. More research is however needed to reveal the “mode of action” of some AMPs and to determine how they might affect the fish host and the gut microbiota. Also, more research is needed to solve lingering issues related to their efficient production, proteolytic instability and oral delivery before these small remarkable peptides can be used as practical disease prevention/treatment applications in commercial aquaculture.

5. “Omics”-Studies In Bacterial Fish Pathogen Research

The fast development and now common application of next-generation sequencing (NGS) related technologies have revolutionized molecular microbiology and therefore research into infectious fish disease. Armed with this technology and the rapidly expanding “tool box” for bioinformatics analysis of “big data”, scientists have made “quantum leaps” in fish pathogen genetics, research into virulence mechanisms not to mention our understanding of fish host immune responses to infections. A recent review (Sundaray *et al*., 2022) provides an overview of ‘omics’-applications successfully used to resolve several fish productive and reproductive issues. Draft genomes of most farmed fish species have been generated and successfully used to map a large number of single nucleotide polymorphisms (SNPs) and to develop marker gene panels/other genomic resources. Transcriptome profiling by RNA-seq has helped to unravel metabolic pathways and gene regulatory/protein interaction networks. This technology has also lead to a profound understanding of biological processes including stress responses, adaptive evolution besides fish host immune responses (Qian *et al*., 2014). NGS can also be used to describe microbial communities in aquaculture-related environments such as hatchery tank biofilms through 16S rRNA-gene profiling (Turgay *et al*., 2019) and to determine the ecological role of microorganisms through functional genomics analysing either metagenome datasets or the mentioned community profiles with special purpose microbiome software.

Whole genome sequencing (WGS), metagenomics and comparative genomics are used to identify novel/emerging fish pathogens, to characterize isolates but also uncultivable bacterial pathogens and to elucidate their evolution including the distribution of known virulence factor genes in strains/isolates. A recent study sequenced the genome of a *Lactococcus garvieae* isolate recovered from the lower intestine of rainbow trout (*Oncorhynchus mykiss*) farmed in the northwest Himalayan region India (Shahi and Mallik, 2020). This bacterium is an

emerging bacterial pathogen that causes fatal hemorrhagic septicemia in cultured fish species. The genome was approximately 2 Mbp and thus very similar in size to those of the six other isolates used for comparative genomics. Analysis revealed that the pan-genome of *L. garvieae* included 2239 putative protein-coding genes of which 1850 are core genes. A small set of 221 genes were unique to the Indian isolate. It was also found that the Indian isolate had no plasmids and that the genome lacked a 16.5 Kb virulence associated capsule-gene cluster but included 39 other confirmed virulence-associated genes (VAGs) and a large complement of 29 antibiotic resistance genes (ARGs). Notable virulence factors of the Indian *L. garvieae* isolate are encoded by several genes for cell-cell or cell-surface adhesion (adhPav, adhPsa, adhC1 and adhCII), three hemolysin genes (Hly 1, 2 and 3), an iron acquisition gene (fagA), a gene for host-tissue invasion (inv) and two systems for secretion of proteolytic enzymes (Yp1A, porP and porS).

The study of obligate intracellular fish pathogenic bacteria is complicated as they either are very difficult or impossible to culture in the laboratory. It is often not possible to isolate enough high quality genomic DNA to sequence and assemble a complete genome. A mini-metagenomics sequencing approach was thus used to build draft genomes of uncultured epitheliocystis agents causing emerging infectious disease in the Mediterranean farming of gilthead seabream (Qi *et al*., 2016). Extracted DNA from multiple cysts were sequenced and genome drafts assembled for a novel beta-proteobacterial lineage, *Candidatus* Ichthyocystis. Reduced genome sizes and metabolic capacity are features typical of pathogenic bacteria with an obligate intracellular lifestyle. These intracellular bacteria have to scavenge amino acids from the fish host as reconstruction of metabolic pathways reveals that amino acid synthesis pathway genes are missing from *Ca*. Ichthyocystis genomes. But, all genomes include a large gene repertoire for predicted effectors considered to be virulence factors, required for adherence, invasion, host manipulation and at several effector secretion systems (type II, III or IV). Finally, the cell wall structure of these bacteria is unusual as they apparently lack genes for lipopolysaccharide (LPS) synthesis. A second study to use culture-independent genomics, investigated another chlamydia-like agent of epitheliocystis in Orange-spotted grouper (*Epinephelus coioides*) farmed in Australia (Taylor-Brown *et al*., 2017). The name *Ca*. Similichlamydia epinephelii has been proposed for this intracellular bacterium. An estimated 68% of the genome were sequenced which could reveal that this chlamydial pathogen shares pathogenic hallmarks with members of the Chlamydiaceae, including an intact type III secretion system and several chlamydial virulence factors such as chlamydial protease-like activity factor (CPAF) and putative cytolytic toxins. It was concluded that *Ca*. S. epinephelii

dedicates a large portion of its genome to pathogenic mechanisms and that they were likely acquired early in the evolution of this unique bacterial phylum.

In a recent study, comparative genomics was used to identify genomic areas and genes unique to two high virulent (HV) isolates not present in two low/non-virulent isolates of *Flavobacterium columnare* (Declercq *et al*., 2021). Only the genomes of virulent *F. columnare* isolates encoded a unique methyl-accepting chemotaxis protein (MCP) likely playing a part in tissue colonization and bacterial competition proteins belonging to the VgrG family. Unique virulence genes of one HV-trout isolate were located in a region identified as a genomic island. This large element included the nodT gene that are likely involved in antimicrobial resistance, as NodT family members are outer membrane lipoproteins of the RND-type efflux systems, as well as the luxR-gene linked to bacterial quorum sensing. The HV-trout isolate carries unique sugar-transferases genes presumably necessary for bacterial adhesion to host cells and other chemotaxis-involved genes cheA, cheB, cheY but also cheW-like proteins. But, the authors could not conclude that chemotaxis is active in *F. columnare* based on these findings. Finally, global analysis of *F. columnare* genomes demonstrated that several unique genes involved in virulence are present only in the genomes of high virulence carp and trout isolates.

Other approaches like transcriptomics and proteomics are used to understand fish immune responses to infection and to elucidate fish host-pathogen interactions including virulence mechanisms. RNA-sequencing (RNA-seq) on NGS-platforms soon replaced microarrays in transcriptome studies, because this approach is capable of identifying and quantifying transcripts without prior knowledge of specific genes. A weakness of RNA-seq based transcriptomics is that it does not take into account post-transcriptional and translational regulation of protein expression. This is the strength of proteomics analysis where gene expression products are analysed through for example two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS).

The first and only study to investigate infection specific mRNA transcripts in European seabass (*Dicentrarchus labrax*) relied on expressed sequence tags (EST) analysis (Sarropoulou *et al*., 2009). It describes the analysis of cDNA libraries from different European seabass tissues following infection either with *V. anguillarum* or with Nodavirus and the identification of possible markers for bacterial and viral infection. More than 100 immune related transcripts were annotated including one encoding the antimicrobial protein hepicidin. The expression of a handful of these immune related transcripts were analysed by qPCR. The qPCR revealed hepicidin up-regulation only in *V. anguillarum* infected European seabass, which suggested

that expression of this AMP could be a good marker for bacterial infections. Fructose-1,6-bi-phopshate aldolase (B isoform) upregulation in the spleen could be second exclusive marker for bacterial infection. It was also found that 14 kDa apolipoprotein expression in the spleen is a potential marker of Nodavirus infection. Finally, the differential expression of chemokine receptor 4 was also found to be a putative biomarker for *V. anguillarum* infection.

A custom designed immune-targeting microarray and RT-qPCR have been used to characterize the late immune response in the spleen and head kidney of rainbow trout to lactococcosis (Castro *et al*., 2019). The main difference observed between tissues was the robust inflammatory response in the spleen and granulomatous lesions containing macrophages with internalized *L. garvieae*. Formation of gramulomas could readily be correlated with up-regulation of chemokine IL8 and its receptor, other inflammatory cytokines, macrophage marker genes and increased transcription of genes related to neutrophils. IL8 plays a crucial part in macrophage phagocytosis initiation as well as neutrophils and monocytes recruitment. The adaptive immune response could be initiated by the up-regulation of TLR22 in the spleen following *L. garvieae* infection. RT-qPCR confirmed up-regulation of secreted IgM in spleen/head kidney as well as the up-regulation of BT cells only in the spleen of *L. garvieae*-infected trout could indicate an antibody response by both IgM (+) and IgT (+) spleen B cells to systemic infection. IgT is involved in mucosal immunity as well as systemic immunity in trout. After *L. garvieae* infection, chemokines/interleukins like CK5B and IL11 were up-regulated in the trout spleen. Reduced innate inflammatory responses 72 hours post infection was indicated by the down-regulation of the inducible nitric oxide synthase (inos) and an important pro-inflammatory cytokine IL1-β. At the same time, regulatory T cells were induced in both tissues with a simultaneous down/up-regulation of complement pathway factors C3 and C6, respectively. Moreover, protein degradation and apoptosis related genes that correlate with a late phase of the inflammatory process were up-regulated in both organs. It was concluded that the study contributes to a better understanding of the host–pathogen interaction during *L. garvieae* infection and likely will aid in the design of new vaccination strategies to prevent disease in rainbow trout.

A recent transcriptomic study assessed splenic immune responses to *Y. ruckeri* infection in rainbow trout (Wang *et al*., 2021). This organ plays central roles in orchestrating both innate and adaptive immune responses in fish. Seventy-eight differentially expressed genes in the spleen, associated with the immune system, were assessed in more detail. These almost exclusively upregulated genes are associated with 20 immunological pathways especially related to NOD-like receptor signalling, RIG-I-like signalling and Toll-like receptor signalling. Several genes belonging to TLR, NLR, and RLR gene families of pattern recognition receptors (PRRs) are

differentially expressed 24 hours after *Y. ruckeri* infection and may be important mediators of the initial induction of immunological responses to the infection in rainbow trout. Consistent with pathogen-induced regulation, thirty-one differentially expressed genes (DEGs) were assigned to the cytokine-cytokine receptor interaction pathway. They included genes encoding the chemokine receptor 9 (CCR9), the chemokine ligand (CXCL11), pro-inflammatory cytokines (IL-12, IL-1β, IFN and TNF) and the caspase recruitment domain-containing protein (CARD9). Upregulation of a regulator of apoptopic cell death caspase 8 (CASP8) but also the NF-κB inhibitor (IκBα) were observed. The intricacy of spleen tissue responses is emphasised through compensatory activation of apoptotic cell death inhibitors as suggested by the upregulation of IAPs, RIPK1 and IkBα following *Y. ruckeri* infection. Finally, the authors identified 22 upregulated genes involved in the MAPK-signalling pathway, including significant MAPK8 upregulation in spleen tissue upon *Y. ruckeri* infection in rainbow trout, suggesting that it plays a key role in the rainbow trout response to infection with this bacterium.

A bioinformatics approach involving comparative proteomics has been used to evaluate the outer membrane proteome of the fish pathogen *Yersinia ruckeri* (Ormsby *et al*., 2019). This etiological agent of enteric redmouth (ERM) disease is responsible for significant economic losses primarily in rainbow trout but increasing also so in Atlantic salmon. Outer membrane proteins (OMPs) play an important role in virulence at the host-pathogen interface but, they are poorly characterised for *Y. ruckeri*. The study identified 141 putative OMPs from four *Y. ruckeri* genomes but only 77 were considered as “core” outer membrane proteins as they were identified in several genomes. A function could be assigned to 121 predicted OMPs based on published literature and had a role in outer membrane biogenesis and integrity, adherence, enzymatic activity and motility. The authors concluded that some of the OMPs expressed in *Y. ruckeri* under laboratory conditions could form the basis for studies aimed at development of improved vaccines. High-throughput proteomics have also been applied to elucidate the defensive role of the rainbow trout liver towards infection with *Aeromonas salmonicida* (Causey *et al*., 2018). The authors hypothesized that the liver serves a dual role in supporting host defence in parallel to metabolic adjustments that promote effective immune function. A label-free method was used for protein abundance profiling on a mass spectrometer. After *in silico* identification, 109 proteins showed significant differential abundance following *A. salmonicida* challenge. Many were upregulated complement system and acute phase response (APR) proteins, in addition to several proteins that likely result in metabolic re-adjustments in the rainbow trout liver.

As exemplified, NGS-based applications have, in combination with bioinformatics analysis, become a leading approach in infectious fish disease research. Immune system responses

to bacterial infection in European seabass and gilthead seabream is far less studied than in salmonids like rainbow trout and should therefore be prioritized given their importance in Mediterranean and European aquaculture.

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CHAPTER 16

PATHOGEN TRANSMISSIONS BETWEEN FARMED AND WILD FISH

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DOI: 10.26650/B/LS32LS24.2024.005.016

1. Introduction

Which came first: the chicken or the egg? Like this old conundrum, the spread of pathogenic fish diseases in nature is complex, are they transmitted to the wild population through aquaculture or vice versa?

Microorganisms can exist in the natural environment without living organisms, but the presence of living organisms' existence increases their chances of survival as a food source. Microorganisms, presence in the water, are major component of nutrient cycle but some of them are pathogenic for aquatic organisms (Wommack and Colwell, 2000).

Before going into the subject of how pathogen transmission can occur, it would be appropriate to briefly give information about the condition of microorganisms in their natural environment and how the disease occurs in fish. Afterward, addressing the problems that can be encountered in two different environments, aquaculture and wildlife, and exemplifying with the studies in the world will be the way to be followed in terms of understanding the subject.

2. The Roles of Microorganisms in Nature

Measurement of bacteria concentrations in aquatic environments is carried out intensively in terms of monitoring the health of both human and ecological systems. Quantification of

viruses has also been added to these studies from time to time. Moreover, these studies have shown that microorganisms are abundant and free in natural aquatic ecosystems (Wommack and Colwell, 2000). In other words, the abundance of microorganisms, especially pathogenic ones, in their natural environment does not always depend on the presence of living organisms. For example, it has been shown that *Flavobacterium psychrophilum*, the causative agent of cold water disease, which is an important disease in trout, can live free in water for at least 300 days (Madetoja et al., 2003). Of course, the high organic load ensures the continuation of the presence of microorganisms (Hennes et al., 1995). Additionally, the amount of dissolved organic carbon driven through rapid bacterial production can be as much as 50% of primary output in marine systems (Hansell et al., 2009).

Although fungus play an important role in decay in nature, we cannot say that parasites are beneficial (Barber, 2007). Many aquatic fungi have been found, and there are also species that cause nitrite accumulation in the seas, but they mostly survive by infecting animals (Richards et al., 2012).

In the oceans, viruses play an important role in the dynamics and structure of the ocean's host populations and communities. They also affect geochemical changes in the oceans; for example, viruses can alter the carbon cycle pathways as a result of the lysis of the cells they infect and even help release inorganic elements out of lysed cells (Fermin and Tennant, 2018).

Additionally, the contribution of viruses to aquatic ecosystems cannot be ignored. It is surprising that viruses are the most abundant plankton in waters, but it has been reported that they can control microbial communities even in small quantities. In these environments, bacteriophages mediate numerous mechanisms among themselves that affect gene flow, adaptation, population control, and others. Bacteriophages that infect bacteria, especially in water, will create an epidemic of viral infection to prevent their rapidly multiplying hosts from dominating the community (Fermin and Tennant, 2018; Hennes et al., 1995; Wommack and Colwell, 2000). In other words, these creatures, which are an integral part of the natural ecosystem, will cause diseases in animals and plants whether they are cultured or not.

3. Mechanism of Disease Formation In Fish

In order for pathogens to cause disease, the physical, chemical and biological parameters of the ecosystem must deteriorate and the living thing must be negatively affected by these conditions for a certain period of time. This proven theory of stress also provides us with the knowledge that illness does not actually happen very quickly.

Aquatic organisms, like all other living things, have a stress mechanism. The environment they live in exposes them to many chemical, physical and biological stressors. Let us briefly consider the deterioration of ecosystem parameters, which is called the environmental stress factor. Worsening of the chemical and physical properties of water, namely poor water quality, such as high levels of pollutants (toxic chemicals, such as heavy metals, pesticides, and oil spills etc.) or low levels of oxygen, can weaken the immune system of fish and increase their susceptibility to diseases. Differences in temperature change the growth rate of microorganisms, the amount of dissolved oxygen in the water, and the rate of metabolite excretion. Being sick of living things that are exposed to heat stress acutely or chronically can be sudden or gradual depending on these factors. The suddenness of these changes or fluctuations is the most challenging the host's defense mechanism (Roberts and Ellis, 2012).

And the availability of food and habitat can also contribute to the health problems of wild fish. The presence of other wildlife, such as predators, can increase stress and risk of disease in fish. In cultured fish, a balanced diet with proper nutrition can help maintain the health of fish and reduce their risk of disease. Whereas handling, transport, or crowded living conditions, can increase the risk of disease in fish (Smith, 2019). In aquaculture, husbandry procedure is a stressor, and in nature, human effects are less, except for species with prey pressure.

Genetic factors also play an important role in disease formation. Certain genetic traits can also make wild fish more susceptible to certain diseases and diseases, especially when populations are small or isolated. In addition, the age of the fish is another biological factor that affects maturation of adaptive immune system and disease resistance (Makesh et al., 2022). In senescence, fish immune system can become weaker and they may become more susceptible to diseases (Roberts and Ellis, 2012). But it should not be forgotten that some pathogens can only affect young fish (Makesh et al., 2022). As an opposing of view, a study on the effect of senescence on big buffalos (*Ictiobus cyprinellus*), known as long-lived fishes living in North America, have shown that their immunity is better than younger fish (Sauer et al., 2021).

The issue of whether fish are genetically resistant to diseases has been the focus of researchers for many years. Looking at the correlation of the disease with the genetic structure, for example, in trials on Atlantic salmon, some studies in Atlantic salmon have indicated positive genetic correlations between resistance to furunculosis, while others indicated that there is a negative correlations (Yáñez et al., 2014). In fact, it has been observed that a new variant can cause disease in salmon that are genetically resistant to IPN, which have started to be produced as a result of studies (Hillestad et al., 2021). Therefore, there is no clear judgment on genetic resistance yet. But we also know that some special genes of fish come into play

during stress. For example, stress in fish leads to overproduction of a group of proteins called heat shock proteins (HSPs). Genetically strong fish can survive stress thanks to these stress-responsive proteins (R.J. Roberts and Ellis, 2012).

4. Living in the Wild

Determining if a fish is sick in nature can be challenging and some of these symptoms may not be noticeable to the untrained observer, and while diseases in a wild fish population are difficult to observe, more effort and further analysis should necessary to determine the cause of diseases. This identification should include laboratory testing of tissues, water samples and other environmental factors to detect the presence of pathogens, just as is done to fish in aquaculture. Of course, when the word disease is mentioned, the presence of pathogens always comes to mind. However, many diseases that are not related to pathogens, such as ocular disorders, are also encountered in nature (Smith, 2019). Since pathogen transmission is the main issue in this chapter, the subject of disorders and non-infectious diseases will not be mentioned.

A sick fish may display abnormal swimming behavior, have abnormal skin coloration, may have visible skin lesions, such as ulcers (Figure 1) or tumors, or show signs of injury or deformity. A sick fish may not be feeding properly or may be more sluggish or inactive and lethargic than healthy fish. In nature, sick fish may face a variety of outcomes depending on the severity of their sickness and the specific pathogens involved. If we want to understand physiologically how fish in their natural environment are affected by the disease, we can first start with their swimming behavior.

Under optimum conditions in nature, that is, the fish swimming continuously in perfect synchronization to form a series of two-dimensional diamonds means saving up to five times the locomotor energy. The fish that make up this flock are of similar sizes, moving at the same speed and moving at regular intervals. In this way, they reduce the drag force by using some of the eddy energy created behind the fish in front of them. In addition, the mucus secreted from the fish epidermis has important reducing drag properties. Thanks to the mucus, the fish can increase their speed. The increase in mucus during flight or chase is to accelerate swimming (Keenleyside, 1979). Mucus secretion regulation is impaired in sick individuals, so swimming speed becomes uncontrollable. Additionally, due to weakened body resistance, they may become more passive or lethargic and lose swarm synchronization in swimming behavior, the drop in their bodies preventing them from keeping up with the pack. Therefore, they leave the herd and the fish that are left alone either become food or hide in a corner, waiting for the disease to end. This end will be either death or salvation.

However, not all sick fish will leave their school, and the outcome will depend on the specific pathogen involved, the severity of the illness, and the behavior and response of the healthy fish in the population. In some cases, sick fish may continue to swim with the rest of their school (Figure 1), even as they are suffering from an illness, which can increase the risk of pathogen transmission to other fish in the population.

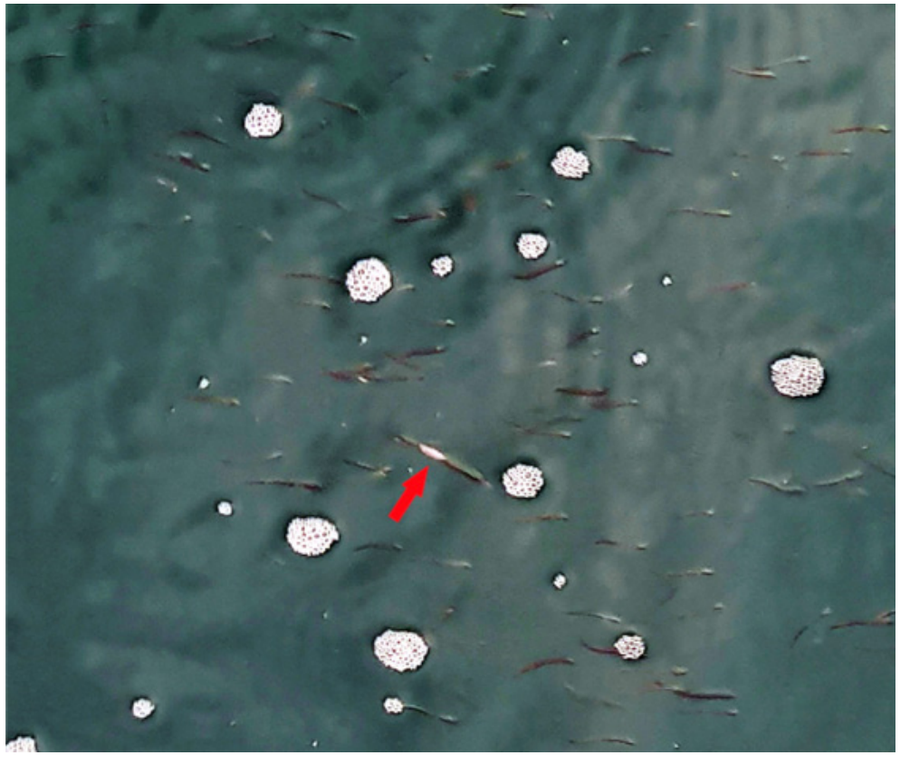

Figure 1. A diseased individual with ulcer (arrowed) in wild mullet school at Gerence Bay, İzmir (Original photo by M.D. Demircan, 2023)

For nutrition, which is another physiological behavior, it is appropriate to say that being separated from the school when the disease occurs in individuals who are accustomed to feeding in school has negative effects on nutrition. Many fish species are carnivores, and fish that feed actively in this way will have problems swimming towards and catching food due to energy losses. Fish that eat sick but live individuals, dead fish, or fish that feed on benthic organisms may first absorb pathogens into their bodies and then transmit pathogens to other

individuals. For sick fish with grazers behavior, feeding may be easier due to less energy expenditure, after all, the algae cannot escape. Since filter feeding fish also feed on plankton, they do not need to spend great energies. However, such fish are also easier to ingest bacteria (Keenleyside, 1979). The sick individual who has problems in nutrition loses his energy more and the disease may progress more.

Overall, the outcome for a sick fish in nature will depend on the specific pathogen involved, the severity of the illness, and the strength and resilience of the fish's immune system. To briefly summarize the question of what happens when fish in nature get sick;

1. Death: In some cases, sick fish may die from their illness, especially if the infection is severe or the fish's immune system is weakened. This can result in a decline in population numbers and reduced reproductive success, which can have cascading effects on the ecosystem.

2. Recovery: If the fish's immune system is able to effectively fight off the infection, it may recover from its illness and return to good health. However, some fish may be left with lingering health problems or weakened immune systems that can make them more susceptible to future infections. And also they can carry the pathogens for their entire life.

3. Spread of pathogen: Diseased fish may also spread pathogens to other fish in the population, leading to a higher incidence of sickness and further declines in population numbers.

4. Predation: Diseased fish may be more vulnerable to predation by other animals in the ecosystem, such as birds and other fish.

5. Carrying the pathogen: In some cases, sick fish may carry pathogens without exhibiting any noticeable symptoms, acting as a reservoir for the pathogen and increasing the risk of transmission to other fish populations.

Although infectious diseases are said to cause losses in natural fish populations, the mechanism and scale of these losses are still unknown (Chapman et al., 2021; Jeffries et al., 2014).

In recent years, large-scale deaths in marine animals due to the effect of temperature have been encountered quite frequently. It is reported that the interaction of the Mediterranean, which is especially relevant to our country, is affected by climate change accelerated and deaths increased in this sea basin (Garrabou et al., 2022). In the study, it was determined by the observations that the increase in mortality rate has a linearly relatedto the increase in temperature, but since other biological factors such as the presence of disease-causing agents were not investigated in this study, they did not find it correct to attribute deaths only to temperature.

The major hurdles in natural fish disease epidemiology are the logistical constraints of observing the disease and collecting samples from freely roaming fish. Therefore, screening of infectious agents has been included in recent telemetry studies (Chapman et al., 2021). Chapman's review argues that even a cursory understanding of the infectious agent community of individual tagged fish at the time of release can improve the interpretation of factors influencing post-release survival, particularly in the presence of highly pathogenic agents.

One of the most important questions here is "WHY DO WE ENCOUNTER MORE CASE OF DISEASE IN CULTURE FISHES BUT FEW WILD FISHES THAT ARE SICK?". The answer to this question may depend on many factors. Overall, the differences in population density, genetics, nutrition, and drug exposure between aquaculture and wild fish populations can help explain why common and high mortality diseases in cultured fish are not as frequently observed in wild fish. If we want to compare two ecosystems, it may be appropriate to look in more detail at what factors affect health.

1. Dense population and stressful conditions in aquaculture facilities can create ideal environments for disease transmission and increase the likelihood of epidemics. Higher density increases the chances of horizontal transfer between fish. In contrast, wild fish populations are generally more dispersed and have more diverse habitats, which can reduce the spread of disease.

2. In culture conditions, it is important that the conditions in which the fish are kept provide environmental parameters suitable for all the requirements of the particular species, since they have limited opportunity to choose the external environmental conditions. Infected fish cannot leave the environment and are more vulnerable to diseases. Natural populations are more free to change their environment than fish living in cultured conditions. When faced with a negative environment change such as a decrease in oxygen level or an increase in temperature, they can change their place and move to the appropriate environment. And they may even swim to warmer areas to protect themselves, helping their bodies increase the inflammatory response (Roberts and Ellis, 2012).

3. Pathogens, even in small amounts in culture conditions, easily find enough food to multiply in the dense fish population and intensify, thereby creating a large reservoir of pathogens. In contrast, pathogens are not found in abundance if there is not enough nutrients in the natural environment. Besides, in the absence of the host, they can be destroyed due to the physical and chemical properties of the natural environment. so that in nature, fish may not encounter some pathogens at all.

4. In aquaculture facilities, fish are often genetically homogeneous and have limited exposure to pathogens through precautions in hatcheries can make them more susceptible to disease in unprotected environmental conditions such as the open-water net cages which they are tranferred. In contrast, wild fish populations are typically more genetically diverse and have longer exposure to various pathogens from the hatching. This increases their immunity and they may be more resistant to disease.

5. Cultured fish are fed artificial diets with a specific formulation. If the health status of the fish is regularly monitored, different nutritional supplements can be added to the feed and the immune system of the fish can be supported. In contrast, wild fish can access a variety of foods depending on what they find in nature, which can be both an advantage and a disadvantage.

6. Cultured fish are usually vaccinated and treated with antibiotics and other chemicals. This is an unfeasible factor for wild fish populations, the fish have to fend for themselves.

As a result, the likelihood of fish caught in nature being protected from disease can vary depending on the type of pathogen, the characteristics of the natural environment, and the characteristics of the fish.

5. Pathogen Transmission Route

Many scientists all over the world investigating the transmission of pathogens between farmed fish and natural populations for many years. None of these studies have found evidence that fish farming contributes to detectable adverse changes in wild fish populations, but the issue is a hot topic of discussion in the media (McVicar et al., 2006). In fact, indirect evidence of wildlife transmission among farmed fish is strong (Beveridge, 2010). It is understood from recent publications that this view is still defended today (Bouwmeester et al., 2021).

For viruses, studies stating that both parties can be donors were compiled in 2011 (Kurath and Winton, 2011). As an example, Rhabdoviruses appear as a family of various fish pathogens and are important to both natural and cultured fish. Some also have salinity and temperature tolerance, for example, they can be transported to all different salinity aquatic environments with salmon and survive there. In this way, they are one of the groups least affected by climate change.

Since the aquatic environment has a displacement feature compared to the terrestrial environment, the environmental characteristics are constantly changing for all living things. In a review, five potential dynamics about farmed fish may alter wildlife disease identified

(Bouwmeester et al., 2021). To summarize, farmed species may have entered parasites to new environment and infect same species (intraspesific parasite spillover) or other species (interspesific parasite spillover) in nature. Parasites from other wild host species can reinfect farm species after multiplying in the same wild fish species (interspecific parasite spillback) or different wild fish species (interspecific parasite spillback). The last dynamic is transmission attempt about that farmed species as carriers can transmit parasites from farmed species to wild host species.

Researchs so far show that there are many pathogens that can be transmitted between wild fish and cultured fish, including; bacteria such as *Aeromonas salmonicida*, *Vibrio* spp., *Streptococcus* spp., and *Edwardsiella tarda*, virus such as such as Infectious Hematopoietic Necrosis Virus (IHNV), Infectious Pancreatic Necrosis Virus (IPNV), and Piscine Reovirus (PRV), fungus such as *Saprolegnia* spp. and Achlya spp., parasites such as *Ichthyophthirius multifiliis*, *Dactylogyrus* spp., and *Gyrodactylus* spp., protozoans such as *Cryptobia* spp. and *Ichthyobodo necator* (Colorni et al., 2002; Glover et al., 2013; Ruane et al., 2007; Noga, 2010; Smith, 2019; Timur and Timur, 2003).

The presence of these pathogens in wild fish populations can lead to declines in population numbers and reduced reproductive success, which can have cascading effects on the ecosystem. In addition, pathogens can also be transmitted to humans and other wildlife, leading to additional health problems and impacts.

It is important to note that the specific pathogens that are present in wild fish populations and that can be transmitted to cultured fish will vary depending on the geographical location and the species of fish involved. Especially, some species of fish may be resistant to the diseases and can infect other susceptible species by carrying disease agents (Figure 2). In addition, invasive fish species are ubiquitous in nature and are often resistant to diseases. Pathogen transmission due to invasive species is also highly possible (Gozlan et al., 2010).



Figure 2. Wild mullets with parasites (Original photo: M.D. Demircan, 2023)

Several cases have been reported that affected wild populations and caused them to collapse suddenly. It is thought that there is another cyprinid species, *Pseudorasbora parva*, caused a 96% decrease in *Leucaspius delineatus* in Europe in 2005, as was the case with *Cyprinus carpio* in Illinois, USA (Gibson-Reinemer et al., 2017; Gozlan et al., 2005). Research continues on the origin of the intracellular eukaryotic fungal-like organisms, namely the rosette-like agent (RA), carried by the invasive *P. parva* (Sana et al., 2017).

Pathogens can be transmitted between wild and cultured fish in a two ways, including through:

1. Direct contact: Pathogens can be transmitted from wild fish to cultured fish when they come into direct contact with each other, either through feeding or through physical interactions. A study with molecular genetic analyzes may be one of the clear evidence for direct contact. In the study, a salmon that was determined to be cultured in the stomach content of natural cod was found and it was determined that this salmon was infected with piscine reovirus (PRV) (Glover et al., 2013). Also we observed using live feed for breeding Tuna fish in last year (Figure 3.) then the idea of preparing this publication on pathogen transmission came up. Wildlife, such as birds, seals, and sea otters, can also carry pathogens that can infect

fish. If these animals come into contact with cultured fish, they can transmit the pathogens to the fish. Pathogens can also be transmitted through the movement of fish from one location to another, such as through the transport of wild fish for use as broodstock or through the transfer of cultured fish from one farm to another. Commercial transport of aquarium fish can also be mentioned here, in which pathogen transport has been proven especially by the presence of cysts of parasites (Şahin Taner et al., 2022; Yildiz, 2005). Escapees form fish farm can also carry pathogens with own body, which can then spread to other wild fish (Arechavala-Lopez et al., 2013). Of course, vectors are also transmitted especially bacteria from one fish to another even to very long distances. For example, sea lice play a major role in the spread of diseases in pacific salmon population (Jansen et al., 2012; Rees et al., 2014).



Figure 3. Imported freezed chub mackerels from Moritanya to Türkiye, for Tuna fish feeding at Karaburun, İzmir (Original photo: M.D. Demircan, 2023)

Indirect contact: Commercial fish facilities have access to water from streams, rivers, ponds, lakes or oceans. Water from nature should be carefully monitored, and it may be necessary to treat incoming water to reduce or eliminate pathogens (Smith, 2019). Pathogens can be transmitted through water to farm thereby spreading the infection to other fish (Meyer et al., 2021). In this way, both aquaculture and wild fish are exposed to released infectious agents, increasing the possibility of disease transmission (Diamant et al., 2000). If the aquaculture system is not designed appropriate, wild fish can also enter the system. For example, it has been reported that in the Milas earthen pond region (Muğla,Türkiye), fish can enter the pond backwards through the channels and transmit the pathogen to the aquaculture fish (Ercan et al., 2015). Pathogens also can be transmitted through the use of fish farm equipment and tools, such as nets and ponds, which can carry pathogens from one place to another. And also ballast water of international ships is an important factor.

6. Other Aquatic Organisms

Disease is still a primary constraint to the culture of many aquatic species, hindering both economic and social development in many countries. And interactions between wild and cultured fish populations are an important concern (Bondad-Reantaso et al., 2005). When we say aquaculture, it is not enough to mention only fish. Today, many different aquatic organisms such as invertebrates, mussels, oysters and aquatic plants are cultivated. In fact, applications of integrated multi-trophic aquaculture are increasing, in which it is possible to breed shellfish, mollusc and seaweed cultures together with fish. However, among these applications, non-fish species, especially invertebrates, are likely to transmit many pathogens that can infect local species, and help them to complete their life cycle faster as an intermediate host of parasites (Bouwmeester et al., 2021). It is considered not to be too much. As for their capacity to carry bacterial pathogens, it has been reported that *Renibacterium salmoninarum* can be found in tissues of freshwater mussels and may be reservoirs for fish (Starliper and Morrison, 2000). In a study we conducted (Demircan et al., 2022), it was observed that there was a large amount of bacteria (x10^7 CFU) in the tissues of *Unio crassus*, and the bacterial density was tried to be reduced with many applications. As a result of the study, which aims to be able to culture fish and mussels together and to reduce fish pathogens from the water by filtration method, it was revealed that fish pathogen motile Aeromonads already exist in its body and lead a symbiotic life with them. In addition, in a study conducted, sea lice larvae, which are pathogenic for Salmon, were found in the stomach of *Mytilus galloprovincialis* and it was emphasized that mussels could save salmon from this big sea lice problem (Molloy et al., 2010). Considering these results, it should not be forgotten that molluscs to be placed in IMTA systems have benefits, but one should be careful about the possibility of carrying fish pathogens.

Pathogen transfer is the common problem of all living things forming the ecosystem. Especially parasite translocations occur frequently with host movements. *Haplosporidium* sp., which has caused massive mortalities among eastern oysters (*Crasostrea virginica*) along the eastern coast of the United States in 2000. It is known that this parasite appeared in *C. gigas* and caused low mortality in Japan in 1976. The parasite carried by *C. gigas* as it spread first to the west coast and then to the east of the United States was disastrous for the more sensitive *C. virginica* species.

Vibrios are also pathogenic for many crustacean species and have been reported to be pathogenic in *V. anguillarum* oyster species (*Ostrea edulis*) and *V. alginolyticus* red abalone (*Haliotis rufescens*), which are known as fish pathogens (Anguiano-Beltrán et al., 1998; DiSalvo et al., 1978). Therefore, many different species living in the same habitat can infect each other with disease agents.

7. Global Examples

While some researchers talk about the increased release of pathogens to nature due to culture (Araujo et al., 2022), it is also argued that pathogens in natural fish populations are a threat to aquaculture (Ercan et al., 2015; Garseth et al., 2013). There are also those who think that it is necessary to accept that both sides are a danger to each other (Olivier, 2002). Perhaps it would be a more accurate approach to address this issue according to the type of microorganism an geographical area. For example, while *Aeromonas salmonicida* was a big problem in the UK in 1935, it only started to appear in Norway by 1991 (McVicar, 1997).

Actually, pathogens infecting both wild and aquaculture organisms are generally the same at the literature. The spread of *Gyrodactylus salaris* in Norway in 1975 led to the questioning of the relationship between aquaculture and natural populations. Similarly, the fungus that is the causative agent of Epizootic Ulcerative syndrome has spread very quickly in both cultured and natural fish in Asia (McVicar, 1997). While some diseases, such as IPN, are on the list of notifiable diseases, they have lost their importance due to their increasing prevalence and being seen everywhere and are not even included in the OIE (WOAH) list (Ruane et al., 2007).

Of course, the factor that causes this situation is that viruses can spread very quickly and their pathogenic properties can change. In order to know the pathogenic properties of viruses, it is important to know how long they can survive in water. It is thought that the Tilapia lake virus, which has become one of the new problems with a similar structure, may show similar characteristics. This virus is a disease agent with a high mortality rate for both natural fish and cultured fish. In Thailand, Nile tilapias were affected by this disease within 1 month after being taken from the hatchery to the rearing cages in the rivers (Tang et al., 2021).

The interactions of cultured fish and wild fish were investigated in two different studies conducted in Tasmania. In the first, a protozoan parasite *Neoparamoeba pemaquidensis*, Amoebic gill disease (AGD) agent, which is a big problem in salmon farms, was sampled from different species of fish roaming the farms to see the interaction of causes, but it was reported that none showed this infection. In the laboratory studies, they compared this parasite with some species and it was seen that the species could be infected with this parasite. Based on this, it was concluded that wild fish cannot be an important reservoir for this pathogen (Douglas-Helders et al., 2002).

In another study, the emergence and spread of the parasite *Myxobolus cerebralis*, which causes Whirling's disease, contributed to the collapse of wild trout populations along the intermountain west of the Americas. Of concern is the risk the disease may have on the conservation and recovery of native cutthroat trout (*Oncorhynchus clarkii*). For this reason, studies have been carried out using risk assessment modeling and it has been suggested that the construction of barriers that can prevent fish movements can reduce the spread among natural fish (Ayre et al., 2014).

Global warming, which is one of the most important events that affects both populations and increases the effects of pathogens, has not been forgotten. In some studies, long-term follow-ups of both global warming effects and pathogen transmissions are performed using modeling and tagging (Clark et al., 2016; Groner et al., 2014, 2018; Teixeira Alves and Taylor, 2020).

8. Status of Türkiye

Despite the value given to science in our country, every publication that contributes to science is gold and every scientist who makes a contribution is appreciated. The mentioned studies were obtained during the literature review and are given as sample studies, so it is certain that there are publications and researchers that are not mentioned or overlooked. Due to the difficulty of reaching the first printed sources, we do not know their exact contents. According to the studies we could reach, the first wild fish study was conducted in 1985, and hemorrhagic septicemia with high mortality caused by *Pseudomonas* sp. was reported in wild pike perch (Timur and Timur, 1985).

Demircan and Candan (2006) were sampled from cultured sea bass and wild mullet caught around the farm in their study and found that Vibriosis disease affected both fish species. The results of this study show that two different populations in the same environment can be affected by each other, although it is not clear whether Vibriosis has been transferred from wild

to cultured fish, or whether there has been a transmition from cultured fish to wild. In addition, it is known that Vibrios isolated as disease agents can maintain their viability and grow for a long time without being alive in nature (Noga, 2010). Septicemia caused by *Aeoromonas hydrophila*, which is also a bacterial infection, was detected wild common carp in Balıklıgöl and published in 2007 (Tel et al., 2007).

After *Trichodina* spp. was detected in 2005 in whitings, which is an economical fish species with a high distribution in the Black Sea, VHSV was detected in the same species in 2010 (Altuntaş and Ogut, 2010; Ogut and Palm, 2005). In the studies conducted, VHSV and IHNV were not found in the trout cultured in the eastern Sea between 2006-2007, but VHSV was found in many wild fish species in the samples between 2009-2011 (Ogut et al., 2013; Ogut and Altuntas, 2014). It is said that VHSV is endemic in this region and can be diagnosed in winter months. In addition, it has been noted in the studies that the virus can be transmitted to cultured fish. It is said that this virus has not yet been found in freshwater and it is important to prevent trout transfer from the Black Sea to freshwater (Altuntaş and Ogut, 2010). Especially considering that Turkish salmon farming is an important economic source in this region, VHS-oriented studies should be increased. Again, according to the study conducted in the eastern Black Sea region, IPN virus was found in cultured trout between 2006 and 2007, but not in wild populations (Ogut et al., 2013). According to the results of this publication, the IPN virus was isolated in the tests performed from hatcheries and it was proven that it was transported from hatcheries to the sea. These studies emphasize the importance of health screening during fish transfers. Besides, the presence of IPNV was examined in the samples collected between 2005 and 2014, and the researchers said that the interaction between wild and freshwater transmission of IPNV was clearly indicated because IPNV was detected in both cultured and wild turbot samples taken in 2010 (Tamer et al., 2022).

It is not surprising that the *Benedenia* sp., which was only detected in 2019 (Turgay et al., 2019) in the shark living in the Black Sea, was seen in the meagre cultured in the Mediterranean in 2005 (Toksen et al., 2007). Considering that the Meagre (*Argyrosomus regius*) grown in the Karaburun peninsula of İzmir still feeds on sardines (*Sardina pilchardus*) caught in the wild, it can be said that parasite transmission to cultured fish is more effective in this way (Figure 4).

There are more studies on parasites than other microorganisms in natural fish in Turkish waters. Other than those mentioned above, some examples of studies conducted in Türkiye are given in Table 2.



Figure 4. Meagre fed with Sardines (caught in the wild) in netcage at Karaburun peninsula, İzmir (Original photo: M.D. Demircan, 2022)

Table 1. Some researchs about fish pathogens in wild fish populations conducted in Türkiye

Fish Species (Latin)	***Common Name***	***Pathogen***	***References***
Mugil cephalus *Chelon labrosus*	Mullet Thicklip Grey Mullet	*Photobacterium damselae subsp. piscicida*	(Tanrıkul and Çağırgan, 2001)
Merlangius merlangus	Whiting	*Trichodina spp.*	(Ogut and Palm, 2005)
Dicentrarchus labrax *Mugil cephalus*	Sea Bass Mullet	*Vibrio anguillarum Vibrio ordalii*	(Demircan and Candan, 2006)
Alosa immaculata, Mullus barbatus Gaidropsarus vulgaris, Scorpaena porcus, Trachurus mediterraneus, Merlangius merlangus Uranoscopus scaber Belone belone Neogobius melanostomus, Raja clavata Engraulis encrasicolus	Pontic shad Red mullet Three-bearded rockling Black scorpionfish Mediterranean horse mackerel Whiting Stargazer Garfish Round goby Common Stingray Anchovy	Viral Haemorrhagic Septicaemia Virus (VHSV): *Novirhabdovirus*	(Ogut and Altuntas, 2014)

Table 1. Continue

Fish Species (Latin)	***Common Name***	***Pathogen***	***References***
Dasyatis pastinaca	Common Stingray	*Benedenia sp.*	(Turgay et al., 2019)
Coryphaena hippuru	Common Dolphinfish	*Caligus quadratus Shiino*	(Özak, 2020)
Epinephelus aeneus	White Grouper	*Gnathiid* isopod	(Genc et al., 2005)
Rutilus rutilus *Cyprinus carpio* *Silurus glanis* *Esox lucius* *Leuciscus cephalus* *Vimba vimba* *Perca fluviatilis* *Scardinus erythrophthalmus* *Carrassius gibelio*	Roach Common carp European catfish Pike European chub Vimba Perch Rudd Prussian carp	*Trichodina spp.* *Ancylodiscoides vistulensis* *Tetraonchus monenteron* *Dactylogyrus spp.* *Paradiplozoon homoion* *Bothriocephalus acheilognathi* *Caryophyllaeus laticeps* *Raphidascaris acus* *Eustrongylides excisus* *Dactylogyrus spp.* *Ergasilus spp. Glochidia* larvae	(Yardımcı et al., 2018)
Cyprinus carpio	Common Carp	*Trichodina acuta,* *Trichodina mutabilis,* *Trichodina nigra* *Trichodina subtilis* *Apiosoma piscicola* *Epistylis sp.*	(Özer and Erdem, 1998)
Mugil cephalus	Mullet	*Myxobolus episquamalis* *Myxobolus ichkeulensis*	(Özak et al., 2012)
Merlangius merlangus	Whiting	Viral Haemorrhagic Septicaemia Virus (VHSV): *Novirhabdovirus*	(Altuntaş and Ogut, 2010)
Belone belone	Garfish	*Caligus adanensis sp. nov.*	(Özak et al., 2019)
Cyprinus carpio	Common Carp	*Argulus foliaceus*	(Ozer and Erdem, 1999)
Anguilla anguilla	European eel	*Shewanella putrefaciens,* *Aeromonas sobria* *Aeromonas veroni*	(Korun et al., 2019)
Gaidropsarus mediterraneus *Trachurus trachurus* *Spicara flexuosa* *Zosterissesor ophiocephalus* *Neogobius melanostomus*	Shore rockling Atlantic horse mackerel Blotched picarel Grass goby Round goby	*Scolex peuronectis*	(Güneydağ et al., 2017)
Anguilla anguilla	European eel	*Anguillicoloides crassus*	(Innal et al., 2019)
Stizostedion lucioperca	Zander/Pikeperch	*Pseudomonas sp.*	(Timur and Timur, 1985)
Cyprinus carpio	Common Carp	*Aeromonas hydrophila*	(Tel et al., 2007)
Atherina boyeri Risso	Big-scale Sand Smelt	*Diplostomum sp.* *Tylodelphys clavata* *Bothriocephalus acheilognathi* *Eustrongylides excisus*	(Özesen Çolak, 2013)

Table 1. Continue

Fish Species (Latin)	***Common Name***	***Pathogen***	***References***
Lagocephalus spadiceus *Lagocephalus suezensis*	Pufferfish	*Taeniacanthus lagocephali*	(Özak, Demirkale, and Yanar, 2012)
Cyprinus carpio	Common Carp	*Dactylogyrus vastator* *Gyrodactylus elegans* *Argulus foliaceus* *Ichthyophthirius multifiliis* *Trichodina nigra* *Schistocephalus* sp. *Caryophylaeus* sp.	(Cengizler et al., 2001)

7. Recommendation and Conclusion

As suggestions, what can be done in our country is discussed under this heading, first for aquaculture and then for the protection of natural resources. The first thing we should pay attention to should be fish caught from nature to be used as broodstock or live organisms brought from abroad. Cultivation of non-native fish species will also lead to the introduction of new pathogens (FAO, 2023). Due to global trade, livestock transportation plays a major role in the transmission of diseases from nature to culture.

To prevent the transmission of pathogens between wild fish and cultured fish, it is important to implement biosecurity measures, such as quarantine procedures, the use of disinfectants, and the proper disposal of waste materials.

There is a requirement for quarantine and monitoring in appropriate periods for fish and aquatic creatures collected from nature all over the world. This period varies according to national or regional regulations and there are different applications for different species (Arthur et al., 2008). In addition to these, there are also international standards that every country must comply with. In fact, if necessary biosafety measures are taken before each new species is cultivated, the damage that will occur afterwards will be minimized. In particular, the use of specific pathogen free (SPF) stocks is the first and most effective safety measure. At this point, it is necessary to mention the importance of advanced breeding systems. SPF stocks are stocks that are determined by sensitive and appropriate diagnostic methods and must be kept under constant control. When fish are obtained from SPF stocks, raising them in a system that provides the same biosecurity conditions ensures that these characteristics are preserved. Of course, it is not possible to maintain these conditions in offshore systems. These conditions are possible with recirculating systems, which are also valid for inland fish, primarily for marine fish. With these systems, both the contamination that can spread to the environment can be prevented, and the pathogens that may come from nature can be

prevented. In particular, the use of ozone, which is a unit of these systems, has been proven to be effective for the destruction of pathogens in water (Gonçalves and Gagnon, 2011).

There are natural problems when streams or reservoirs are used as water sources in aquaculture facility. Wild fish, including fry and eggs harboring parasites, pathogenic bacteria or viruses, may be native to these sources. It is the best way is to disinfect these waters with ozone or ultraviolet light, however, the effectiveness of the treatment rely on the physical nature of the water (Plumb and Hanson, 2011).

After disinfection of the water of fish in aquaculture or in net-cages where disinfection is not possible, nutrition should be given importance and a husbandry procedure should be carried out to minimize stress. In addition to the nutrients (amino acids, fatty acids, polysaccharides, minerals and vitamins) needed by the fish depending on their species, it would be appropriate to supplement their diet with prebiotics, probiotics, immunostimulants, herbal additives when their immune systems are weakened, that is, when environmental conditions suddenly change (Oliva-Teles, 2012). Of course, prophylactic vaccination is of great importance in the prevention of diseases and should not be forgotten.

Although one of the methods that can be applied is to use challenge tests in the selection of genetically resistant individuals, it is also known that the cost of making these trials for each rootstock will be very high and time-consuming (Yáñez et al., 2014). It should not be forgotten that it will be an important and less costly method to prevent the occurrence and spread of the disease, if the producers follow their own rootstocks (indirect measures) and ensure the continuation of the strains with high disease resistance.

It is important to monitor the health of wild fish populations and to address any issues that arise in order to conserve these valuable resources and maintain their populations. Some common methods used in these types of studies include:

1. Observational studies, where the researchers observe and document the incidence and spread of diseases in wild fish populations.

2. Experimental studies, where the researchers manipulate variables in controlled conditions to study the effects of different factors on disease dynamics.

3. Sampling and monitoring studies, where the researchers collect and analyze samples from wild fish populations to determine the prevalence of disease and the presence of pathogens.

4. Mathematical models, where the researchers use mathematical models to simulate the spread of diseases in wild fish populations and to evaluate the effects of different management strategies.

It is difficult to determine which method is used the most among these publications, as it varies depending on the specific study. Some studies may use a combination of methods, while others may focus primarily on one method. The choice of method will depend on the specific research questions, the availability of data and resources, and the objectives of the study.

The fact that there is an important need to increase the surveillance of wild fish in our country is understood from the increase in research on this subject in recent years. Increasing the feasibility of research conducted in the world for our country can be possible with the follow-up of developing technology. For example, advanced diagnostic methods are used to detect important diseases in fish in the wild at an early stage of development. Field diagnostic systems POCKIT™ (manufactured by GeneReach Biotechnology Corp.) was developed and is in use for TILV diagnosis. This fast commercial kit with results in 45 minutes has been approved by the OIE (WOAH) (Tang et al., 2021).

As a result of many studies, researchers refer to the importance of the preventive role of monitoring. Health monitoring of aquatic organisms should be started in nature and a common digital platform that shows which pathogen are found in which region, should be created where everyone can access. To minimize the risk associated with fish diseases at farm level or in wild fish, both parties, scientists responsible for aquaculture and fisheries, business owners and government managers should communicate and collaborate better. In Türkiye, there is a need to increase surveillance of wild fish using advanced diagnostic methods to detect significant major disease problems at an early stage of their development. As a result of many studies, researchers refer to the importance of the preventive role of monitoring (Gozlan et al., 2014; Olivier, 2002).

eDNA studies are now recognized as a widely used technique for pathogen screeninig in nature (Chapman et al., 2021). Pathogen detections can be made with this technique in aquaculture or wild aquatic environments without touching the living things (Sana et al., 2018). Various sensor techniques are being used to develop systems that give warning without the proliferation of microorganisms.

Risk assessment and modeling approaches are useful tools that have recently come into use to predict the spread of disease. In order to prevent the spread of *Myxobolus cerebralis* in the USA and Canada, many water sources are monitored (Alberta Environment and Parks, 2018). It is very interesting that *Myxobolus cerebralis*, which is mentioned as a carrier of birds, has not yet been reported in the Australian continent (Department of Agriculture, 2020).

As can be seen from the examples, we continue to search for a solution to the still not fully understood enigma of whether the chicken or the egg comes from the egg. We need to do more detailed studies to prevent this ecologic problem, which we cannot decide which is more infective. In recent years, we see that the foreign fish species entering our country are increasing day by day. Consequently, it is stated that puffer fish, which is one of the many new fish species entering our waters from the Red Sea, migrated from the coasts of Japan and India and carries the parasites on them to the Mediterranean, and that these species may threaten economically important species (Özak, Demirkale, and Yanar, 2012) (A.A. Ozak, personal communication, July 13, 2023). This incident is a good example for the subject we are trying to explain in chapter. Knowing that every pathogen that will affect our country economically, whether it is cultured or wild fish, is of great importance for us, and identifying and preventing the transport routes of these pathogens should be at the forefront of our future research.

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CHAPTER 17

THE OLD AND NOVEL TECHNOLOGIES FOR DIAGNOSIS OF FISH DISEASES

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DOI: 10.26650/B/LS32LS24.2024.005.017

1. Introduction

Modern microbiology studies began in the 17th century by Robert Hooke and Anton van Leeuwenhoek was the first person to remark and describe bacteria with his microscope. In 1875, Ferdinand J. Cohn proposed the science of bacteriology as a branch of microbiology. Louis Pasteur found that the fermentation and spoilage stages of food are caused by bacteria and linked bacteria to human illnesses in his “germ theory”. This theory was later proved by Robert Koch and his findings which we still follow are called as the “Koch’s Postulates” (Fader et al., 2019).

Fishes, even those living in a wild population or in any type of a culture ambiance, can get sick due to the interactions of various factors. The presence of a pathogenic organism alone is insufficient to induce an outbreak. The “Sniezko circle” explains fish diseases in the simplest way (Plumb and Hanson, 2006). Fish individuals can get sick when there are pathogens around only if there is a deficiency in the environmental conditions and/or the immune system of the fish itself. Depending on the ecological harmony, in the disease cases of wild populations, fishes recover owing to their immune system functions or after the environmental conditions improve. Even if they don’t die, they become more vulnerable to predator attacks

and become an easier prey. If the strength of the immune system or the enhancement of the environmental conditions is not sufficient, the fishes die and they are “recycled” in the environment via the saprophytic organisms. But in culture conditions, there is at least a commercial concern and due to its concept, fish are kept in a closed area with a dense population. Due to this fact, if a disease case is induced, it is spread faster and may affect a high percentage of the population. Hence, researchers and companies from all over the world have focused on the prevention, identification and treatment of fish diseases.

2. Disease Diagnostics

Aquaculture showed a great improvement worldwide as well as in Türkiye, which will be discussed in relevant chapters of this book. As the sites for aquaculture and the species cultured diversified, also with the interaction of other environmental factors, infectious diseases showed an increase and diversification of pathogens was observed. In this chapter, traditional and novel methods used for the isolation, detection and characterization of fish pathogens, prevention and treatment strategies and future trends will be discussed.

A great number of techniques are available to detect pathogens in fish and the aquatic environment. However, a combination of the old and novel techniques is often recommended for a reliable and explanatory diagnosis of the problem. Various basic traditional methods were utilized for fish disease diagnostics which include phenotypic and biochemical identification of fish pathogens and histological examination of fish tissues. Traditional methods and immunodiagnostic methods are still very important and commonly used, but molecular technologies, in particular, PCR-based methods and some other methods, are also widely used and still being developed. There has been a rapid expansion of rapid diagnostic techniques for use in aquaculture over the last decades, as methods used in clinical and veterinary medicine have been customized to be used for aquatic microbiology, commercial reagents and kits have become available and the extent of laboratory facilities and expertise of available staff to perform the tests. General procedures and methods in use were listed by the OEM (World Organisation for Animal Health, formerly Office International des Epizootics) in Aquatic Animal Health Code (Table 1) as a guideline for aquatic animal health specialists also known as fish pathologists. Modern methods provided real progress in the understanding of the taxonomy of fish pathogens and have led to an insight into the epidemiology of aquatic animal diseases (Snow, 2011). Recently, the focus in clinical medicine has been on the development of multiplex assays so that large numbers of samples from a series of pathogens can be rapidly processed. Some techniques are currently too expensive for use in aquaculture, but clearly have potential for the future (Adams and Thompson, 2011).

The ideal specimens for a successful disease investigation or health monitoring are live fish (Ferguson, 2006; Roberts, 2012). Right after anesthetic application and the external examination, fish must be dissected for internal examination, bacterial and virological inoculation or histological sampling. Anamnesis and clinical field observations (behavioral changes or external symptoms) are helpful for diagnosis. Samples can be taken either *on-site* or transported live to the laboratory under strict conditions. Invasive and non-invasive methods are used for the detection of fish health status and identification of fish diseases and disorders. Many of these techniques were first invented for human diseases and later applied in the veterinary sciences and aquatic microbiology. The basic difference here is the difference in the ways for microbial specimen collection as the fishes live in a completely different environment than other vertebrates. An important step for infectious disease diagnosis in fish is the isolation of the pathogen. Skin mucus or the gills are the targets for parasitic or fungal pathogens. Skin lesions can be selected for various bacterial agents. For a great percentage of bacterial and viral pathogens, hemopoietic organs such as the liver, kidney and spleen are selected for inoculation on the solid media. For some specific parasitic or fungal pathogens, the eyes or the brain can be examined. Intestines and blood are reliable targets for some internal parasitic pathogens (Roberts, 2012; Austin and Austin, 2016; Sitjà-Bobadilla et al., 2021).

Each technique developed for diagnosis, classification, prevention or treatment has its own advantages and disadvantages by means of applicability, reliability, distinguishability, standardization possibility, duration and cost. Besides, it should be environmentally friendly and should not treat the researcher’s health. Especially in the 21st century, environmental and ecological concerns became abundant, and new environmental-friendly methods and techniques that reduce the use of plastics and/or harmful chemicals became more favorable (Moshood et al., 2022).

Table 1. Diagnostic methods in aquaculture

Field diagnostic methods	Clinical signs (external examination of the fish to observe wounds or lesions on the skin, pigmentation, lethargy etc.) Behavioural signs (slow swimming, abnormal swimming, jumping etc.)
Clinical diagnostic methods	Gross pathology (internal examination of the fish sample to detect visual changes in the organs) Smears and wet mounts (smears prepared from fish mucus, blood smears and wet mounts prepared from fish gills or intestines to detect the presence of pathogens) Microscopic pathology of fixed sections (Histopathological examination of fish tissues for the detection of the effects of the disease case) Transmission electron microscopy (TEM) (Electron microscopic examination of the fish tissues to detect the effects of the disease case at the cell level)

Table 1. Continue	
Agent detection and identification techniques	Techniques aiming at direct detection or identification of the pathogen Microscopic methods (rapid staining of bacteria or fungi and motility tests) Agent isolation (isolation of the bacterial, viral or fungal pathogen on an appropriate media) Biochemical testing for bacteria and fungi Serological methods (detection of the pathogen based on the antibody-antigen reactions such as agglutination methods, ELISA, iFAT and immunohistology) Immune electrophoretic methods (characterization of the pathogen depending on its various antigenic structures such as membrane proteins or lipopolysaccharides via SDS-PAGE protein profiling or Western blotting) Molecular methods (detection, identification or characterization of the pathogen via its nucleic acids)
* This table was created after the written information from Ferguson, 2006; Plumb and Hanson, 2006; Roberts, 2012; Buller, 2014; Austin and Austin, 2016; Zrnčić, 2020; Sitjà-Bobadilla et al., 2021 and Aquatic Animal Health Code of WHO (2022)	

2.1. Methods used in the diagnosis of parasitic fish diseases

Major fish pathogenic parasite groups are the protozoans, helminths, nematodes, acanthocephalans, and crustaceans (Roberts, 2012; Patarnello and Vendramin, 2017; Sitjà-Bobadilla et al., 2021). Parasitological examination of fish is performed via the smears prepared from live fish mucus, and wet mounts prepared from the gills or intestines under a microscope. Blood smears or histological slides stained with various methods also help the detection of internal parasites. As the fish pathogenic parasites are bigger than the bacteria in size, they can be identified morphologically (Timur et al., 2009; Roberts, 2012; Zrnčić, 2020; Sitjà-Bobadilla et al., 2021).

2.2. Methods used in the diagnosis of fungal fish diseases

Representatives of the genera *Saprolegnia*, *Fusarium*, *Branchiomyces* and *Aphanomyces* generally cause secondary infections in fishes (Hoole et al., 2001; Plumb and Hanson, 2006; Roberts, 2012; Buller, 2014). Fish pathogenic fungi can directly be examined on skin smears under a microscope or they can be isolated from fish skin and eggs in the hatchery onto a solid medium (generally Sabroud-Dextrose Agar) and the hypha are stained with lactophenol cotton blue and identified under a microscope morphologically. Histological slides are also another way for the detection of fungal structures in fish tissues (Timur and Timur, 1984; Sitjà-Bobadilla and Alvarez-Pellitero, 1990).

Besides bacteria, aquatic fungi are also the main microorganism group affecting fishes. Raymond Jacques Adrien Sabouraud, who mainly worked in dermatology and mycology, invented a medium for the selective isolation of fungal pathogens in 1892. Sabouraud Dextrose Agar (SDA) is an agar growth medium that contains peptones, developed for the cultivation of dermatophytes and other types of fungi *in-vitro*. The acidic pH (5.6) of traditional Sabouraud agar and the addition of antibacterial substances, such as chloramphenicol, inhibits bacterial growth where dextrose concentration supports the survival of fungi (Odds, 1991). Also, various other media formulations for the isolation and cultivation of aquatic fungi other than SDA are available (Buller, 2014).

2.3. Methods used in the diagnosis of viral fish diseases

Viruses are small infectious agents which can multiply solely in an alive cell by utilizing their organelles for their own reproduction. Once penetrated inside the new cell, the genomic structures of the virus are created and virus replication begins. This process is also known as an infection. By the late 19th century, Martinus Beijerinck and Sergei Winogradsky and also Dimitri Ivanosvski worked on microbial physiology, ecology and diversity which paved the way for the discovery of viruses and the fundamentals of virology. Martinus Beijerinck referred to the sifted, irresistible substance as an “infection,” and this discovery is regarded as the beginning of virology. These studies and discoveries reached an advanced stage with the invention of the electron microscope by Max Knoll and Ernst Ruska in the 1930s. As the virions are as small as the nanometer level in size, the invention of the electron microscope allowed scientists to visualize their morphology. Despite it is not among the routine laboratory diagnostic procedures proposed by OEM, electron microscopy is used when necessary, especially for the diagnosis of virus infections (Timur et al., 2008) as well as for revealing some physiological processes in fish such as pinocytic activity in the intestines (Akaylı et al., 2017).

Cell culture or tissue culture is a technique where animal cells are maintained in controlled conditions in the laboratory. It was invented by American pathologist Montrose Thomas Burrows. After the target cells were isolated from tissues, they can be maintained *in-vitro*. These conditions may be changed for different types of cells but generally contains a rich cell culture medium. Hence the essential nutrients (minerals, vitamins, amino acids and carbohydrates), hormones, growth factors and gases (O_2 or CO_2) are supplied and the incubation ambient (temperature, osmotic pressure and pH) is under total control. Minimal Essential Medium (MEM) is a synthetic cell culture medium that is used for the maintenance of cells in tissue cultures *in-vitro*, and it was developed by Harry Eagle in 1959. It is based on calcium, potassium, magnesium and salts, glucose, essential amino acids and vitamins. Many variations of this medium (by adding different amino acids, vitamins and nutrients) have been developed that are named as DMEM (Dulbecco’s Modified Eagle’s Medium), α-MEM (Minimum Es-

sential Medium Eagle - alpha modification) and Glasgow's MEM, Hank's MEM (HMEM) and Leibovitz L-15 medium (Lakra et al., 2011; Roberts, 2012).

Wolf and Quimby (1962) developed RTG-2 (rainbow trout gonad), the first established cell line from a fish species. Since then, the number of fish-originated cell lines in use has expanded and several salmonid cell lines such as CHSE – 214 (from chinook salmon embryo) were developed (Lannan et al., 1984). The most widely accepted approach to viral fish disease case diagnostics has been the culture of the virus in a tissue culture *in-vitro*. Apparent cytopathic effects (CPE), such as necrosis, retraction, rounding, syncytial formation and detachment, are the expected reactions of virus growth in the cell monolayer. The majority of fish viruses cause several degenerative changes in cell culture to be monitored under an inverted microscope. Viruses produce characteristic types of CPE and the CPE observed can help to make an initial diagnosis, but the final diagnosis must be performed with further techniques. The viral culture must always be confirmed with another diagnostic method, such as the detection of viral nucleic acid or viral antigens (Roberts, 2012; Zrnčić, 2020).

Following the success and failures of traditional salmonid cell lines, various cell lines were also initiated from tropical marine fish. Recently, more than 300 types of fish cell lines were listed by Lakra et al. (2010). Later on, 3-D cell culture technologies were developed at the end of the 20th century and this brought the possibility of tissue engineering and regenerative medicine development (Cacciamali et al., 2022). IHN, IPN, VHS, lymphocyctis, viral erythrocytic infection, spring viremia of carp and infectious salmon anemia have been the main viral infections of fishes up to now. The study of viral fish infections continues to evolve, especially in the establishment of enhanced cell lines, cell culture media, comparative molecular biology of viruses, novel virus detection techniques, experimental pathogenesis studies and viral classification (Ferguson, 2006; Roberts, 2012; Austin, 2012; Patarnello and Vendramin, 2017; Zrnčić, 2020).

2.4. General bacteriology studies

Besides the harmless and beneficial ones, some bacteria are able to take advantage of temporary weaknesses in the host immune system to cause diseases (Liu, 2011; Tortora et al., 2019). A great number of bacterial species were identified as opportunistic or obligate pathological agents of fish diseases so far (Austin and Austin, 2016). Bacteriology studies mainly contain the isolation of bacterial cells from the sample material (fish, water etc.) on a pre-sterilized culture media, obtaining pure cultures and performing biochemical and phenotypic tests for identification. The classification of bacteria is based on their morphology

(e.g., rod, cocci, spirilla, and filament), cell wall properties (Gram staining characteristics), growth requirements (aerobic or anaerobic growth), biochemical profiles (enzymatic profile) and genetic characteristics (16S/23S rRNA) (Liu, 2011; Tortora et al., 2019). General traditional bacteriological procedures are still applied but serological, immunochemical and molecular biologic methods are also commonly used for the identification, classification and characterization of bacteria (Liu, 2011; Roberts, 2012; Austin and Austin, 2016). All of these techniques and chemicals are also available in Türkiye and carried out successfully in laboratories working on fish diseases diagnostics.

In some cases, such as *Flexibacter* infections (microorganisms with elongated larger cells) or *Renibacterium* infections (microorganisms that are hard to cultivate *in-vitro*), wet mounts freshly prepared from the wounds of the infected fish are useful. The general approach is the direct recovery of the bacterium, especially the ones that colonized the internal organs of the host. Besides, various techniques that aim the identification via the detection of the microorganism while it is still in fish tissues, based on the antibody-antigen reactions or the detection of their nucleic acids are also rising (Jaramillo et al., 2017).

Fish pathogens (mostly bacteria) can be classified as culturable and non-culturable (Liu, 2011) and generally the most reliable procedure for disease diagnosis is the isolation of the agent and culturing it *in-vitro*. Since Petri dishes, needles and the culture media formulations were first invented, researchers developed various formulations depending on the ecology, needs and behavior of the target microorganism. As the pathogens show species diversity in time, semi-selective or selective culture media formulations were developed after the general ones. For example, some general bacteriological media, namely Tryptic Soy Agar (TSA), Nutrient Agar (NA) and Brain Heart Infusion Agar (BHIA) (with small modifications in NaCl content or etc.) that contain basic nutrient substances are mostly adequate for the primer isolation of common aquatic bacteria. Later on, the need for the rapid or selective isolation or presumptive identification of these bacteria emerged, and scientists investigated different culture media. Selective media contain ingredients that suppress the growth of competing organisms and encourage the growth of desired ones, and differential media allow the desired organism to form a colony that is somehow distinctive (Tortora et al., 2019).

During the historical development of bacterial fish disease studies, various media specialized for a genus of species were proposed for presumptive or selective isolation and identification. Anacker and Ordal (1959) developed a selective medium for the *Cytophage*-group bacteria. Kobayashi et al. (1963) proposed TCBS (Thiosulfate – Citrate - Bile salts – Sucrose) agar medium for the selective isolation of both *Vibrio cholera* and various aquatic pathogenic

halophilic bacteria. Shotts and Rimler (1973) developed a selective bacteriological medium for the identification of *A. hydrophila*. Waltman and Shotts (1984) developed an inexpensive, easily prepared agar media that increase the chance of the recovery of *Yersinia ruckeri* from clinical material and infected fish. Bullock (1986) promoted a medium that is selective for *Flexibacter*-group bacteria. Waltman and Shotts (1990) formulated a bacteriological medium for the selective isolation of *E. ictaluri* and *E. tarda*. Alsina et al. (1994) (1994) developed a selective medium for the presumptive identification of *Vibrio anguillarum*. Lowenstein-Jensen Medium (LJ) is selective for the *Mycobacterium* representatives which was developed by Lowenstein and modified by Jensen (Austin and Austin, 2016). Glutamate Starch Phenol Red Agar (GSP-A) is used for the differentiation of fish pathogenic aeromonads and pseudomonads (Balta, 2020). Aeromonads produce yellow colonies whereas pseudomonads produce violet colonies on this agar. Some of these media are more widely used and also research on the isolation and culture media are still an important issue. Various bacteriological media that are used for the recovery and cultivation of important marine bacteria were evaluated by Austin (2019).

Two main issues for the recovery of bacterial fish pathogens are the content features and proportions (salinity, pH and other nutrients) of the isolation media and the incubation temperature. The isolation media should contain a similar salinity percentage to the environment that the fish to be sampled is living in. After isolation, the media should be incubated at a temperature similar to the environment that the fish to be sampled is living in (Roberts, 2012; Austin and Austin, 2016).

Once the bacteria are isolated from a moribund fish sample, the rest of the study relies on general microbiological procedures. The streak plate technique is obligately used to obtain the isolates in pure cultures. Visual examination of the colonies is the first guide for selecting the necessary diagnostic tests and reducing the time. Some of the criteria for colony description are color, size, shape, margins, elevation, brightness, opacity and viscidity (Roberts, 2012; Buller, 2014; Austin and Austin, 2016; Tortora et al., 2019).

Gram staining is still the first test to be performed for diagnosis but it was also shown that a non-staining 3% KOH solution method is also useful for the detection of the Gram group of a bacterial isolate (Buck, 1982). However, the main objective here is the visual detection of the cell and the cell-cluster morphology. Stained smears also allow the detection of possible contamination of the colony. Ziehl – Nielsen staining which aims the differentiation of bacteria into acid-fast and non-acid-fast groups is also useful in most Gram-positive cases. Bacteria that are not stained by the Gram staining method, particularly the mycobacterial

samples can only be visualized by acid-fast staining (Roberts, 2012; Austin and Austin, 2016). The “acid-fastness” or resistance of the cell wall to acid-alcohol decolorization after staining with carbol-fuchsine is characteristic of *Mycobacterium* species (Zrnčić, 2020).

Following the determination of the colony and cell morphology and Gram-stain characteristic of a freshly cultured isolate in pure colonies, selected distinguishing biochemical and physiological tests should be performed for identification. These tests generally involve biochemical testing for the presence of specific enzymes, utilization of various sugars as a carbon and energy source, ability to grow on different salinity or temperature ranges and resistance against certain antibiotic substances (Roberts, 2012; Buller, 2014; Austin and Austin, 2016; Tortora et al., 2019).

At the first step, some initial tests such as motility, oxidation/fermentation, cytochrome oxidase and catalase should be performed for the determination of the genus. Susceptibility to O/129 and growth on TCBS are also necessary tests when studying with aquatic Gram-negative rods. Coagulase is used for Gram-positive bacteria, staphylococci especially. A 20% KOH solution is used for the detection of flexirubin pigment. IMViC ([I] indole production, [M] methyl-red, [Vi] Vogues-Proskauer, [C] citrate) tests are required and useful for the biochemical identification of almost all groups of fish pathogenic aquatic bacteria. Enzymatic activities are widely used to differentiate bacteria. Even closely related bacteria can usually be separated into distinct species by subjecting them to biochemical tests (Tortora et al., 2019). With the addition of different amino acids (arginine dihydrolase, ornithine and lysin decarboxylase), production of acids from carbohydrates (glucose, arabinose, lactose, mannitol, inositol, sorbitol, sucrose, rhamnose, melibiose etc.), enzyme tests (hemolysis on blood agar, urease, gelatinase, amylase, lipase, ß-galactosidase etc.), utilization of citrate, reduction of nitrate, growth characteristics on various selective or semi-selective agars (TCBS, MacConkey agar, mannitol-salt agar, Baird-Parker agar, chromogenic agar, *Pseudomonas* selective agar etc.), growth on different salinity percentages (from 0% to 10%) and temperature levels (form +4°C to +40°C), around 30-50 tests, including phenotypic criteria and genus-level tests are used for the biochemical and phenotyping identification of fish pathogenic bacteria. Depending on the microorganism (reproduction cycle especially), the tests and the laboratory conditions, the duration of biochemical identification may take several days (for enterics) to several weeks (for mycobacteria) (Roberts, 2012; Buller, 2014; Austin and Austin, 2016).

As the “need for speed” in disease diagnostics has increased, scientists developed the idea for rapid diagnostic kits in different ways. Almost all of these kits were first developed for the rapid detection of human pathogens and later adopted in diseases of other organisms such

as fish. The analytical profile index or API is the classification of bacteria based on biochemical tests, allowing rapid identification which was developed for identification of clinically important bacteria. It was invented in 1970s by Pierre Janin and today it is produced as a standardized, miniaturized version of existing techniques, which can be time-consuming or complicated to prepare or read. Such tools are designed to perform several biochemical tests simultaneously and can identify bacteria within 4 to 24 hours via colorimetric instruments. These strips allow scientists to identify a freshly cultured pure bacterial strain belonging to a previously known species without a long and complicated apparatus cleaning and sterilization, chemical purchase and storage and test media preparation stages. Besides the most widely known and used Biomerieux API kits (API 20E, API 20NE, API-Staph, API-Strep, API ZYM, API 50CH and API RapidID32) there are various types of these kits available such as Biolog MicroPlatesGN2, GP2, AN, Enterotubes, BBL Crystal E/NF, commercially produced by various brands, and designed for the identification of different bacterial groups or genera. This is sometimes called numerical identification because the results of each test are assigned a number. After performing initial tests to determine the genus, these kits generally contain 15-20 biochemical tests that enable researchers to identify the organism at the species level. To increase the identification ability of these kits, the database of the results should be enriched as the strains of the same bacterial species isolated from different locations can generate different API profiles. Besides bacteria from different genera can also generate the same profile in these kits which will be confusing. Hence the results obtained from these kits should be compared and supported with other additional distinguishing tests.

Following the initial applications in the world (Grisez et al., 1991; Romalde and Toranzo, 1991; Sakai et al., 1993) and in Türkiye (Karataş, 1996), commercial kits became a widely used easy solution for the identification of fish pathogenic bacteria in aquaculture. API 20E, the most widely applied kit was used for the identification of the representatives of the genera *Vibrio* (Ercan et al., 2013; Çanak et al., 2014; Topçu et al., 2017), *Aeromonas* (Karataş, 1996), *Pseudomonas* (Kayış et al., 2009), *Yersinia* (Altun et al., 2010), *Photobacterium* (Yardımcı et al., 2020), *Shewanella* (Altun et al., 2014), *Citrobacter* (Altun et al., 2013a; Akaylı et al., 2021) and *Hafnia* (Akaylı et al., 2021) in Türkiye. Some other kits such as API 20NE (Akaylı et al., 2011a; Akaylı et al., 2011b; Balcı et al., 2023), API ZYM (Akaylı et al., 2011b; Turgay et al., 2015a; Yardımcı and Timur, 2016), API-Staph (Turgay et al., 2015a; Akaylı et al., 2019; Çanak and Timur, 2020), API-Strep (Ürkü and Timur, 2014), Rapid ID 32 (Kubilay and Uluköy, 2004) and Microgen GN-ID A + B (Balcı et al., 2023) were also applied in Türkiye.

The main inadequacy of these kits is the lower database information on the fish pathogenic bacteria, especially the enteric ones. Generally, *Hafnia alvei* and *Yersinia ruckeri* strains are misidentified and *vice-versa* (Topić-Popović et al., 2007) and also incubation duration and temperatures should be optimized for *Y. ruckeri* (Candan and Yazıcı, 2000; Altun et al., 2013b). Also, there are more than 20 profile codes in the API 20E database for *V. anguillarum* whereas there are fewer codes for other fish pathogenic vibrios and in some strains, *V. anguillarum* can be misidentified as *A. hydrophila* (Buller, 2014). Analysis of the results of commercial identification kits for the identification of *Vibrio*'s demands a great deal of caution (O'Hara et al., 2003). Besides, many motile aeromonads of distinct species are identified as *Aeromonas hydrophila* and *P. damsela* subsp. *piscicida* strains can be misidentified as *Pseudomonas fluorescens*/*putida* with API 20E (Topić-Popović et al., 2007).

Besides online databases that are intended for the analysis of the results obtained with identification kits (such as ApiWeb), some independent software were also launched. *Identax* is a computer-assisted microorganism identification software by using only results obtained from conventional biochemical tests (Flores et al., 2009). ABIS (Advanced Bacterial Identification Software) is a newly launched online powerful tool for microbiology laboratories and its encyclopedia connection provides essential information about the ecological significance, pathology and other features of the identified strains (Sorescu and Stoica, 2021).

3. Fish Histology and Histopathology for Disease Diagnosis

Histology is defined as the scientific study of the microscopic structure (microanatomy) of cells and tissues and its roots back to the 17th century, to the first findings and descriptions by Marcello Malpighi and Marie François Xavier Bichat. Histology and histopathology have been very useful as a diagnostic tools in fish health studies (Ferguson, 2006; Meyers, 2009; Roberts, 2012). To reveal the histological appearance of injured tissues, the researcher must be familiar with the normal histology of the animal under examination. Organs and tissues respond to stimuli, either physiological or pathological, in various ways, many of which can be identified and studied by histology. These morphologic changes can provide an important tool for diagnosticians. Certain morphologic changes in diseased tissues are useful for presumptive diagnosis (Ferguson, 2006; Meyers, 2009; Roberts, 2012). While sometimes histology is sufficient by itself for diagnosis, it can become more powerful if supported by other diagnostic methods such as bacteriology, virology, serology, and toxicology. Accumulation of heavy metals like cadmium (Cd), chromium (Cr), cobalt (Co), iron (Fe), lead (Pb), nickel (Ni), titanium (Ti), zinc (Zn), and their mixtures in fish tissues (Mahino et al., 2014) or the histopathological alterations caused by cyanobacterial toxins (Panagiotis et al., 2014) can be demonstrated by fish histopathology studies.

The five steps of preparing slides for histology are fixation, processing, embedding, sectioning and staining. Only tissues of newly dead or moribund individuals must be sampled for a successful histopathological examination. While phosphate-buffered formalin solution fixation is widely used as it conserves histological structures with minimum deformation, some special tissues require fixatives other than formalin. Bouin's fixative is commonly used for bonny tissues to dissolve $CaCO_3$ followed by an ethanol washing step for 1-2 days. During processing, generally, increasing ethyl alcohol series and chloroform are used. But these chemicals dissolve fat droplets in the tissues and this step can also be modified according to the tissue to be studied (Ferguson, 2006; Meyers, 2009; Roberts, 2012).

Generally, two counter stains are used in histology which have opposite characteristics for electric charge, pH level, solution media and subsequently target component in a cell. The type of tissue to be studied is also the main decisive factor in stain selection. Numerous variations of Haemotoxylin and Eosin staining are present for general histology and histopathology examination, which is the most commonly used method for staining of any type of tissue. Generally, the Giemsa stain protocol is used for the differentiation of parasites in fish tissues and Ziehl-Nielsen staining protocol is followed for the demonstration of mycobacterial infections. Periodic acid – Schiff (PAS) is used for the demonstration of carbohydrates, the Feulgen reaction is used for the visualization of DNA and Van Gieson is used for the demonstration of collagenous tissue (Ferguson, 2006; Meyers, 2009; Roberts, 2012; Timur, 2013). As it is a general stain to demonstrate basic degenerations in fish tissues, Haemotoxylin and Eosin was used in numerous studies on fish histology and histopathology in Türkiye (Ürkü and Timur, 2014; Çanak and Timur, 2020; Akaylı et al., 2021).

Although the stains are developed to examine the normal cells or the cellular degenerations that occurred in the tissue itself, some methods for the detection of pathogens present in the tissue were also developed according to the development of the concept of pathogenandhost relations. A good example of this aim is the variations of tissue Gram staining methods (Table 2). They generally stain the tissue cells with a pale color and an opaque color to highlight the bacterial cells. After comparative use of these four above-mentioned tissue Gram staining methods on moribund fish tissues infected with Staphylococci, Hucker and Twort's staining method was recommended by Çanak and Timur (2020) as it gives better results for distinguishing Gram-positive bacterial cells and the hemosiderin pigments accumulated in fish tissues. Despite the fact that they are non-species specific, they can distinctly demonstrate the presence of Gram-positive and -negative bacterial cells in a sample. Species-specific techniques that combine histology and immunology will be discussed later in this chapter.

As a more popular concern in the late 20th century, various modified staining methods that reduce plastic-dissolving xylene steps were developed for the detection of microplastics in the tissue. In a method proposed by Gonçalves et al. (2018), Davidson's fixative is used to reduce fixation time; xylene and chloroform are eliminated as they damage, deform or completely dissolve microplastics; isopropanol solutions with a significantly lower toxicity level compared to xylenes are used in processing and staining steps which don't react with polystyrene throughout processing process. Commercial mounting media contain strong solvents (mostly xylene), hence the slides were mounted with aqueous glycerol (50% v/v). Since it is a rather new technique, it is not yet widely used in fish histology studies, but it was used to demonstrate the presence of microplastic particles in the gastrointestinal tract of wild mullet populations in Türkiye (Çanak et al., 2019).

Table 2. Comparison of various tissue Gram staining methods

	Brown and Brenn	**Modified Brown and Hopp**	**Hucker and Twort**	**Modified Brown and Brenn**
Stain	Aqueous 1% Crystal violet	Aqueous 1% Crystal violet	Alcoholic 2% Crystal violet with ammonium oxalate	Alcoholic 2% Crystal violet with ammonium oxalate
Counter stain	Aqueous 0,25% Fuchsin	Alcoholic 1% Fuchsin	Twort Solution (a mixture of 1% alcoholic Neutral Red and 1% alcoholic Fast Green FCF)	Aqueous 0,25% Fuchsin
Cell wall	brownish yellow	blackish purple	dark blue	brownish yellow
Cytoplasm	pale yellow	red	pale turquoise	pale yellow
Nucleus	brown	purple	blue	brown
Gram-positive bacteria	black	black	black	black
Pigments	black	black	reddish brown	black
Reference	Brown and Brenn, 1931	Engbæk et al., 1979	Bancroft and Stevens, 1990	Carson and Hladik, 2009

Apart from the techniques and chemicals; automated tissue processing, sectioning and staining machines were developed during the late 20th century. Also, powerful microscopes were developed and they are equipped with high-resolution digital cameras and software which allow researchers to take better pictures of their results with additional measurement features or filtering functions. Once the picture is digital, there is no limit to additional analyses. On the other hand, automation may reduce researcher-related mistakes during processing and/or staining, but the whole histology processes still need the touch of an experienced staff.

As light microscopy has a relatively limited magnification power, electron microscopy is also used for diagnosis in fish diseases. Courses in histology focus on the preparation of histology slides, relying on previous mastery of anatomy and physiology. Light and electron microscopy techniques and their equipment and chemicals are almost completely different, but they are all performed to examine cellular degenerations or the presence of foreign structures in the body. This procedure requires chemical fixation of the tissue, epoxy resin embedding, ultra-microtome equipped with a diamond knife, lead or uranium staining and electron microscopic imaging steps. Despite most of the chemicals used in this method being hazardous and installation and running an electron microscope laboratory might be very expensive, this method provides visual images of viral particles and cell damage where only general cytopathic effects can be observed in light microscopy (Roberts, 2012).

4. Fish Haematology, Serology and Immunologic Methods for Disease Diagnosis

As most of the cells and chemicals related to immune reactions are served via the blood in the fish body, the study of serology, immunology and haematologic properties are of crucial importance for understanding the fish physiology and health status. Haematology is the study of the cause, prognosis, treatment, and prevention of diseases related to blood and blood disorders and blood properties. Serology is the science that studies serum and immunological reactions and properties such as antibodies against a specific antigen that are present in serum. Immunology deals with the immune system and the cell-mediated and humoral aspects of immunity and immune responses in detail. Edward Jenner, an English doctor who is also known as the father of immunology developed the world's first immunization against the smallpox virus and used the terms "immunization" and "inoculation" in 1775. After the discovery of factors in the blood serum that destroy bacteria, Jules Bordet and his colleagues at Pasterus Institute developed the complement-fixation test for the detection of the presence of infectious agents in a blood sample (Fader et al., 2019; Tortora et al., 2019).

The application of non-lethal and cheap techniques for monitoring fish health is regarded as a major issue to increase aquaculture production. Haematology is among the key tools to assess the health of different fish species since it allows a reliable non-lethal evaluation (Satheeshkumar et al., 2012). Thus, the establishment of haematological values should be developed as a reference for health evaluation in fish. Fish haematology is generally used for the determination of fish health status. Interpreting the results obtained from fish haemotology combining the knowledge on fish physiology, environmental conditions and fish diseases provides valuable information. Various factors are affecting haematologic parameters in fish

such as age, weight, sex, temperature, environmental conditions, stock density, and pathogens (Whitehead et al., 2019). Fish blood can be sampled from live fish for haematology, blood biochemistry, plasma parameters and serologic analysis for the detection of viral or bacterial pathogens. The automated haematological analysis devices developed for mammals are usually not applicable for fish blood evaluation because of the differences in erythrocyte structure. Hence many fish blood cells count analysis methods are carried out manually (Fazio, 2019).

Blood collected from the live fish sample can be immediately smeared on a slide without the addition of any anti-coagulant and stained with various protocols such as May Grunwald and Giemsa or Dif-Quick. These protocols include counter-stains for the differentiation of the blood cell types and their special structures under a light microscope. Fish blood samples can also be collected into tubes containing various anti-coagulants such as EDTA or heparin for further blood count methods which should be performed manually since the automated systems mostly designed for mammalian blood samples may not differentiate fish blood cells accurately (Ferguson, 2006; Roberts, 2012; Fazio 2019). Packed cell volume (PCV) is the quantitation of the percentage of erythrocytes in blood in a microhematocrit tube in spun a microhaematocrit centrifuge. On the other hand, haematocrit is a value obtained with an automated blood analyzer using the concentration of red blood cells and mean cell volume (MCV) (Voigt and Swist, 2011). Since these values are not well listed for fish, PCV is the correct term for fish blood. Natt and Herrick solution is generally recommended for dilution and staining of fish blood during manual leukocyte counts in hemocytometry on a Neubauer counting chamber. This solution stains erythrocytes pink or red and all types of leukocytes are stained dark purple with distinct margins.

A decrease in the PCV of fish blood can be observed due to hemorrhages caused by an infectious disease or other environmental factors such as toxins, nutritional deficiencies or low water temperature while the increase in PCV has been associated with the increased activity of fish or acute handling stress (Ferguson, 2006; Roberts, 2012). A higher leukocyte count is generally observed during bacterial infections due to increased immune response (Ferguson, 2006; Roberts, 2012). While the cells of some bacterial pathogens or blood parasites can be detected in fish blood smears, detection of the cytopathic effects of *Ricckettsia*-like organisms (Timur et al., 2013) or some fish pathogenic viruses (Timur et al., 2008) provide useful information for disease diagnosis. Haematological examination of blood chemistry can also explain many of the pathological aspects of diseases in fishes (Del-Pozo et al., 2010) since many stress factors in fish can be tracked via blood sampling (Yavuzcan-Yıldız et al., 2017; Yavuzcan-Yıldız and Bekcan, 2020; Akaylı et al., 2022). When used together with other

routine diagnostic techniques, haematology is useful for the identification of stress-causing factors or infections that are limiting success in aquaculture (Fazio, 2019). Some nutritional deficiencies such as folic acid deficiency causes the development of abnormally shaped erythrocytes in fish (Seibel et al., 2021).

Fish defend themselves against pathogens with their immunological strategies namely humoral and cellular pathways, alteration of membrane permeability/fluidity and antimicrobial peptides or enzymes (Khalil et al., 2023). Antibody, also called immunoglobulin is a protective protein produced by the immune system due to the presence of a foreign structure, known as antigen. The antibodies recognize and latch onto antigens in order to remove them from the body. A wide range of substances is regarded by the body as antigens, including disease-causing organisms and toxic materials. When an alien substance enters the body, the immune system is capable of identifying it as foreign because molecular structures on the antigen surface differ from those found in the body. To eliminate the invader, the immune system activates various mechanisms, such as antibody production. Antibodies are produced by B lymphocytes (or B cells). When an antigen binds to its surface, a B cell is stimulated to divide and mature into a group of identical cells called a clone. The mature B cells, called plasma cells, secrete millions of antibodies into the bloodstream and lymphatic system (Branson, 2008; Lieschke and Trede, 2009; Fletcher and Secombes, 2015).

Serological and immunologic methods for prompt diagnosis developed rather steadily in fish. These methods may detect the presence of antigens or antibodies against a particular antigen within fish tissues and blood or identify the pathogen (Roberts, 2012). Especially in asymptomatic infection cases, a presumptive diagnosis can be performed with polyclonal antibodies in any serologic method, namely FAT, IFAT, whole-cell (slide) agglutination, precipitin reactions, complement fixation, immunodiffusion, latex agglutination, passive haemagglutination or ELISA (Austin and Austin, 2016).

Antigen-Antibody interaction-based agglutination reactions are easily applied immunological tests which provide rapid detection of bacterial fish pathogens. When the species-specific antibody is present, slide agglutination is the simplest and the fastest way to perform this test as the visible agglutination (clumping of the pathogen and the antibody in a transparent saline solution) is observed in 10 seconds. For some fish pathogenic bacteria, antisera induced against different serotypes can be used and the characterization of the pathogen can also be performed. *V. anguillarum* isolates of cultured gilthead sea bream (Çanak, 2011) and European sea bass (Akaylı and Durna, 2017) were shown to be serotype O1 by using slide agglutination and the latter was also shown by a similar technique, Dot-Blot assay (Akaylı and Durna,

2017). Also *T. maritimum* isolates of European sea bass were identified as serotype O1 by Dot-Blot (Yardımcı and Timur, 2016). The BIONOR Mono-kit R commercial kits, based on a similar principle, latex agglutination of bacteria, were used for quick and accurate detection of *V. anguillarum*, *Y. ruckeri*, and *R. salmoninarum* (Romalde et al., 1995; González et al., 2004b). Agglutination-based kits Mono-Va (for *V. anguillarum*) and Mono-Pp (for *Photobacterium damselae* ssp. *piscicida*) and their versions to be used for identification of these bacteria in fish tissues Aquarapid-Va and Aquarapid-Pp were used successfully in a long-term monitoring study (Korun, 2004). These kits are regarded as inexpensive and suitable agents for the study of mass sample groups in fish health laboratories but are limited for global use due to the serological differences among strains (Plumb and Hanson, 2011).

A test called the enzyme-linked immunosorbent assay (ELISA) is widely used for determining specific antibodies or pathogens in fish serum. In a direct ELISA, known antibodies are placed in the wells of a 96-well ELISA microplate, and a reaction between the known antibodies and the bacteria in the serum provides identification of the bacteria. ELISA also allows researchers the quantitation of fish antibodies against a specific antigen with high sensitivity (Roberts, 2012; Austin and Austin, 2016). Besides determining the presence of an antigen or antibody in the specimen, the quantitative analysis provides useful information for the protective status of vaccines used in aquaculture. As the enzyme-labeled antibody used in this technique is bound to an indicator substrate (such as streptavidin- horseradish peroxidase complex), colorimetric change analyzed under a spectrophotometric device provides an exact numerical value. Humoral antibody production against *Y. ruckeri* in rainbow trout (Kubilay and Timur, 2001), against *V. alginolyticus* (Akaylı et al., 2008) and *V. ordalii* (Akaylı et al., 2010) in gilthead sea bream, against *L. garvieae* in rainbow trout (Ürkü and Timur, 2014) and against *T. maritimum* (Yardımcı and Timur, 2016), *V. anguillarum* serotype O1 and *P. piscicida* (Akaylı and Sönmez, 2021) in European sea bass was demonstrated by using ELISA.

One of the Gel-Diffusion (GD) methods, described by Ouchterlony (1949) has been extensively used in fish immunology. It is based on antigen-antibody interaction that they are allowed to move towards each other by passive diffusion on a solid agar gel medium. A visible opaque insoluble complex is formed as a precipitin line indicating a positive result of an antigen-antibody reaction.

Serological methods are indirect methods to indicate infection and have proved very useful in fish diagnostics. To date, few have been validated and it is likely that they will be most useful for viral infections (Adams and Thompson, 2012). Serological testing can differentiate not only among microbial species but also among strains within species. Strains with different

antigens are called serotypes, serovars, or biovars. Rebecca Lancefield, a prominent American microbiologist was able to classify streptococcal serotypes by founding that the different antigens in the cell walls of various serotypes of streptococci stimulate the formation of different antibodies. A bacterial pathogen found commonly in Mediterranean marine aquaculture, *P. damselae* subspecies *piscicida* is serologically homogeneous, with no O-serotypes present (Bakopoulos et al., 1997) and being uniform antigenically, serologic rapid identification kits could be produced (Korun, 2004; Zrnčić, 2020). But in the case of *V. anguillarum* with 23 O-serotypes depending on the European serotyping scheme of this species (Pedersen et al., 1999), antibodies against the possible fish pathogenic O1, O2 and O3 serotypes were included in a rapid commercial agglutination kit which allowed the kit to identify *V. anguillarum* but could not provide information about the serotype (Korun, 2004). Some other above-mentioned techniques that use serotype-specific antigens or SDS-PAGE protein profiling studies should be carried out for the exact determination of the serotype (Çanak, 2011).

There are also techniques that are applied on the fish tissue slides which combine immunological reactions with histology, also known as the immunohistochemical methods. The fluorescent antibody technique (FAT) is used for the detection of antigens or antibodies in fish. The smear from the kidney is treated with the fluorescein isothiocyanate (FITC) labeled species-specific antisera (generally induced in rabbits) and the positive antigen-antibody complexes are observed as bright green areas under a fluorescence microscope. In the indirect fluorescent antibody technique (IFAT), the first species-specific antibody is overlaid with the smear and later a fluorescent-labeled secondary antibody targeting the first one is added. Viral and bacterial fish pathogens can also be detected via immunoperoxidase methods in fish tissues. In this method, antibody and horseradish peroxidase-labeled substrate steps are added to the histological staining procedures to demonstrate antigen-antibody reactions. This method shows the presence of the pathogens in fish tissues and also the location of the pathogens can be detected. One other advantage of this method against similar methods, namely FAT and IFAT is, this method does not require a fluorescent microscope, but the slides should be protected from direct light after staining. While IFAT was utilized for the recognition of *L. garvieae* (Ürkü and Timur, 2014) and *Vagococcus salmoninarum* (Yardımcı et al., 2016) in moribund rainbow trout tissues, *T. maritimum* in moribund European sea bass tissues (Yardımcı and Timur, 2016) and *Enterococcus casseliflavus* in cultured meagre (Ürkü and Timur, 2019), streptavidin-biotin staining was applied for the identification of *Vag. salmoninarum* (Yardımcı et al., 2016) and *V. anguillarum* (Akaylı et al., 2018) in moribund rainbow trout tissues.

5. Immunelectrophoretic Methods

Methods that combine serological structures, antibodies and their antigenic components and electrophoretic methods are called immune-electrophoretic methods. In these methods, the antibody is treated with various chemicals to extract the proteins or lipopolysaccharides present in their cell wall and later they are separated in a medium (generally a gel) electrophoretically.

Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS PAGE) is an electrophoretic method to separate large molecules such as proteins and lipopolysaccharides on an SDS gel and run in a vertical electrophoresis tank (Laemmli, 1970). Under the constant electric current, lightweight molecules can be pushed for a longer distance and heavier molecule stays on the upper parts of the gel. The profile obtained on the gel can be visualized with Coomassie brilliant blue staining for proteins and silver nitrate staining for lipopolysaccharides. There are manual methods for protein extraction from different types of bacteria and some kits are also commercially available in which the solutions are provided ready to use. This method was used for outer membrane proteins (OMP) and lipopolysaccharides (LPS) analysis of *V. alginolyticus* (Akaylı et al., 2008) and *V. ordalii* (Akaylı et al., 2010) isolated from gilthead sea bream in Türkiye. LPS profiles of *V. anguillarum*, *A. hydrophila*, *A. schubertii*, *P. fluorescens* and *Y. ruckeri* recovered from diseased rainbow trout were also revealed (Akaylı et al., 2015).

This method is mainly used for the characterization of bacterial strains. More than 20 serotypes were described for *V. anguillarum* which is among the most widely spread bacterial fish pathogen and mainly 2 or 3 serotypes were isolated from moribund fish samples. The OMP weight of this species is the distinguishing characteristic for serotyping. Recently, *V. anguillarum* isolates of gilthead sea bream cultured in Türkiye were shown to be of serotype O1 by using an SDS-PAGE method (Çanak, 2011). Also, a membrane protein with a molecular weight of 140 kDa was shown to be essential for colonization and biofilm formation in the host ability for human clinical strains of *Staphylococcus epidermidis* (Hussain et al., 1997). Strains of this species isolated from moribund fish samples in Türkiye were shown to have a protein molecule in their cell wall with the same weight. Despite this function was not proved, *S. capitis* subsp. *capitis* strains isolated from moribund fish in Türkiye have a protein molecule in their cell wall with the same weight and their biofilm formation ability was demonstrated by an *in vivo* experimental study (Çanak and Timur, 2020). Hence, since they are among the main antigenic structures, the information provided by the protein profiling studies is crucial for vaccine or serologic rapid identification kit development studies.

Another serological test, Western blotting, a technique that combines immune electrophoresis of bacterial cell-wall proteins and antigen-antibody reactions is also useful for the identification of antibodies in serum. After the vertical electrophoretic separation of proteins on a gel, the antigens are transferred to a nitrocellulose paper and probed with a specific enzyme-conjugated antibody (Towbin et al., 1979). Western blotting has been shown to be an effective method for the detection of proteins after electrophoretic analysis, particularly those with low abundances. The effectiveness of this method is based on its capability to simultaneously resolve multiple antigens in a sample which can be detected by specific antibodies (Kurien et al., 2011). Besides, Western blotting has various applications for studying the regulatory mechanisms that underpin energy metabolism and protein turnover, as well as chronic physiological changes. (Bass et al., 2017).

6. Molecular Methods in fish Diseases Diagnostics

During the 1930's Arne Wilhelm Kaurin Tiselius developed a method called electrophoresis to separate a substance from one another and this invention brought him the Nobel Prize in chemistry in 1948. If an electrical charge is laid over a sheet of damp paper on which lies a few drops of a solution containing electrically charged molecules, then the molecules will begin to migrate along the electric field. In 1869, Friedrich Miescher discovered the molecule "nuclein", which is also known as DNA. James Watson and Francis Crick's discovery of the double helix, or the twisted-ladder structure of DNA in 1953 gave rise to contemporary molecular biology studies (Lamm et al., 2020). Gel electrophoresis was developed in the 1970s to separate nucleic acids and revolutionized the research of proteins, lipopolysaccharides, DNA, RNA and, the whole molecular biology studies. But the revolutionary invention of the American biochemist Kary Mullis in 1983, the polymerase chain reaction (PCR) totally changed everything in all fields of biological sciences. Molecular methods based on identifying macromolecules (fatty acids, complex carbohydrates, proteins, and/or nucleic acids) that are common within the species yet unique to that species are used for identifying parasites, bacteria, and viruses *in-vivo* and *in-vitro*. Molecular methods with high specificity are generally applied to confirm the initial diagnosis but may also be applied to tissues of the fish sample. Thus the presence or absence of the targeted pathogen can be demonstrated.

Bacteria have a single circular chromosome and may possess a tiny extrachromosomal DNA which is known as the plasmid. The plasmid contains genes associated with antibiotic resistance or various virulence factors. The early molecular methods such as G+C (Guanine + Cytosine / Adenine + Thymine percentage) content determination for taxonomy, DNA–DNA hybridization, fluorescence *in situ* hybridization (FISH), ribotyping *etc.*, are largely non-amplified. During the last decades, comparison of DNA sequences has led to great strides in re-classifying known species and identifying new species. The genetic sequences of hundreds of organisms are compiled in NCBI Genome Database which can be used online. Ribotyping is also used for determining the phylogenetic relationships among organisms The rRNA genes in the amplified fragments can be sequenced to determine evolutionary relationships between organisms. This technique is useful for classifying a newly discovered organism into a domain or phylum or to determine the general types of organisms present in one environment.

The use of restriction enzymes enables researchers to compare the base sequences of different organisms via DNA fingerprinting for the determination of relatedness. Recently, they are only used for the characterization and description of bacteria due to their various disadvantages in rapid identification (Liu, 2011). The 16S rRNA-based PCR detection has been successfully used in Türkiye for the identification and characterization of various fish pathogens (Altınok and Kurt, 2003; Karataş et al., 2010; Altun et al., 2013a; Ercan et al., 2013; Altun et al., 2014; Turgay et al., 2015b; Steinum et al., 2016; Topçu et al., 2019; Yardımcı et al., 2020; Akaylı et al., 2021). This approach has been successfully used for the identification of fish-pathogenic *Staphylococcus pasteuri* (Atanasoff and Ürkü, 2022), *Citrobacter freundii* and *Hafnia alvei* (Akaylı et al., 2021), but it may not be adequate for the identification of the *Lactococcus* (Altınok et al., 2022) and *Pseudomonas* (Saticioglu et al., 2022) genera. Hence whole genome sequencing (WGS) is becoming an increasingly popular method for distinguishing closely related bacteria which provides a high-resolution characterization. The recent technological advances in nucleic acid sequencing, called next-generation sequencing (NGS), which provide a huge amount of molecular information at a low cost in a relatively short period of time, have revolutionized the field of genomics and have also influenced viral research (Nkili-Meyong et al., 2016).

In situ hybridization (ISH) uses a labeled nucleic acid probe that anneals to a complementary viral antigen sequence in well-conserved cells or tissues (Roberts, 2012). Sample material (tissue sections) are treated with molecular probes labeled with digoxigenin (DIG) or another similar signal molecule and they hybridize the target gene. Probe's position is visualized by colorimetric staining or fluorescence by fluorescent microscopy, and the antigen present in the tissue around the damaged area can be detected (Roberts, 2012).

The more recent methods often involve nucleic acid amplification where only a specific complementary sequence can bind to the nucleic acid. These mainly include polymerase chain reaction (PCR) and its variations. PCR is a technique by which small samples of DNA (extracted from the moribund fish tissues or from the overnight *in-vitro* culture) substrate can be quickly amplified and generate copies of a specific fragment of DNA, that is exponentially increased

to quantities (10^6 – 10^7 copies) that are large enough for analysis. Following optimizations of standard PCR, various other types were evolved such as nested PCR (two consecutive PCR reactions are carried out), multiplex PCR (more than one primer sets are occupied for simultaneous amplification of several genes), arbitrarily primed PCR (a single ~10-base oligonucleotide amplify random regions in a genome), and reverse-transcriptase PCR (RT–PCR) (targeting RNA). Faster and easy-to-use variations are necessary for on-site screening and early and immediate recognition of pathogens in aquaculture. Development and use of PCR-based molecular methods have increased exponentially over the last decades and without doubt, led to a significant improvement in fish and shellfish disease diagnosis. These highly sensitive methods are ideal for detecting low levels of pathogens and characterizing them.

Conventional PCR is used to amplify a single gene target for identification at the genus (Çanak and Timur, 2020) or species (Çanak et al., 2013) level, or for revealing various characteristics of the pathogens (Teker et al., 2019). Whereas multiplex PCR involves amplifying multiple gene products in a single reaction to identify fish pathogens (González et al., 2004a; Altınok et al., 2008; Altınok, 2011; Tsai et al., 2012) then the products are run on an agarose gel. However, a more selective examination of the products can be made with DNA microarrays (González et al., 2004a). Advances in microfluidics offer a higher precision than traditional methods (Liu et al., 2022). Microfluidics technology has recently been developed for use in aquaculture using RT-PCR (Lien et al., 2009) to detect NNV, Iridovirus, and *V. anguillarum* and RT-LAMP to detect NNV in grouper (Wang et al., 2011). A fluorogenic loop-mediated isothermal amplification-based dual-sample microfluidic chip allowed the simultaneous detection of multiple bacterial and viral aquatic fish pathogens (Zhou et al., 2021; Hu et al., 2023). In real-time PCR, or quantitative PCR (qPCR), the newly made DNA is tagged with a fluorescent dye, so the levels of fluorescence are measured after every PCR cycle. qPCR provides quantitative monitoring of DNA copies and recognizes bacterial growth in different antibiotic concentrations (Maugeri et al., 2019).

7. Non-invasive Imaging Technologies Used for Fish Diseases Diagnosis

As fishes are very sensitive to the surrounding environmental factors, continuous fish health and behavior monitoring may reduce economic losses due to stress and disease development (Saberioon et al., 2017). Depending on technological development, digital camera systems provide underwater pictures or videos of higher resolution even in waters with high turbidity (Hung et al., 2016; Chang et al., 2022). Underwater camera systems are occupied especially in marine offshore cages to watch the fish during feeding, but later, software was added for image analysis to obtain detailed data on the population. All these data are also

related to fish health and welfare tracking. Software-assisted camera systems can provide data about the length, weight, sex, maturity and skin color of individuals and many others without any stress development in the population (Saberioon et al., 2017). Size estimation is made easier thanks to these systems, especially in bigger fish species such as Bluefin tuna (Costa et al., 2009). These systems are also useful for post-harvest processes. Traditionally, fish are inspected by experts for quality parameters in an inconvenient and imprecise way for hours (Balaban et al., 2008). Automated computer vision systems offer this work to be done in a cheaper and more accurate way (Saberioon et al., 2017). A computer-assisted underwater imaging system was also designed to detect uneaten fish feed pellets in the culture site for waste management and obtaining a cleaner culture environment (Li et al., 2017). Hyperspectral camera systems supported with image analysis software were also used for the digital scoring of operational damages such as fin erosions for tracking the fish welfare in cultured Atlantic salmon (Lindberg et al., 2023). As a novel technology of developing stages, software used in these systems is now supported and enhanced with the use of artificial intelligence (Chang et al., 2022).

Diagnosis of skeletal deformities during early stages will improve quality and production amount in hatcheries and besides the simple methods, various other procedures (X-ray imaging, double staining, and computer tomography) have also been used in scientific studies. Besides tracking the bone and gas bladder development in hatcheries (Boglione and Costa, 2011), or for the detection of skeletal deformities in market-sized individuals (Çolak and Çanak, 2020), Planar X-ray imaging provided precise estimation of the weight in herring (Veliyulin et al., 2011. Another method where X-ray imaging and image analysis software were used together was reported for the tracking of individual feed intake in Atlantic salmon (Difford et al., 2023). All these applications also reduce handling stress in fish and may increase the fish welfare of relatively younger and vulnerable individuals in hatcheries.

8. Future Trends

The one pathogen–one disease paradigm is moving to the pathobiome concept to characterize disease dynamics. In this concept, the combination of multiple pathogens, host, and environment is working together to disease or the health of the organism (Vayssier-Taussatet al., 2014). The number of diagnostic methods potentially available appears endless, as technologies in clinical medicine expand, but not all are suited for application in fish disease studies, mainly due to cost and complexity. As explained and exampled above, despite the traditional methods are still in use, the research studies on fish diseases, fish health management and fish welfare mainly concentrated on the rapid detection, and characterization of pathogens,

eco-friendly prevention strategies like new vaccine formulations, probiotics and prebiotics, use of novel antibiotics and anesthetics (Adams et al., 2008; Newaj-Fyzul and Austin, 2015; Akaylı et al., 2021; Nguyen et al., 2022; Robinson et al., 2022). Also, there are a lot of studies on the understanding of the host-pathogen host-environment relationships with different approaches. High and novel techniques such as proteomics have become promising tools for pathogen identification, virulence mechanisms and pathogenesis processes in association with host-pathogen interactions that may help to control, prevent, and treat diseases in farmed fish (Moreira et al., 2021). Also, new non-invasive applications are suggested for use in aquaculture to avoid stress in fish and to provide biosecurity in aquaculture sites. Besides, digital web-based tools and software are intensely used to maintain the sharing the knowledge.

Although this technology was proposed more than 30 years ago, another trending method for pathogen detection in clinical diagnostics is the matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS), a cost-effective and accurate technique for routine identification of bacteria (Seng et al., 2009). This method enables rapid implementation of targeted ameliorative measures following outbreaks of bacterial fish diseases (Jansson et al., 2020). Recently it was used for the identification of fish pathogenic *Vibrio* (Moussa et al., 2021; Yavuzcan-Yıldız et al., 2022), *Pseudomonas* (Kačániová et al., 2019; Saticioglu et al., 2021), *Photobacterium*, (Yavuzcan-Yıldız et al., 2022), *Tenacibaculum* (Fernández-Álvarez et al., 2017) *Flavobacterium* (Fernández-Álvarez et al., 2018), *Mycobacterium* (Puk et al., 2018), *Streptococcus* (Assis et al., 2017) representatives and environmental aquatic bacteria (Topić-Popović et al., 2022) as well. As the Main Spectra Profile (MSP) databases of fish pathogenic bacteria are enriched, this system is a promising rapid identification tool for the future (Piamsomboon et al., 2020) as it is shown to have a limited performance for the identification of aeromonads at the species level (Pérez-Sancho et al., 2018).

9. Conclusions

Since the initiation of the fish disease diagnosis studies, traditional microbiology and bacteriology procedures, where the pathogen is recovered and identified with phenotypic and biochemical characteristics, and observation of histological sections from moribund fish tissues are still widely used. By the time, biochemical identification test strips were developed for rapid diagnosis of the common bacterial pathogens. Later on, serologic methods were involved for accurate diagnosis. After the development of molecular methods and PCR especially, taxonomy, characterization, and identification studies were added to the routine diagnostic studies. For each method, as the accuracy and reliability are proved, the more the method becomes widely used and the more the chemicals and/or kits became more easily accessible. After so many years of fish disease diagnosis history, researchers are now aware of the need for the combined use of many techniques for an accurate and reliable disease diagnosis as there is no “one super technique” that is able to give this result. A disease case should be explained with field observation, internal, external and pathologic symptoms, identification of the causative microorganisms which is supported by phenotypic, biochemical, serological or molecular methods and a treatment method should be proposed. In conclusion, as seen in the above-mentioned data, old and traditional methods are still in use and of great importance, but novel techniques for rapid and more accurate diagnosis are developing. Depending on the requirements of the study and opportunities of the laboratory, multiple choices of the above-mentioned tests, both traditional and advanced ones should be used for reliable identification.

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PART V

ECONOMY AND INDUSTRY 4.0

CHAPTER 18

IMPACT OF TURKISH AQUACULTURE SECTOR ON ECONOMIC DEVELOPMENT

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DOI: 10.26650/B/LS32LS24.2024.005.018

1. Introduction

Mankind has used the terrestrial area on which they have settled since the beginning of life, in agriculture, settlement and activities such as industry depending on technological developments. Due to the increasing population day by day, a large part of the terrestrial areas is used for settlement purposes, and the share of agriculture has gradually decreased. As a result, new areas were needed for the production of foodstuffs. Among these areas, the first source that comes to mind was the aquatic environment.

Water has always been used for different purposes since the beginning of humanity and continues to be used. The most important of the sectors where water is used intensively is the aquaculture sector. Fishery products, especially fish, have an important place in human nutrition as one of the animal protein sources. The aquaculture sector constitutes one of the important branches of economic activity in terms of both the added value and employment created and its place in foreign trade (Doğan, 2010; Atar, 2019; Ceyhan, 2019). The demand for seafood is increasing day by day due to the rapid increase in the world population, the changes in consumption habits, the increase in the standard of living and the fact that fish is a healthy food. Aquaculture has been one of the most important food production areas in which water has been used since ancient times in order to meet the demand as a result of the difficulty of accessing fisheries and the gradual decrease of natural resources.

Türkiye's geographical location and available natural resources offer investors superior opportunities for fisheries and aquaculture production. Türkiye has a huge fishing area of 23.475.000 hectares with its 8.333 km coastline, including the Black Sea, the Aegean Sea, the Mediterranean Sea, an inland sea, the Sea of Marmara and the shores, and the shores of the island, where each have unique characteristics and production opportunities with their different ecological structures. In addition, Türkiye has a great inland fishing potential with 320 natural lakes, around 861 dams and over 500 ponds (DSİ, 2023 [General Directorate of State Hydraulic Works]). Despite this potential, the commercial history of fish farming which dates back to 3000 BC in the world, is quite new in Türkiye (Çelikkale et al., 1999; Güven et al., 2001; Doğan, 2003).

Entrepreneurs and scientists in Türkiye started to work in 1950's to evaluate the potential of aquaculture. It was stated that the lakes could be enriched in terms of fish population by breeding carp and trout in inland waters for the first time in Türkiye. For this purpose, fertilized *Coregonus maraenoides* eggs were brought from Germany and hatched in the hatchery established in Lake Iznik (Kosswig, 1952; Kosswig, 1954). In the following periods, the first small-scale trout hatchery was established in Lake Abant and eggs were taken from a local subspecies, Abant trout *Salmo trutta abanticus* in a controlled manner (Akşıray, 1957). The juvenile Abant trout obtained here were released into Lake Abant and the lake was fished. Such studies were carried out for both scientific and experimental purposes, but a commercial production farm establishment could not be realized by that time.

R&D studies on aquaculture in Türkiye have focused on the breeding possibilities of alternative species with economic value other than inland and marine fish farming. The first experimental study on aquaculture of alternative species in Türkiye was carried out on sponge cultivation in Gökçeada (Gökalp, 1974). These trials paved the way for starting a commercial aquaculture business at the end of the 1960's in Türkiye and inland fish farming has been started by a private enterprise established in Bilecik (Bozüyük) with the controlled production of rainbow trout (*Oncorhynchus mykiss*) (Baran, 1977; Çelikkale et al., 1999; Güven et al., 2001; Memiş et al., 2002; Doğan, 2003; Aydın and Baltacı, 2017; Çanak, 2017). Apart from rainbow trout, a very small amount of carp and tilapia production in inland waters in very few places have started to be grown locally.

Marine fish farming in Türkiye started with the growing of gilthead sea bream (*Sparus aurata*) juveniles collected from nature in net cages in 1980's. After the first applications, from the beginning of the 1980's, the breeding of sea bream juveniles collected from the nature was started especially in the wooden cages (approximately 5x5 m) placed in the closed bays and gulfs

around Muğla Bodrum (Figure 1); Immediately after that, European sea bass (*Dicentrarchus labrax*) culture activities were also initiated (Alpbaz, 2005; Çanak, 2017). With the development of aquaculture technologies, gilthead sea bream and European sea bass farming activities were started in 1984 for the first time in commercial terms (Alpbaz, 2005). These applications have increased rapidly over the years, new production facilities have been established and the culture of various marine fish species, especially gilthead sea bream and European sea bass have increased in production amount. In 2000's, experimental and commercial trials on the cultivation of meagre (*Argyrosomus regius*), sturgeon (species of the genera *Acipenser* and *Huso*) and various shrimp, crab and mussel species were carried out (Tokşen et al., 2006).

As of today, it is seen that Türkiye is in an important position in global aquaculture production and presenting these products to the country and world market. According to the latest TUİK (Turkish Statistical Institute) data available, Türkiye's aquaculture production in 2022 was 849.808 tons. 335.003 tons of this production was provided by fisheries activities and 514.805.686 tons was provided by aquaculture activities (BSGM, 2022b [BSGM: Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture]; TUİK, 2023). Aquaculture production have been increasing rapidly in recent years in Türkiye as well as in the world, thanks to new technological developments. In addition to production amounts, studies and investments have been made for the economic development and sustainable operation of aquaculture. In this respect, it has been possible to make aquaculture sustainable without harming the environment. The production of aquaculture sector in Türkiye continues to increase steadily every year and studies are being carried out on the introduction of new alternative species to the market. In this sense, every work done provides added value to the country's economy.

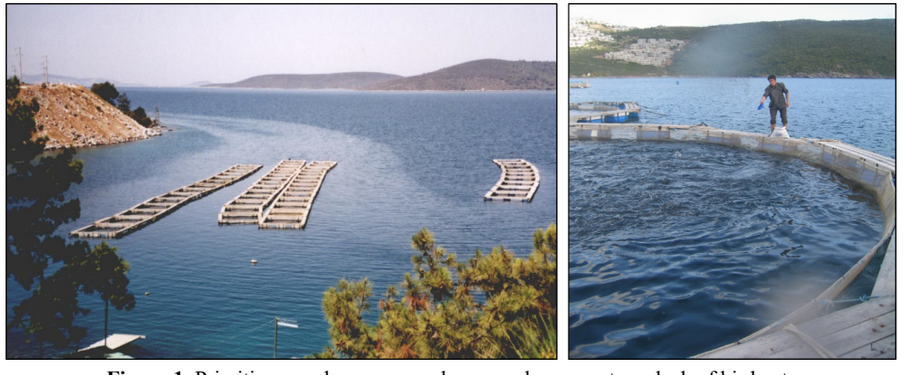

Figure 1. Primitive wooden square or hexagonal cage systems lack of bird nets operated with hand feeding

In this section, aquaculture will be discussed and the benefits of aquaculture on the economic structure of Türkiye (primarily on rural development) and the region will be emphasized. The contributions of the aquaculture sector to socio-economic life and economic development will be analyzed under sub-titles. Also in this section, various benefits of aquaculture such as meeting the rapidly increasing demand for aquaculture and preventing hunger, balanced and healthy nutrition, providing raw materials to various industrial sectors, reducing the hunting pressure on natural fish stocks and using resources effectively, the protection of biological diversity, its contribution to rural development and creating employment, high export potential and enabling the development of Türkiye's economy will be discussed.

2. World Aquaculture Sector

Aquaculture sector, which has developed rapidly in the world in recent years, shows itself as a driving force in the global economy with its share in national and international trade. People's need for valuable and high quality protein has brought the aquaculture industry to an important position in international trade. In this international trade, aquaculture products with high economic value are in the first place. The export of fish and fish products has an important place in the economy of many countries (Tolon, 2019; FAO, 2022a [FAO: Food and Agriculture Organization]).

In addition to the gradual decrease in natural stocks in the world, the importance of aquaculture is increasing more and more in meeting the nutrition and protein needs of the rapidly increasing population around the world. It is seen that there has been a decrease in the species diversity and amount of fish caught due to overfishing and pollution over the years. On the other hand, the increase in aquaculture production over the years is considered as the replacement of fishing with aquaculture in the demand for fishery products.

The production of fishery products, which is of great importance as a quality and healthy human food, was approximately 177.768.543 tons in 2020 according to FAO data; 90.265.933 tons of this production was obtained by fishing and 87,502,609 tons by aquaculture (FAO, 2022a). According to FAO data on fisheries and aquaculture production for 2020 (Table 1), China comes first with 35% followed by India (8%), Indonesia (7%), Vietnam (5%) and Peru (3%) (FAO, 2022a).

While the caught-fisheries production has decreased in recent years, a steady increase has been recorded in aquaculture production (Table 1). Globally, 74.3% of fisheries production was obtained through caught-fishing in 2000, of which 90% was marine fishery products. Also, in 2000, 42.3% of the total aquaculture was obtained from the seas. In 2020, the share

of cautght-fishing decreased to 50.8%. In addition, the share of aquaculture production obtained from inland waters increased to 62.2% of the total aquaculture production in 2020 (FAO, 2022c).

Table 1. World fisheries and aquaculture production (2000-2020 / shortened) (metric tons/year)

Year	Fisheries			Aquaculture			General Total
	Marine	Freshwater	Total	Marine	Freshwater	Total	
2000	84.985.331	8.593.411	**93.578.742**	13.726.706	18.694.353	**32.421.059**	**125.999.800**
2005	83.097.538	9.435.438	**92.532.976**	18.369.380	25.960.003	**44.329.383**	**136.862.360**
2010	76.337.350	10.867.693	**87.205.043**	21.870.539	35.949.144	**57.819.683**	**145.024.725**
2015	80.438.718	11.134.146	**91.572.864**	27.068.357	45.861.641	**72.929.997**	**164.502.862**
2016	78.206.872	11.314.670	**89.521.542**	28.609.670	47.960.247	**76.569.918**	**166.091.460**
2017	81.491.055	11.877.368	**93.368.423**	30.083.077	49.545.170	**79.628.248**	**172.996.671**
2018	84.520.639	11.985.462	**96.506.101**	30.877.296	51.601.032	**82.478.328**	**178.984.429**
2019	80.105.137	12.090.295	**92.195.432**	31.862.628	53.349.551	**85.212.180**	**177.407.612**
2020	78.795.376	11.470.557	**90.265.933**	33.118.115	54.384.495	**87.502.609**	**177.768.543**

Source: FAO, 2022a; BSGM, 2022

According to the FAO data, aquaculture in the world is classified as inland water and marine production. This production amounted to 87.502.609 tons in 2020. 62.2% of this production was obtained from the production in inland waters and 37.8% from marine aquaculture (FAO, 2022a). Between 2000 and 2020, the aquaculture production amount in the World increased by an average of 4 million tons per year. The total aquaculture, which was 32.4 million tons in 2000, reached to 57.8 million tons in 2010, 72.9 million tons in 2015 and 87.5 million tons in 2020 (FAO, 2022b). World aquaculture continues to make significant contributions to local economies in developing countries. In 2020, the contribution value of the sector to the world economy is estimated to be 424 billion USD. China, India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, Chile and Myanmar are the leading countries in the world's total aquaculture. Most of these Asian countries are important producers since they have a crowded population and their agricultural areas are rather limited. Also aquaculture in Norway is an important sector since it has a mountainous geography surrounded with marine areas. According to the world aquaculture projection, it is predicted that in the coming years, the demand for healthy nutrition will rise and the aquaculture production amount will be higher with the increasing population. It is predicted that the amount of aquaculture in the world will exceed the amount of fishing in 2030 and that almost all aquaculture products for human consumption will be provided through aquaculture in the coming years. It is vital that this growth can occur by protecting ecosystems, reducing pollution, protecting biodiversity and

ensuring social equality. Contrary to what was previously thought, in FAO's 2030 projection for fisheries and aquaculture, its total production is expected to reach 202 million tons in 2030, thanks to the rapid growth figures achieve (FAO, 2022c).

3. Turkish Fisheries and Aquaculture Sector

In Türkiye, the fisheries and aquaculture sector operates as a sub-sector of the agricultural sector and consists of fisheries products caught from inland waters and seas, and fisheries grown by the culture method in inland waters and seas. Aquaculture sector also provides economical buoyancy and employment support to other related sub-industries. Many sub-industries are positioned within the aquaculture sector in the areas where fishing boats are operated and aquaculture is carried out. While fishing activities were dominant in the past, aquaculture production has increased its share in total production in recent years. The gradual decrease in natural stocks has increased the importance of aquaculture in meeting the protein needs of the rapidly increasing population around the world.

The visible increase in Türkiye's fisheries and aquaculture production was shown in Table 2 (TUİK, 2023). While the amount of caught-fisheries production follows a fluctuating course, a continuous upward acceleration is observed in aquaculture production and as of 2022 60.6% of this production was provided by aquaculture products. The economic contribution arising from the total production of fishery products was about 2.5 billion dollars in 2021.

Table 2. Turkish fisheries and aquaculture production (1980-2022) (metric tons/year)

Years	Fisheries			Aquaculture			General Total
	Marine	Inland	Total	Marine	Inland	Total	
1980	397.321	32.255	**429.576**	-	-	-	**429.576**
1985	532.602	45.471	**578.073**	-	-	-	**578.073**
1986*	539.565	40.286	**579.851**	35	3.040	**3.075**	**582.920**
1990	342.017	37.315	**379.332**	1.545	4.237	**5.287**	**385.114**
1995	582.599	44.993	**627.548**	8.494	13.113	**21.607**	**649.200**
1996	474.243	42.202	**516.445**	15.241	17.960	**33.201**	549.646
2000	460.521	42.824	**503.345**	35.646	43.385	**79.031**	**582.376**
2001	484.410	43.323	**527.733**	29.730	37.514	**67.244**	**594.977**
2002	522.744	43.938	**566.682**	26.868	34.297	**61.165**	**627.847**
2003	463.074	44.698	**507.772**	39.726	40.217	**79.943**	**587.715**
2004	504.897	45.585	**550.482**	49.895	44.115	**94.010**	**644.492**
2005	380.381	46.115	**426.496**	69.673	48.604	**118.277**	**544.773**
2010	445.680	40.259	**485.939**	88.573	78.568	**167.141**	**653.080**

Table 2. Continue

Years	Fisheries			Aquaculture			General Total
	Marine	Inland	Total	Marine	Inland	Total	
2015	397.731	34.176	**431.907**	138.879	101.455	**240.334**	**672.241**
2016	301.464	33.856	**335.320**	151.794	101.601	**253.395**	**588.715**
2017	322.173	32.145	**354.318**	172.492	104.010	**276.502**	**630.820**
2018	283.955	30.139	**314.094**	209.370	105.167	**314.537**	**628.631**
2019	431.572	31.596	**463.168**	256.930	116.426	**373.356**	**836.524**
2020	331.281	33.119	**364.400**	293.175	128.236	**421.411**	**785.811**
2021	295.018	33.140	**328.158**	335.644	136.042	**471.686**	**799.844**
2022	301.747	33.256	**335.003**	368.742	146.063	**514.805**	**849.808**

Source: DİE**, 1980-2000; BSGM, 2022, TUİK, 2023
*: First data on aquaculture production
**DİE: formerly Turkish Statistical Institute

3.1. Aquaculture in Türkiye

Aquaculture in Türkiye started with the breeding of species with high economic value and commercial enterprises were established. Aquaculture, which first started with carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), in the first commercial aquaculture facility located in Bilecik (Bozüyük) in 1970. The first commercial marine aquaculture farm was established in 1984 in the Aegean Sea region (İzmir-Çesme) for gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). In the late 1980's, trout and salmon culture in cages were started in the Black Sea, and in the mid-1990's, shrimp and mussel farming started in Antalya. It could not be successful because the water temperature in the Black Sea is high for salmon in summer. In addition, in 1997, a controlled turbot (*Scopthalmus maeoticus*) production study started in Trabzon Fisheries Research Institute with the cooperation between the the Ministry of Agriculture and Rural Affairs of Türkiye and the Japan International Organization (JICA). In the following years, the production of turbot continued in a private enterprise established in Kocaeli (Kefken) (Baran, 1977; Anonym, 1993; Çelikkale et al., 1999; Güven et al., 2001; Memiş et al., 2002; Doğan, 2003; Aydın and Baltacı, 2017; Çanak, 2017).

Apart from the above-mentioned fishes, breeding studies of mussels, shrimp, *etc*., which are widely cultivated in the world were also carried out in Türkiye. After physical, chemical and biological studies that lasted for two years, a private enterprise (Marsan A.Ş.) was established for mussel (*Mytilus galloprovincialis*) farming in the Dardanelles, the Marmara Sea for the first time in 1982 in Türkiye (Bilecik, 2023). In the following years, a private enterprise started mussel farming in Kocaeli (Kefken), but they have been unsuccessful. Despite

these negativities, mussel farming activities have not been abandoned and are still continuing (Çelikkale et al., 1999; TUİK, 2022). Shrimp farming, which is a seafood of high economic value, has been carried out in the Mediterranean and Aegean regions since the 1990's, but due to various reasons, successful economic results could not be obtained from these initiatives.

While research on aquaculture continues in the world, R&D studies of alternative species suitable for aquaculture continued in Türkiye as well and successful results were obtained. Among these species, meagre and various porgy fish species were presented to the market as fresh or filleted, tuna as canned fish, sturgeon as canned caviar and shrimp as frozen packaged pud prawns. In addition, due to the increasing interest, there have been developments in the aquarium fishery sector, and the production of mainly imported species has begun in Türkiye.

According to 2022 data, total aquaculture production amount of 514.805 tons has been reached with 13 marine and 7 inland water species in Türkiye. 71.5% of aquaculture production was carried out in the seas and 28.5% in inland waters (TUİK, 2022). In terms of numbers, there are 2.223 fish farms with a project capacity of 604.095 tons and 18.6% of them are operating as marine enterprises and 81.4% as inland water enterprises (BSGM, 2022). In addition, 73.6% of the enterprises produce rainbow trout, 8.0% European sea bass and 7.4% produce gilthead sea bream. Rainbow trout (145.649 tons) in inland waters, European sea bass (156.602 tons) and gilthead sea bream (152.469 tons) take the first places among the species that are cultivated. In recent years, important developments have occurred in terms of aquaculture in Türkiye. The current aquaculture potential has been evaluated and the amount of production has increased every year by using new technologies. The increase in this production is due to the continuous increase in the number of aquaculture facilities. The share of aquaculture in Türkiye's total fisheries production increased from 13.6% in 2000 to 35.8% in 2015 and 60.6% in 2022.

Türkiye is divided into seven regions in terms of geographical locations. Each region has different characteristics in terms of climate, water resources and agricultural productivity and as a result, they all have different socio-economic values. A wide range of marine and inland water sources with different salinity and temperature levels produce various ecological environments that allow the production of distinct aquaculture fish species with a great potential. Existing farms that are operating within the borders of 76 of the total 81 provinces are mainly located in the Aegean (29.5%), Black Sea (19.8%), Mediterranean (17.1%) and Eastern Anatolian (16.4) regions (Table 3, Fig 1). Among the provinces, approximately 15% (339) of theese farms are located in Muğla followed by 7% (164) in Elazığ,%5 (101) in Denizli, 4% (93) in İzmir, 4% (92) in Antalya, 4% (81) in Isparta, 3% (69) in Aydın, 3% (58) in Mersin, 3%

(58) in Trabzon and 2% (52) in Burdur. The distribution of fish farms also affected the distribution of fish feed factories and seafood processing facilities. For instance, 43.7% of the fish feed factories and 32.9% of the seafood processing facilities are located in the Aegean region.

Table 3. Distribution of fish farms, fish feed factories and seafood processing facilities to the geographical regions of Türkiye

Regions	Fish farms	%	Fish feed factories	%	Seafood processing facilities	%
Aegean region	659	29.5	14	43.7	83	32.9
Black Sea region	444	19.8	4	12.5	40	15.9
Mediterranean region	383	17.1	3	9.4	32	12.6
Eastern Anatolian region	366	16.4	4	12.5	4	1.6
Central Anatolian region	179	8	1	3.1	15	6
Marmara region	125	5.6	4	12.5	76	30.2
South-Eastern Anatolian region	81	3.6	2	6.3	2	0.8
Total	**2.237**	**100**	**32**	**100**	**252**	**100**

Source: BSGM, 2022; TOB, 2022b; GGBS, 2023
*TOB: Republic of Türkiye Ministry of Agriculture and Forestry
*GGBS: Food Safety Information System of Türkiye

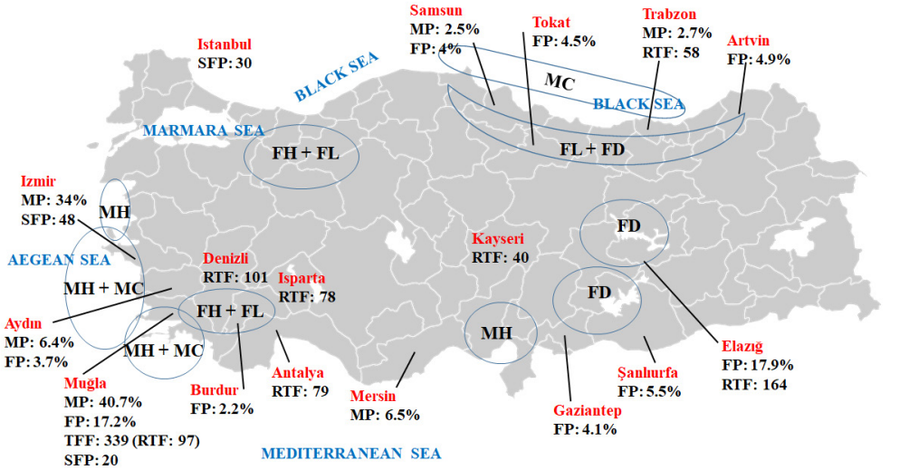

Figure 1. Distribution of different types of aquaculture facilities in Türkiye. *MC: Marine cages, MH: hatcheries for marine fishes, FD: Freshwater fish farms in dam lakes, FH: hatcheries for freshwater fishes, FL: Land-based pools for freshwater fishes, FP: share of the province in freshwater fish production, MP: share of the province in marine fish production, FF: number of fish farms, RTF: number of rainbow trout farms, SFP: seafood processing facilities*

As the most widely cultured fish species, rainbow trout farms are distributed all around Türkiye which is affected by the presence of high quality water resources, year-round water temperatures, and the presence of dam lakes. Useage of the land and water resources for other purposes such as agriculture and industrial production and the land purchase prices also effects this distribution. Among provinces, 164 of these rainbow trout farms are located in Elazığ, followed by 101 in Denizli, 97 in Muğla, 79 in Antalya, 78 in Isparta and 58 in Trabzon.

It is aimed and encouraged to operate the rural economy in the places where the fishery production enterprises are established and to contribute to the local people in socio-economic terms. However, enterprises engaged in marine fish farming are large-scale integrated enterprises and they supply the fish they produce to the market either fresh or by processing. Hence, people in the countryside benefit indirectly by working in these enterprises and as suppliers of some intermediate goods. On the other hand, enterprises in inland waters are mostly small or medium scale enterprises that produce rainbow trout. These enterprises are mostly local small-scale enterprises and they employ entrepreneurs and local people in the region.

There are many factors among the reasons for the increase in aquaculture production. Development of technology, economic value of fishery products, country targets and strategies are some of them. With the introduction of new dams in aquaculture systems and inland waters in Türkiye in recent years, the project capacities of family-type enterprises have been increased and production in net cages has begun. Thus, an increase in production was achieved and an increase in the number of integrated enterprises was recorded. Especially in marine fish farming, with the transfer of production away from the coast to the open sea, the need to use larger cages arose, and this brought an increase in production capacity. The fact that the products produced have a high added value economically and that they are exported at high prices have created an increase in economic profits.

Aquaculture is not only fish production, but also the activities carried out in the sub-sectors that contribute to this sector make a wide contribution to the country's economy. Sub-sectors contributing to the aquaculture sector are feed production, hatcheries, processing facilities, fish health services (disease prevention, disease control, vaccination), aquaculture mechanization, production of cage construction materials and their marketing.

3.1.1. Inland aquaculture in Türkiye

Although commercial aquaculture activities started relatively lately in Türkiye, the economic evaluation of aquaculture is based on previous years. The oldest legislation for aquaculture is the Regulation of the Municipal Police of 1882 during the time of the former Ottoman

Empire era (Called as *Zabıta-i Saydiye Nizamnamesi* – Regulation for Fishing Activities), and there are regulations regarding the breeding of bivalve mollusks such as mussels, oysters and scallops in the seas. Research and scientific studies on aquaculture were also continued. However, commercial aquaculture facilities could not be put into operation.

The place and importance of fisheries and aquaculture in the agricultural sector in Türkiye gained vitality with the entry into force of the Fisheries Law No. 1380 in 1971. Intensive studies have started within the scope of the Fisheries Regulation issued by the former Ministry of Agriculture and Rural Affairs and contributed to the establishment of new enterprises through the state. Türkiye is one of the leading countries in aquaculture in the world with the potential of inland water resources with different temperatures. Abant trout (*Salmo trutta abanticus*) was raised in the hatchery that was first established by the state in Yedi Göller (Bolu, Black Sea Region) at the end of 1969, and the fry obtained here were released into the lakes for the purpose of supporting fish and sport fishing (Yürüker, 1970). Later, scientific studies were carried out on trout farming in net cages at the Konuklar State Breeding Farm (Çelikkale et al., 1981). Researchers from Istanbul University and Former Federal Germany conducted the "Marmara Region Inland Fisheries Development Project". In the project carried out within the framework of Turkish and German Technical Cooperation, trout farming practices were initiated in Sapanca Fish Production Unit (Aydın and Baltacı, 2017). Today, this facility is still operated with the name of Istanbul University Faculty of Aquatic Sciences Sapanca Inland Fisheries Production Research and Application Unit especially for academic purposes besides commercial egg, fry and wholesale and retail market sized fish selling. Commercial inland aquaculture production, which started with carp and rainbow trout, later continued with trials of alternative species on the following years (Doğan and Güven, 2005) in Türkiye is currently being carried out mainly on 7 freshwater species, primarily rainbow trout, salmonids, carp, sturgeon, tilapia, Eurpean catfish and frog (Table 3).

Table 3. Freshwater aquaculture production of Türkiye (2014-2022) (metric tons/year)

Type of Fish	**2014**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Trout (*Rainbow trout*)	107.533	99.712	101.761	103.192	113.678	126.101	134.174	144.347
Trout (*Salmo* sp.)	450	1.585	1.944	1.695	2.375	1.804	1.558	1.302
Mirror carp	157	196	233	212	203	173	171	293
Sturgeons	17	6	13	2	-	14	-	1
Tilapias	32	58	8	12	6	13	6	-
European catfish	-	-	8	5	121	92	84	95
Frog	50	44	43	49	43	39	49	25
TOTAL	**108.239**	**101.601**	**104.010**	**105.167**	**116.426**	**128.236**	**136.042**	**146.063**

Source: TUIK, 2023

As of 2021, rainbow trout ranks first among the species cultivated in inland waters in Türkiye with a share of 35%. Türkiye ranks second after Iran in the world rainbow trout production as of 2020. With the developments in the aquaculture systems in recent years and the introduction of new dams in inland waters, the project capacities of family-operated enterprises have been increased and production in net cages has begun. Thus, increases were recorded in the number of integrated enterprises and in production amount. In Türkiye, enterprises that have economic value and are suitable for breeding operate in all regions. The number and capacity ranges of inland water aquaculture enterprises were given in Table 4.

Table 4. The number, capacity and share in production of the freshwater fish farms in Türkiye

Capacity group (ton/year)	Facility (*n*)	Share in number (%)	Total project capacity (ton/year)	Share in Production (%)
0-50	**1.101**	**66.3**	**19.303**	**9**
51-100	**112**	**6.8**	**9.955**	**4.6**
101-250	**220**	**13.3**	**44.108**	**20.05**
251-500	**126**	**7.6**	**54.399**	**25.3**
501-1000	**97**	**5.9**	**82.357**	**38.3**
1001- >	**2**	**0.1**	**4.900**	**2.3**
Total	**1.658**	**100**	**215.022**	**100**

Source: BSGM, 2022

According to their capacity ranges, enterprises with a capacity of 0-50 tons/year have a high rate of 66.3% in total number followed by enterprises producing between 501-1000 tons range with a rate of 38.3% (BGSM, 2022). In recent years, producers have increased their capacity at a significant level and preferred to do integrated aquaculture in natural lakes and dam lakes in off-shore cage system. Examples from inland fish farming facilities in Türkiye were shown in figure 2. While rainbow trout enterprises constitute 95.2% of the existing inland aquaculture facilities, other species are cultivated in the remaining ones. Detailed information about the hatcheries that provide eggs and fry to these enterprises will be given in the following sections.



Figure 2. Examples of freshwater fish farming facility types (concrete pools on the left and lake cages on the right)

3.1.2. Marine aquaculture in Türkiye

Since the mid-1980's, there have been important initiatives in marine fish farming in the Aegean and Mediterranean regions in Türkiye. With the beginning of gilthead sea bream and European sea bass farming on the shores of the Aegean Sea, trout and salmon farming in cages in the Black Sea in 1990, meagre, dentex, sharpsnout sea bream and tuna farming in the Aegean Sea and the Mediterraneans at the beginning of 2000's, aquaculture in Türkiye gained a great momentum (Deniz et al., 1997; Alpbaz, 2005; Saka et al., 2007; Doğan, 2010; Çanak, 2017; Altay and Maltaş, 2020; Tosun, 2020; Bilecik, 2023). As a result of the establishment of technology-supported hatcheries instead of collecting fry from nature, the supply of fry fish has become easier, continuous and economical. This has led entrepreneurs to invest and thus the number of enterprises (to a total of 432) and production volumes (to a total capacity of 389.074 tons/year) have increased over the years. In the majority of these enterprises, gilthead sea bream, European sea bass and other alternative species are cultured in marine net cages. Land based pools close to the sea shore that are operated with ground water with a high salinity were also established in the last two decades for growing marine fishes. Apart from these, there are also farms for tuna fattening and enterprises that grow mussels. The number of active marine fish farming enterprises and their project capacities were given in Table 5. Especially after 2007, in accordance with the legislation enacted by the Ministry of Environment and Urbanization, the characteristics of the aquaculture sites have been re-defined with the "Communiqué on the Determination of Closed Bay and Bay Areas, which are Sensitive

Areas, Where Fish Farms can not be Established in the Seas". Thus, re-location of the fish farms to a minimal distance of 0.6 nautical miles from the shore, to a depth of at least 30 m and to points where the current velocity is at least 0.1 m/second has been imposed (Anonym, 2007). This re-location necessitated the use of new techniques suitable for the tougher conditions here. Accordingly, improvements have been made in cage sizes and structures, net systems and automated feeding systems with a technology at the world standards. Examples from marine fish farming facilities in Türkiye were shown in figure 3.

Table 5. The number, capacity and share in production of marine fish farms in Türkiye

Capacity group (ton/year)	Facility (*n*)	Share in number (%)	Total project capacity (ton/year)	Share in Production (%)
0-50	133	28.9	3.685	0.9
51-100	14	3.0	1.165	0.3
101-250	19	4.1	3.104	0.8
251-500	52	11.3	17.956	4.6
501-1000	131	28.4	117.724	30.3
1001- >	112	24.3	245.440	63.1
Total	**461**	**100**	**389.074**	**100**

Source: BSGM, 2022



Figure 3. Examples of marine aquaculture facility types. Off-shore marine cages with various sizes, barge systems (top left), crane systems (top right), automated fish feed distribution systems (middle left), mussel farm (middle right), land based sea bass pool (bottom left) and fish feed transportation with a crane (bottom right)

The first official records in marine fish farming in Türkiye were published in 1986 statistics with the production of 1 ton two-banded sea bream and 34 tons of gilthead sea bream. Other species were also included in the aquaculture records in the following years. In 1990, 1 ton two-banded sea bream, 102 tons of European sea bass, 300 tons of salmon, 1.031 tons of gilt-head sea bream and 111 tons of other alternative species were produced in Türkiye. There has been an increase in the number of species cultivated and production amounts over the years, and in 2000, 17.877 tons of European sea bass, 15.460 tons of gilt-head sea bream, 1.961 tons of salmon, 321 tons of mussels and 27 tons of shrimp were produced. Since successful results were obtained in aquaculture, there was an increase in both the number of species and the number of production facilities, and in connection with this, the total amount of production compared to the beginning years also increased. In 2005, 37.290 tons of European sea bass, 27.634 tons of gilthead sea bream, 1.249 tons of salmon, 1.500 tons of mussels and 2.000 tons of alternative species; In 2010, 50.796 tons of European sea bass, 28.157 tons of gilthead sea bream, 7.019 tons of salmon, 340 tons of mussels and 2.201 tons of other species were produced. Production amounts of marine aquaculture in Türkiye between the years 2014-2022 were shown in Table 6. As can be seen in the table, gilthead sea bream, European sea bass and rainbow trout took the first three place. Of the total marine aquaculture production of 304.135 tons (46.2%) is European sea bass, 39.8% is gilthead sea bream and 9.4% is rainbow trout.

Table 6. Marine aquaculture production of Türkiye (2014 – 2022)

Species	2014	2016	2017	2018	2019	2020	2021	2022
Rainbow trout (*Oncorhynchus mykiss*)	4.812	4.640	4.972	9.235	9.411	18.182	31.509	45.454
Salmo spp.	798	1.073	980	375	281	507	45	-
Gilthead sea bream (*Sparus aurata*)	41.873	58.254	61.090	76.680	99.730	109.749	133.476	152.469
European sea bass (*Dicentrarchus labrax*)	74.653	80.847	99.971	116.915	137.419	148.907	155.151	156.602
Common seabream (*Pagrus major*)	106	225	20	2	5	1	4	28
Bluespotted seabream (*Pagrus caeruleostictus*)	75	61	192	144	140	-	3	5
Redbanded seabream (*Pagrus auriga*)	-	-	66	1	-	-	-	-
Corb (*Sciena umbra*)	39	20	125	30	47	26	2	18
Meagre (*Argyrosomus regius*)	3.281	2.463	697	1.486	3.375	7.428	5.913	4.771
Common dentex (*Dentex dentex*)	113	43	51	24	27	-	-	-

Table 6. Continue

Species	2014	2016	2017	2018	2019	2020	2021	2022
Sharpsnout seabream (*Diplodus puntazzo*)	8	2	-	-	-	-	-	-
Bluefin tuna (*Thunnus thynnus*)	1.136	3.834	3.802	3.571	2.327	4.338	4.952	3.839
Mussel (*Mytilus galloprovincialis*)	-	329	489	907	4.168	4.037	4.585	5.469
Whiteleg shrimp (*Penaeus vannamei*)	-	-	-	-	-	-	4	19
Kum Şirlanı	-	-	-	-	-	-	-	25
Spirulina	-	-	-	-	-	-	-	2
Lahoz	-	-	-	-	-	-	-	1
Total	**126.894**	**151.794**	**172.492**	**209.370**	**256.930**	**293.175**	**335.644**	**368.702**

Source: TUİK, 2023

4. The Effect of Aquaculture on Economic and Social Development

Aquaculture is a form of business that operates to meet human needs. However, economic concerns come to the fore in meeting the needs. In order to meet human needs, it is aimed to obtain the highest efficiency with the least input in. In this section, the socio-economic contributions of aquaculture sector such as meeting the rapidly increasing demand for food, providing raw materials to the other industrial sectors, reducing the hunting pressure on natural fish stocks and protecting natural diversity, creating employment, providing high export opportunities and foreign currency inflow will be evaluated.

4.1. Contribution of aquaculture to economy

Türkiye is a rapidly growing country in the aquaculture sector with its trained manpower and potential. Due to its climate zone and geographical structure, Türkiye offers suitable environmental opportunities for commercial fishing and aquaculture in inland waters and seas. From this point of view, aquaculture contributes to the country's economy primarily by contributing to rural development, creating employment opportunities, economic value and high export potential. Aquaculture is also an economic sector that contributes to different industries. It creates an integrated economy with hatcheries, feed mills, suppliers, vendors and exporters that support aquaculture (Doğan, 2010; Aydın, 2017; Tolon, 2019; Yıldırım and Çantaş, 2022). Every investment made in aquaculture enterprises can also be considered as an investment in health, agriculture and economy. Such investment works to be carried out support rural development and bring socio-economic development. It is a fact that the investment in aquaculture will increase in the coming years with the development of new technologies as

a result of reliable scientific researches. As it is expected that a higher share of the aquaculture products will be supplied to the market, Türkiye will have a greater economic advantage in national and international markets.

High input prices in aquaculture all over the world and in Türkiye increase the cost of production. Especially in aquaculture, the high feed prices affect the farming negatively. Hence, it is obligatory to give incentives and support to the producers of the sector by the state. Incentives provided to producers by the state in Türkiye aim to reach the targets set in the investment programs for the economic and social development of the country. With the incentives in the field of aquaculture, it may be possible for the entrepreneur to establish new businesses or to maintain the already established business in a sustainable way; and to contribute to employment and the national economy. In addition, pre-supplying the feed in order to reduce the feed expenses, which have a high proportion in the operating expenses, will also reduce the pressure on the production cost.

In particular, support and incentives are provided by the state for the purposes of strengthening the desire and desire of entrepreneurs to engage in investment, export and similar economic activities, and to provide necessary guidance for their realization in accordance with the needs of the country. These supports can be summarized as; organic agriculture and good agricultural practices, agricultural insurance premium support (TARSİM), rural development investment support program (TKDK: Ministry of Agriculture and Forestry – Agriculture and Rural Development Support Institution), low interest investment and business loan applications, IPARD II program within the scope of fisheries sector which includes investments in physical assets related to the processing and marketing of agricultural and fisheries and aquaculture products, investments in diversification of farm activities and business development (Çöteli, 2021; TOB, 2022a). Increasing the types of support and the amount of support given in aquaculture will contribute to the increase in exports and social and economic development. It is inevitable for the sector to raise awareness of the public by carrying out studies that aquaculture does not have a negative impact on the environment, and to develop policies that show that aquaculture is beneficial to human health.

According to 2021 data (TUİK, 2021), the agricultural sector in Türkiye constitutes 5.5% of the total GDP (TUİK, 2022). The fishing sector is considered as a sub-sector of the agricultural sector and constitutes 6.1% of the income of the agricultural sector. As an important branch of the agriculture industry, aquaculture represents 59.0% of the total production amount of the whole fisheries sector and 83.6% of its economic value. In 2021, aquaculture production provided an added value of USD 2.074.138.494 to the national economy. Aqua-

culture amount and economic value of Turkish aquaculture between 2005-2021 were given in Table 7. In the calculation, the value is given in USD based on the average TCMB (Central Bank of the Republic of Türkiye) exchange rate of each year. The income of the Turkish aquaculture sector reached 500 million USD in 2010, 1 billion USD in 2014, 1.5 billion USD in 2020 and 2 billion USD in 2021. The place of the fisheries sector in the country's economy, its effects on development and future projections are explained in detail below.

Table 7. Economic value of the aquaculture production of Türkiye between 2005-2021 (USD/year)

Years	Production (ton/year)	Value (USD)	Average TCMB USD/TRY rate
2005	118.277	522.736.584	1.3473
2010	167.141	686.075.374	1.5549
2015	240.334	942.552.127	2.7258
2016	253.395	1.069.894.963	3.0277
2017	276.502	1.107.639.923	3.6557
2018	314.537	1.162.253.769	4.8241
2019	373.356	1.353.979.600	5.6826
2020	421.411	1.546.200.128	7.0234
2021	471.686	2.070.397.749	8.9270

Source: BSGM, 2022; TCMB, 2023

As a result of new scientific research studies, by making use of the experiences of other countries and using new technologies, there has been an increase in the number of enterprises and development from year to year. Small-scale enterprises operate mostly locally in rural areas and those working in them are generally the people living in these regions. As a result of the establishment of these enterprises in rural areas, rural employment and development are also supported.

Aquaculture is not only a food production activity with high economic value, but also a sector where it is applied in investments that will provide rural development and social and economic development of rural areas. The aquaculture sector, which is a way of accessing healthy food as a result of the decrease in natural stocks and the increase in the need for food of the world population, is also an economically important sector. This sector has always contributed increasingly to the country's economy and to raising the level of development. Even if new technology is used in breeding, it is a business line where human labor is used intensively. In this respect, it has a great contribution to Türkiye's economy and country's development as a result of creating large employment, contributing to rural development, contributing to human nutrition, providing raw materials to the industrial sector and having

high export potential. In addition to the economic contributions of the aquaculture sector, there are also socio-cultural and socio-economic contributions. For example, despite the dominant use of cow, lamb and chicken meat in the traditional Turkish kitchen especially in the central and east Anatolia, various dishes made with the fishes produced in the region were added in the menus of the restaurants. Increases in production are also recorded due to the increase of the number of employees. And this is also an important factor for decreasing immigration fron the rural areas of the country to the crowded cities where accomodataion and employment opportunities are tougher.

Aquaculture sector has been the "shining star" of the world economy also in recent years, especially in meeting the nutritive needs of billions during the COVID-19 pandemic period. This sector has a different place among export activities in Türkiye since fish is the main animal product for human nutrition exported from Türkiye. The products exported here are generally fresh and/or processed trout, gilthead sea bream, European sea bass and tuna fish.

4.2. Aquaculture and rural development

The settlements located in the provincial and district centers in Türkiye are called urban, and the rest of them are called rural areas. The areas where agricultural production is made intensively are rural areas. The primary objectives of the countries are to be self-sufficient in agricultural production and to meet the necessary food needs in the rural areas and to distribute them in a balanced way.

The limited job opportunities in the rural areas of Türkiye, but the high population growth accelerated the migration to the metropolitan cities and caused the rural areas to be emptied. Since the establishment of the Republic, rural development projects have been implemented in order to get rid of these negativities and to relieve the people living in rural areas socially, culturally and economically. The aim of the implemented projects is to remove the difference between the economic, social and development levels of the people living in these regions and to ensure the rational use of the resources available in the economic sense. Within the projects implemented in this sense, many models such as village institutes, central villages, village-cities, attraction village practices, efforts to establish agricultural industry in rural areas can be counted (Yeşilbaş, 2011; Akci, 2015; Şahin, 2021).

Within these rural development projects, there have been evolutions in government programs in recent years. Türkiye's relations with the EU, which started with its application for membership in the European Economic Community in 1950, reached a new level when it gained candidate country status with the Helsinki Summit in 2005. With the effect of the EU harmonization

processes, strategy documents have been included in government programs and development plans in order to eliminate the imbalances between the village and the city. In 2006, the National Rural Development Strategy Document (UKKS-I) was prepared in Türkiye, harmonization processes were initiated and a total of 35 negotiation chapters were determined within the scope of EU criteria. Chapters 11, 12 and 13 of these were carried out by the Ministry of Agriculture. Chapter 11 (Agriculture and Rural Development) covers rural development in agriculture and what needs to be done. Having made these preparations, Türkiye has continued its work throughout this integration process and within this scope, the EU has provided financial assistance to candidate countries for rural development (Doğan and Akşahin, 2011).

The people and life preferences of those living in rural areas vary according to the area they live in. For this reason, rural policies should be developed and implemented in line with the needs and expectations of those living in these regions. Policies are developed mostly on agricultural products and technology in rural areas. In this sense, Rural Development and Organization units were established by the Ministry of Agriculture and Forestry and they continue to work. Within the scope of the support program in different fields of agriculture carried out by the Rural Development Department, investments in aquaculture activities are supported. In these investments, it is aimed to raise small and medium-scale farms, which are engaged in aquaculture and will bring aquaculture activities to the level of "good aquaculture practices", according to the legislation of the Ministry of Agriculture and Forestry. It is encouraged to modernize aquaculture farms with new technologies and to expand them in places where aquaculture activities are insufficient. (TOB, 2022a).

Among the economic investments based on agriculture, fixed investments made for aquaculture and fishermen's shelters are within the scope of the grant. In inland waters, catfish, sturgeon, carp, tilapia, blackfish, macro and micro algae, mussel, shrimp, land snail; and fixed investments made for the marine aquaculture of oyster, mussel and trout species are supported financially. With rural development projects in Türkiye, many medium-scaled enterprises have been supported and made financially sustainable. With the support made in this regard, regional developments have been experienced in aquaculture. As a result of the increase in aquaculture and processing activities, regional employment, regional income and socio-economic improvements were achieved.

4.3. Nutritional importance of fisheries

The rapid increase in the world population brings with the problem of nutrition with healthy and valuable foods. Rapid population growth, together with the increase in the need

for housing, causes the shrinkage of agricultural areas. The decrease and insufficiency of the products obtained from the shrinking agricultural areas bring the products obtained from the aquatic ecosystems to the fore. Seafood are among the most important sources of healthy nutrition for the world population. The fisheries and aquaculture sector is not only important for its economic value, but also for the raising of healthy generations. Its role in community health and balanced nutrition make aquaculture important.

Seafood are of great importance for human health due to their high protein content, amino acid diversity and polyunsaturated fatty acids, rich mineral and vitamin content. (Çicek et al., 2020). Fish-meat is a food that consists of water, protein, fat, vitamins and minerals. It is rich in vitamins and minerals such as iodine, Vitamin-D, calcium, phosphorus, potassium, selenium and folic acid and Omega- Fatty acids, which are essential for the body. The bioavailability of all the nutrients it contains, including protein, is high. With this content, fish is a functional food with a high demand (Baysal, 2017; Öksüz et al., 2018; Garipağaoğlu, 2019).

Seafood contain proteins with high biological value for all individuals, especially children in the age of growth and development. In terms of protein quality, it can be converted into body protein equivalent to breast milk. Most high-quality protein foods also contain fat. In addition to the high-value protein it contains, seafood is also rich in many minerals such as fat-soluble vitamins A, D and E, and potassium, selenium, zinc, iodine, which are important for metabolism and health. Iodine is an essential mineral source for thyroid gland and brain development, zinc for reproductive health, selenium for its protective properties against cancer (Şengör and Ceylan, 2018; Çehreli, 2019). In this respect, people highly prefer seafood consumption for a healthy diet. It has been proven in various studies that consuming fish and seafood positively affects human health. Consumption of seafood plays a role in preventing some chronic diseases and changing eating habits. Due to the fatty acids, protein, vitamins and minerals it contains, it is included in the healthy food group by many health institutions in the world (Atar and Alçicek, 2009).

4.4. Aquaculture hatcheries

The production of brood and juvenile fish, which constitute the main source of aquaculture, is of crucial importance. Sustainable aquaculture operation depends on the production of healthy eggs and juvenile fish. At first, aquaculture sector in Türkiye was used to be run by buying fertilized eggs or fry fish from abroad to perform ongrowing stages in inland facilities, and by feeding the fry caught from nature in wooden cages close to the shore in marine aquaculture. The first marine fish hatchery of Türkiye was established by Pınar Holding in Urla, İzmir in 1985 (Anonym,

2023). In recent years, Türkiye has become a country that can produce its own eggs and fry in terms of both freshwater and marine fish in hatcheries established and operated with the newest technology. According to 2021 data (Table 8), 27 marine fish hatcheries with a capacity of 1 billion eggs and 140 million fry per year; 77 inland fish hatcheries with a capacity of 619.286.900 eggs and 283.092.000 fry per year exists in Türkiye (BSGM, 2022). Eggs and fry raised in these hatcheries meet the needs of farms operating in Türkiye and besides they are also exported abroad which provides an other economical income. Hatcheries in Türkiye are modern environmentally friendly facilities that are operated in accordance with the world standards (Figure 4) equipped with technology that can automatically measure water quality parameters and control feeding systems. Marine fish hatcheries of Türkiye are mainly operated on the sea coastline within the provincial borders of Aydın, Çanakkale, İzmir and Muğla and most of them serve as hatcheries and adaptation units as well. On the other hand, rainbow trout hatcheries were established in line with the needs in every region where fish farming is carried out and most intensively in the provinces of Aydın, Bilecik, Elazığ, Kahramanmaraş, Kayseri, Muğla and Trabzon.



Figure 4. Examples of marine fish hatchery unit (on the top), mass phytoplankton production unit (middle left), trout stripping (middle right) and rainbow trout hatchery (on the bottom) operations

Table 8. Egg and fry production capacity of existing hatcheries in Türkiye (2021)

Group	Number	eggs / year	fry / year
Freshwater fish hatchery	77	283.092.000	619.286.900
Freshwater hatcheries operated inside of a production facility	Not known exactly	Appr. 570.3 million	Appr. 934.7 million
Marine fish hatchery	27	140.402.000	1.028.250.000

Source: BSGM, 2022

4.5. Aquculture feed sector

The rapid increase in production in aquaculture in Türkiye has brought the need for fish feed production to the fore. The increase in aquaculture production in recent years to 471.686 tons shows that 850-950.000 tons of feed is needed where the average food convertion rate (FCR) values are around 1,8 (1,5 – 2) for gilthead seabream and European seabass (Yıldırım and Çantaş, 2021). Meeting this feed requirement from the domestic market will contribute to the country's economy. Investments have been made to contribute to economic development by offering new business areas to entrepreneurs in fish feed R&D activities, supply of fish feed raw materials, fish feed production, packaging, storage, marketing and transportation.

As a result of the attempts to produce feed, which is the most important main component of aquaculture, in our country, there have been increases in the number of factories producing only fish feed and new initiatives, separating them from other animal feed production activities. In parallel with the rapid growth of the aquaculture sector and the increase in production capacities, the number and capacities of feed factories (Figure 5) have also increased. Every increasing initiative creates added value for the employment and economy of the country. The inclusion of domestic fish feed factories during the development of the aquaculture sector ensures that the feed is obtained more easily and cheaply. Feed expenses constitute approximately 40-70% of the operating costs in aquaculture facilities (Yıldırım, 2008; Olsen and Hasan, 2012; Han et al., 2018; Galkanda-Arachchige et al., 2019). The fact that the feed is supplied from the factories established and operated with domestic capital in the country will save the fish producers from a significant financial burden in foreign currency. Thus, it will be possible to produce quality fish at more affordable costs and to reduce the foreign dependency of the country.

According to 2022 data (TOB, 2022b), there are 32 factories producing fish feed in Türkiye. It is known that 90% of these fish feed factories only produce fish feed, while the others produce fish feed alongside other animal feeds. In these factories, feeds of species such as rainbow trout, gilthead sea bream and European sea bass, which are widely cultivated in

Türkiye, are produced. Most of the feeds produced in Türkiye are extruder feeds and these are nature-friendly feeds that are digestible and soluble by fish. Besides, most of the marine fish hatcheries have their own algal and zooplankton production units for the first feeding of the fish larvae. Although the manufacturing factories operate all over Türkiye, it is seen that 43.7% of them are located in the Aegean Region and 12.5% in the Marmara and Black Sea regions (see Table 2). Feed factories have important economic contributions such as processing agricultural products, creating employment and providing foreign exchange income with exports.



Figure 5. Examples of fish feed factory (on the left, middle-top and right-top) and storage units and various feed packaging types and sizes (on the middle-bottom and right-bottom)

4.6. Seafood processing industry

Turkish seafood processing facilities represents a sub-sector that provides more added value to the produced fishery products. The facilities where fishery products are stored starting from the raw material, classification, processing, evaluation, making them suitable for consumption or marketing, and where they are stored to be sent to sales points or exported, and the places containing the complementary units of these facilities are called aquaculture processing and treatment facilities. Seafood processing facilities process fish products and make them ready for consumption and add value to the products they process by producing hygienic products that can be consumed quickly.

In Türkiye, there are 252 seafood processing facilities, which are registered in the Food Safety Information System (GGBS) of the Ministry of Agriculture and Forestry in 2023, scattered all over the country. 32.9% of them are located in the Aegean Region, 30.2% in the Marmara Region, 15.9% in the Black Sea Region and 12.6% in the Mediterranean Region

(see Table 2) (GGBS, 2023). In the distribution of processing facilities by province, İzmir ranks first with 48 facilities, followed by Istanbul with 30, Muğla with 20, Çanakkale with 18, Balıkesir with 11 and Antalya with 10. In addition to a small number of large-scale integrated facilities (Figure 6), seafood processing facilities in Türkiye are generally small and medium-sclaed facilities and supply products both for domestic consumption and abroad (Erkeç and Bilgin, 2020). In these facilities, most of the fish are processed as fresh-chilled; they are also processed as frozen, fillet and smoked. In facilities located in provinces such as İzmir, Muğla, Trabzon, Denizli, Kayseri, Elazığ, Kahramanmaraş and Malatya, the fish produced in aquaculture farms are processed and marketed for ready-to-eat consumption. While gilthead seabream and European sea bass are mostly processed as fresh-chilled, frozen and fillets, rainbow trout is also processed as smoked fish. Especially marine fishes, octopus, shrimp and clam are processed in the facilities and a significant amount of sales are made to the foreign market as well as the domestic market. Facilities that process seafood products with EU approval number can export. In the domestic market, products are sold to hotels, restaurants, catering companies, supermarkets, hypermarkets and local markets.



Figure 6. Examples of seafood processing facilities operated in Türkiye.

4.7. Foreign trade of aquaculture sector

Countries with insufficient aquaculture resources import from other countries in order to meet the demand for healthy and high quality seafood products. As a result of the developments in aquaculture production and processing technology in Türkiye in recent years, an increase has been recorded in the export of fisheries and seafood products. Aquaculture sector in Türkiye provides foreign currency input to the country's economy by exporting a significant amount of products, as well as meeting the quality protein needs of the people in domestic consumption. Produced and processed fishery products are exported to approximately 80

countries. Fresh fish, chilled fish, frozen fish, smoked and canned fish can be preferred in the export of aquatic products, as well as live fish are sold abroad. EU countries and other neighboring countries stand out as the most important foreign markets for Türkiye. Rainbow trout, gilthead sea bream and European sea bass rank first among the species exported to the EU. Another important market for Türkiye is Japan. Especially, almost all of the tuna fish grown in Türkiye are exported to Japan. Germany, the United Kingdom, the Netherlands, Iraq, Spain, Italy, Japan, Lebanon, Russia and Greece are among the main exporting countries. Türkiye's aquaculture exports in 2021 amounted to 238.732 tons of products and approximately 1.4 billion USD (Table 9). It is aimed that this export income will exceed 1.5 billion USD in 2022 and approach 2 billion USD in 2023 according to the Ministry of Agriculture and Forestry (Anonym, 2022a; Anonym, 2022b).

Since Türkiye is the largest producer in Europe of rainbow trout, European sea bass and gilthead sea bream and is the second largest exporter of European sea bass and gilthead sea bream in the world, most of the produced fish is exported to provide foreign currency input with higher added value rather than domestic consumption. Among exported products, Europeean sea bass ranks first followed by gilthead seabream. After the Russia-Ukraine tension, there has been a significant jump in the export amount of fish produced in Türkiye, especially "Turkish salmon" products. Exports are made to more than eighty countries, 55% of which is to EU countries (Anonym, 2022a; Anonym, 2022b). With the advantage of being located in the central part of the world geographically and operating an airline company, the Turkish Airlines, that flies to around 128 countries in the World, Türkiye has the potential to transport processed / packaged fish to many different parts of the World. Especially with the support provided by the Turkish Airlines in logistics, significant increases were recorded in the export figures of the Turkish salmon fish.

Table 9. Turkish Fisheries import and export amount (shortened) (metric ton/year) and values (USD/year)

Years	Import		Export	
	Amount (ton/year)	Value (USD/year)	Amount (ton/year)	Value (USD/year)
2000	14.533	46.374.937	44.230	36.647.254
2005	37.655	206.039.936	47.676	68.558.341
2010	55.109	312.935.016	80.726	133.829.563
2011	66.738	395.306.914	65.698	173.886.517
2012	74.006	413.917.190	65.384	176.402.894
2013	101.063	568.207.316	67.530	188.068.388
2014	115.381	675.844.523	77.551	198.273.838

Table 9. Continue

Years	Import		Export	
	Amount (ton/year)	Value (USD/year)	Amount (ton/year)	Value (USD/year)
2015	121.053	692.220.595	110.761	250.969.660
2016	145.469	790.303.664	82.074	180.753.629
2017	156.681	854.731.829	100.444	230.111.248
2018	177.500	951.793.070	98.315	188.965.220
2019	200.226	1.025.617.723	90.684	189.438.745
2020	201.375	1.064.877.338	85.269	156.929.169
2021	238.732	1.376.291.922	104.708	217.179.174

Source: BSGM, 2022

4.8. Employment in aquaculture

Agriculture sector in Türkiye has always played an important role in social and economic development due to its rich soil resources, biological diversity, favorable climate and geological conditions. Agriculture sector is also important in terms of creating employment opportunities and contributing to national income in addition to food production. At the same time, it still continues to be an important employment area for the rural population.

Fisheries and aquaculture sectors contributes to the economy as sub-sectors of agriculture. Despite the use of technological developments in aquaculture sector, the dealed product is subject to human control due to the fact that it is alive. For these reasons, people employed in the aquaculture sector have to know the biological and physiological characteristics of the cultured organism. People to be employed work with physical force, without being mechanized.

Fisheries and aquaculture sector not only produces agricultural food, but also plays a very important role in its social and economic development. In the sector, small, medium and large-sized enterprises directly employ many people. Besides, people, who contribute to the above-mentioned sector and deal with many other jobs in the position of suppliers, establish businesses and create employment just because of the presence of the aquaculture sector. While aquaculture is being carried out in Türkiye, all of the suppliers of the sector such as hatcheries, feed industry, fish health management services, aquaculture processing industry, foreign trade of fishery products, enterprises supplying equipment to the sector, logistics sector and the sub-sectors dealing with the marketing of the produced products all contribute to the aquaculture sector and provide employment and a large economy. According to FAO data, aquaculture and fisheries sector in the world provides direct employment to 59 million people, 35% of which is in the aquaculture sector (FAO, 2022b). Although there is no clear

information about the exact number of employees in the aquaculture sector in Türkiye, it is estimated that more than 25.000 people are employed in aquaculture and related business lines (Aydın, 2017; CUAP 2018; Göncüoğlu -Badur, 2020; TUIK, 2022). Although the exact number of employees employed in aquaculture and its subsidiaries is not known, there is a problem in the number of engineers employed in enterprises. Although it is legally obligatory for enterprises with certain potential to employ fisheries engineers, they are generally employed in large-scale and mostly marine fish farming facilities.

The workers and engineers working for the feed sector, hatcheries, fish health-related companies and supply companies are also regarded as the employees of the aquaculture sector. Besides fisheries technicians, fisheries and aquatic sciences engineers and fisheries technology engineers who carry out the crucial and main stages of the whole production cycle, biologs, civil engineers (who provide support during preparation of facility designing and construction stages) and veterinarians (who provide support during fish health management services) are also employed professionals in this sector as technical support team. In addition, the routine controls of cage net structures and mooring systems are carried out by certified SCUBA divers.

One of the most important obstacles to the total production amount in aquaculture is diseases caused by pathogens. In Türkiye, scientific researches such as diagnosis of fish diseases with traditional and novel technologies, characterization of pathogens, treatment applications, use of probiotics in the prevention of diseases or vaccine development are generally carried out in state universities and research institutes. In addition, there are few private diagnostic laboratories, especially in regions where aquaculture activities are carried out more intensively. Procurement and application of therapeutic drugs or vaccines for prevention in fish farms are carried out by private companies. In the application of vaccines to fish, especially in the Aegean Region, there is an intense employment of women (Fig.7).

Despite aquarium is a relatively old recreational sector for decorating homes, schools etc., the number of big-sized public aquariums are also increasing all around Türkiye. Istanbul is the first province to be established with the 4 big-sized aquariums, others are located in Ankara, Antalya, Eskişehir, Trabzon, Diyarbakır and İzmir. Besides providing employement and economical income in the aquaculture and tourism sectors, they are also useful for raising public awareness at all age groups (Fig. 7).

Today, there are state-affiliated education and research institutions contributing. These institutions also create employment in the fields of education and scientific research, as well as training qualified personnel and knowledge production for the sector. Particularly, through the regional fisheries research institutes affiliated to the Ministry of Agriculture and Forestry,

local aquaculture practices are promoted and encouraged to the public, thus contributing to economic development.

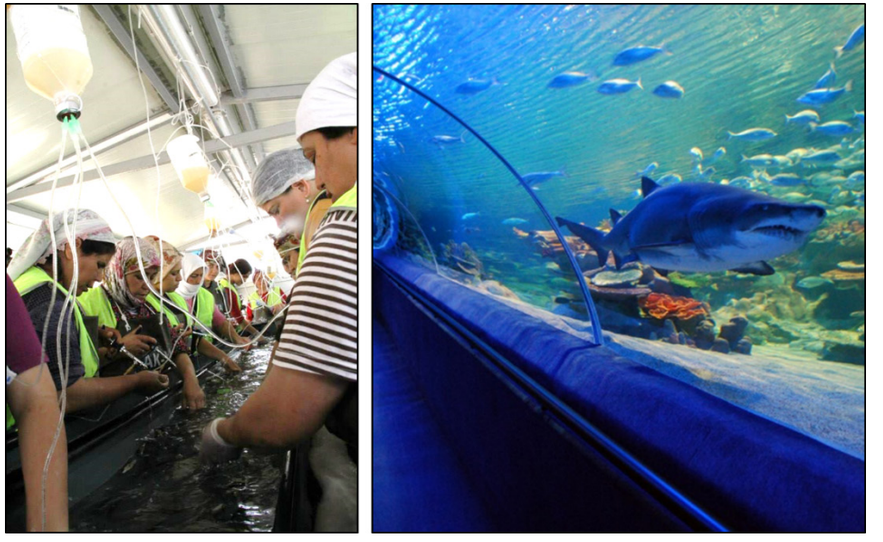

Figure 7. Women employed in fish vaccination operations (on the left);
A big-sized public aquarium with a tunnel operated in Türkiye (on the right)

4.9. Sub-sectors related to aquaculture

In Türkiye, suppliers of fisheries and aquaculture sectors have a very important economic position. The ability to produce good quality and healthy production depends on the quality of the production materials used as well as the water quality. With the introduction of suppliers that facilitate production specific to aquaculture, other technological equipments, especially the cage systems and their equipments, had a great impact on the increase in production. High-capacity off-shore cage units used in marine fish farming are equipped with durable and technological systems, resulting in superior success in aquaculture.

In aquaculture, there are mechanical and electronic equipment that are used for increasing the production efficiency of the facility. These are fixed investments related to direct production and aquaculture and equipment that can be used as working capital for sustaining the workflow of the facility and these are classified as farm, pool, cage, hatchery equipment and equipment related to fish health and safety.

It should be ensured that the water resource to be used for aquaculture purposes is provided from a healthy environment and brought into operation, and the facility should be equipped with up-to-date technological aquaculture mechanization systems. Besides the pools, cages,

fishnets, aquariums and fiberglass tanks; water pumps, water quality parameters monitoring devices, fish feed production machines, fish handling tables, fish grading and counting devices, software-supported underwater camera systems, feeding automation devices, cranes, water aeration equipments, oxygen and ozone tubes, egg counting and selection machines, hatching cabinets and all related software are among the basic basic equipments used in various stages of aquaculture. In addition, security camera systems, electricity generators, transportation and transportation vehicles such as boats and trucks and cold storage units with ice machines are among the needs of an aquaculture facility. All these technical, mechanical and electronic equipment should be available in a facility for faster, more effective, more economical and healthier production. In addition, during the developmet process of aquaculture, new equipment and software are developed as a result of R&D studies.

4.10. Education and research in aquaculture

In Türkiye, the evaluation and operation of activities such as fisheries, aquaculture and seafood processing are carried out by private entrepreneurs with the permission of the central government, Ministry of Agriculture and Forestry. Hence, the approval of the projects and the granting of the application permission are carried out within the legislation of the ministry. The Ministry of Agriculture and Forestry is an organization spread all over the country. In addition, researches on biological monitoring of water resources in the region, detection and evaluation possibilities of existing aquatic organisms in the region, genetic characterization of species specific to the region and fish health studies are carried out in fisheries research institutes that are affiliated to the ministry and established in different geographical regions of the country.

Colleges, vocational high schools, and faculties engaged in education and research activities in the field of fisheries and aquaculture provide educational services in Türkiye and technical people who graduate from these schools at different levels work in this sector. While Istanbul Beykoz Maritime and Fisheries College, which was established in 1977, was in a position to provide practical training on fisheries and aquaculture, the school's fisheries department was closed recently. Vocational High Schools, which were opened in the 1980's, providing a 2 years programme on fisheries and aquaculture and training technicians, were also abondoned in the 2010's. The faculties that give engineering education for 4 years in this field in Türkiye continue their education and research activities at the undergraduate, graduate and doctoral levels under the names such as the Faculty of Aquatic Sciences, the Faculty of Fisheries and the Faculty of Marine Sciences and Fisheries Technology. In addition, some universities have fisheries programs within the Faculty of Agriculture that give an engineer degree. Among the general research topics of these faculties are aquaculture, fish health, freshwater biology, marine biology, water resources management, fishing technologies, aquaculture processing technologies, biotechnology in aquaculture and aquaculture economics (OSYM, 2022 [Republic of Türkiye Directorate of the Centre for Student Assessment, Selection and Placement]). Aquaculture practices, which started with a production of 3.075 tons in 1986 in the first statistical records in Türkiye, reached 471.686 tons in 2021 thanks to the above-mentioned research units and technical staff trained in educational institutions. Beyond that, it is believed that the goal of increasing this production amount in the coming period, thanks to these qualified personnel that the country has raised with its own resources.

5. Conclusions

In conclusion, in this section where the development processes of commercial aquaculture activities in Türkiye are examined, it is seen that an important economic activity branch has been created from scratch and reached an important point thanks to the potential of the country and the presence of institutions that can train qualified personnel. The fact that healthy food production has strategic importance all over the world has led to government incentives and the interest of entrepreneurs to shift to this field, and thanks to continuous R&D activities, great developments have been achieved in the amount of production, employment and economic input. As branches of aquaculture activities, hatcheries, feed factories, aquaculture processing facilities, mechanization companies and fish health services have led to the development of many different business lines as well. It is projected that this growth will continue in the future, due to the increasing need and the water resources, knowledge and labor potential of the country.

Note: Most of the pictures used in this chapter were taken by the authors. The authors would like to thank to Dr. Deniz Devrim TOSUN for his valuable contribution for the figures 1, 3, 4, 5 and 6.

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CHAPTER 19

THE IMPACT OF INDUSTRY 4.0 ON AQUACULTURE

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DOI: 10.26650/B/LS32LS24.2024.005.019

1. Introduction

Aquaculture, encompassing land-based ponds and various water-based systems such as pens, cages, longlines, and stakes in brackish water and marine habitats, has emerged as a significant contributor to global fisheries production. In fact, it currently accounts for approximately half of the total output. This innovative approach to fish production offers a sustainable alternative to the consumption of wild fish, addressing concerns related to resource depletion and environmental impact. By reducing inputs, optimizing outputs, and mitigating pollution, aquaculture has the potential to revolutionize the way we meet the escalating demand for seafood in a rapidly changing world.

Despite its merits, aquaculture is not without environmental consequences. Habitat destruction, reliance on wild fish for feed production, and water pollution are among the notable challenges associated with this industry. Recognizing these issues, researchers and practitioners have turned to digital applications to enhance the efficiency, precision, and sustainability of aquaculture practices. This chapter explores the concept of Aquaculture 4.0, where digital technologies are integrated into fish farming, revolutionizing the field and offering promising solutions to address its environmental impacts (Parra et al., 2018a)

One of the key areas where digital technologies have made a significant impact in aquaculture is in precision stock management. Remote observation and automatic monitoring

technologies have revolutionized the field by providing accurate, detailed, and regular measurements of fish dimensions and positions. These advancements enable the inference of crucial information regarding growth, behavior, and disease prevalence. Computer image analysis, for instance, allows non-invasive and remote monitoring of fish size, shape, conformation, and movement patterns. Additionally, the emergence of robotics and advanced information technology tools has facilitated the assessment of water quality and the evaluation of daily fish feeding practices, further enhancing the precision and efficiency of fish farming operations (Antonucci and Costa, 2020).

The advent of the Internet of Things (IoT) technologies has played a pivotal role in transforming aquaculture into an intelligent and interconnected ecosystem. Intelligent sensors, processing capabilities, and control mechanisms have enabled seamless data collection, real-time image acquisition, wireless transmission, warning information release, remote control, and auxiliary decision-making processes. This integration of IoT technologies has proven vital in establishing effective communication channels between sensors and farmers, promoting informed decision-making and enhancing overall farm management practices (Chen et al., 2017).

Aquaculture 4.0 represents a paradigm shift in the way fish farming is practiced, driven by the integration of digital technologies. By leveraging remote observation, computer image analysis, robotics, and IoT technologies, this new era in aquaculture has the potential to mitigate environmental impacts, increase productivity, and ensure the sustainable production of seafood.

Aquaculture has witnessed substantial growth worldwide, particularly in the context of Industry 4.0. The advancements in heavy industry technology during the 1970s laid the foundation for modern scientific and technological progress. However, the negative environmental consequences of heavy industry have become apparent over time. According to FAO data, economically viable fish species in oceans and seas will reach the brink of extinction by 2050. Therefore, the aim is to achieve 100% resource conversion without wastage and reintegrate them into the economy. As a result, there has been a shift towards environmentally-friendly technologies to minimize these impacts and ensure sustainable practices for future generations. The proper handling and utilization of waste generated during the various stages of transforming raw materials into finished products have become significant considerations (Romer, 1990; OECD, 2015; World Bank, 2016; UNEP, 2017).

Aquaculture, dating back to the 1970s in our country, has evolved significantly over the years. With the emergence of marine fish farming in the 1980s, it has surpassed freshwater

fish farming in terms of production. In recent times, aquaculture has not only outperformed fisheries in our country but has also gained prominence in Europe as a leading sector in aquaculture production. This chapter explores the influence of Industry 4.0 on aquaculture, specifically focusing on the utilization of visual data processing, environmental sustainability, automation, and resource management. In Türkiye, according to TUİK 2023, the production of aquaculture products showed a 6.2% increase in 2022 compared to the previous year, surpassing total production of 850,000 tons. Out of this production, 335,000 tons are obtained through fishing, while 514,000 tons come from fish farming. Thus, 60% of the total seafood production is derived from aquaculture. When looking at fishing, the highest proportions are 125,000 tons of anchovy and 49,000 tons of horse mackerel. On the other hand, aquaculture figures indicate the production of approximately 145,000 tons of trout, 156,000 tons of sea bass, and 152,000 tons of sea bream, generating an income of over 1 billion dollars. This means that one out of every four fish consumed in Europe is produced in Turkish waters and reaches the tables. In 2022, this production was exported to 103 countries, aiming for a superior level of production through standardization to meet the desired quality production conditions by buyers. Many different quality standards recognized globally are certified by companies, enabling exports to these 103 countries. However, fish consumption in Türkiye still varies between 6-7 kilograms per year. It is crucial to protect our developing and potential-rich waters, manage resources, and leave a clean country for the future with the awareness of different sectors serving the aquaculture industry (Gün and Kızak, 2019).

The implementation of programming languages such as artificial intelligence (AI) and others has been introduced to promote and sustainable production techniques, leading to the development of fully automated production processes. Following the stages of automation and digitization in the industry, standardized and high-quality production has been achieved.

Within the food industry, aquaculture has rapidly embraced these processes of digitalization and automation in various fields. Türkiye, with its geographical advantage of being surrounded by seas on three sides and the richness of its inland waters, has quickly realized the high potential and high-quality fish production through the efforts of aquaculture engineers.

Ensuring the protection and strategic security of companies' and countries' know-how and information becomes a paramount concern. The utilization of computer technologies plays a crucial role in safeguarding and processing large datasets, reaching meaningful conclusions, and generating models that shape future perspectives. These strategic insights into potential threats are essential for countries to navigate their environmental and natural challenges effectively.

It is evident that information is no longer merely a numerical value; it has become a dimension that shapes the future. In Europe, aquaculture has reached a production volume of approximately 25 million tons, with an economic value of around 4 billion Euros. It is important to note that this economic value refers to the final product. Aquaculture today serves as a sector that caters to hundreds of different industries, not only limited to aquaculture companies but also benefiting other service-providing firms in the industry, taking advantage of the Industry 4.0 revolution.

According to the innovation news network (2019), one key aspect of Industry 4.0 in aquaculture is the adoption of visual data processing techniques. By leveraging technologies such as computer vision, image recognition, and machine learning algorithms, aquaculture practitioners can efficiently analyze and interpret visual data. This enables them to monitor fish health, growth rates, and behavior, detect diseases and parasites, and optimize feeding practices. Visual data processing also plays a crucial role in quality control and product inspection, ensuring high standards and reducing waste.

In the pursuit of sustainable aquaculture practices, Industry 4.0 offers innovative solutions. Automation and digitization have revolutionized production processes, leading to optimized resource utilization and reduced environmental impacts. By employing sensors, IoT devices, and AI-powered systems, aquaculture operators can monitor water quality parameters, automate feeding regimes, and manage energy consumption more effectively. These technologies enable precise control, minimize the use of chemicals and antibiotics, and support the preservation of natural ecosystems (Antonucci and Costa, 2020; Chen et al., 2017).

The implementation of automation and digitization in aquaculture has paved the way for standardized and high-quality production. Through the integration of robotics, autonomous vehicles, and smart systems, tasks that were previously labor-intensive are now performed with greater accuracy and efficiency. Automated systems can handle feeding, sorting, and harvesting operations, reducing human error and increasing productivity. Standardization ensures consistent product quality, meeting market demands and enhancing consumer trust.

The economic impact of aquaculture within the framework of Industry 4.0 is noteworthy. In Europe, aquaculture has reached a production volume of approximately 25 million tons, contributing to an economic value of around 4 billion Euros. This value encompasses various sectors beyond aquaculture itself, benefiting service-providing firms and capitalizing on the Industry 4.0 revolution. In Türkiye, aquaculture production has exhibited steady growth, with a 6.2% increase in 2022. The country's strategic geographic position and abundance of water resources have facilitated the production of high-quality fish, leading to a significant contribution to exports and meeting global market demands (FAO, 2022).

The management of water resources in aquaculture is carried out by knowledgeable and trained individuals, considering that water has multiple stakeholders. Attention is given to the interactions among these stakeholders and ensuring a clean world for future generations.

Once smart production protocols and standardization are established, the aim is to optimize the utilization of time and resources. The Industry 4.0 revolution has brought about competition between humans and machines; however, this competition is expected to contribute to the advancement of industries and humanity as a whole to achieve standardized production.

The goal of the Industry 4.0 revolution is to achieve the production of high-performance, quality products in more controlled and compact spaces. Europe aims to establish a system resembling smart factories and is making efforts to reach Industry 4.0 levels between 15% and 20% in all areas by 2030 (Innovative news network, 2019).

The transition to Industry 4.0 in the field of aquaculture is increasing both in Türkiye and worldwide. Considering that aquatic resources are not infinite, ensuring sustainability while respecting both humanity and nature becomes crucial. Industry 4.0 has brought unprecedented opportunities and advancements to the field of aquaculture. The adoption of visual data processing, environmental sustainability practices, automation, and resource management techniques have transformed the industry, promoting efficient production, conservation of resources, and preservation of ecosystems. As aquaculture continues to evolve within the context of Industry 4.0, it is crucial to strike a balance between technological advancements and responsible practices to ensure a sustainable and prosperous future for both the industry and the environment.

2. Economic Impact of Industry 4.0 on Aquaculture

Industry 4.0, also known as the Fourth Industrial Revolution, is a concept that refers to the integration of advanced digital technologies and automation in various industries. It represents a paradigm shift in manufacturing and production processes, characterized by the convergence of cyber-physical systems, the Internet of Things (IoT), cloud computing, artificial intelligence (AI), and other emerging technologies. Industry 4.0 aims to create smart factories and interconnected systems that enable enhanced efficiency, productivity, and flexibility in industrial operations.

At its core, Industry 4.0 entails the utilization of data-driven decision-making, real-time monitoring, and autonomous control systems to optimize production processes and respond to dynamic market demands. Through the integration of smart devices, sensors, and connectivity, physical systems, and digital systems are interconnected, enabling seamless communication,

information exchange, and coordination. This interconnectedness facilitates the generation and analysis of vast amounts of data, which can be leveraged for intelligent decision-making, predictive maintenance, and continuous process improvement.

Industry 4.0 has the potential to significantly impact the aquaculture industry by increasing productivity, reducing costs, improving quality, and creating new market opportunities. Here are some specific examples of how Industry 4.0 is being used in aquaculture:

Sensors: Sensors are being used to monitor water quality, fish health, and other factors in aquaculture farms. This data is used to make informed decisions about feeding, breeding, and other management practices (Parra et al., 2018b).

Robotics: Robots are being used to automate tasks in aquaculture farms, such as feeding, sorting, and harvesting. This can lead to increased productivity and reduced costs. The conventional practices of underwater operations in aquaculture heavily rely on manual labor, which not only proves to be labor-intensive but also poses significant safety risks. Compounding this challenge is the industry's current struggle with a severe labor crisis. However, with the continuous advancement of technologies and the decreasing costs associated with manufacturing, the implementation of underwater robots for aquaculture operations has gained substantial traction, driven by the pressing need to address these concerns and meet the industry's growing demands (Li and Bao, 2018).

Artificial intelligence: Artificial intelligence (AI) is being used to develop new aquaculture technologies, such as disease detection systems and feed optimization algorithms.

Key technological components of Industry 4.0 include cyber-physical systems, which involve the integration of physical machines and processes with digital systems and networks, enabling real-time data collection, analysis, and control. The Internet of Things (IoT) plays a crucial role in Industry 4.0 by connecting various devices and objects, enabling data sharing and communication across the production environment. Cloud computing provides the necessary infrastructure for storing, processing, and accessing large volumes of data, while AI algorithms and machine learning techniques enable intelligent data analysis, pattern recognition, and autonomous decision-making (Chen et al., 2017).

The implications of Industry 4.0 extend beyond individual factories or enterprises. It has the potential to transform entire value chains and ecosystems, fostering collaboration and coordination among suppliers, manufacturers, distributors, and customers. Through the seamless integration of information and communication technologies, Industry 4.0 enables supply chain optimization, personalized production, and the creation of innovative business models.

The concept of Industry 4.0 is often discussed in relation to its impact on industrial competitiveness, economic growth, and societal implications. It is recognized as a disruptive force that reshapes traditional production methods, requiring organizations to adapt their strategies, processes, and workforce skills. Scholars and researchers explore the challenges and opportunities associated with the implementation of Industry 4.0, examining factors such as technological readiness, organizational readiness, regulatory frameworks, and the socio-economic consequences of digital transformation.

3. Industry 4.0 Applications in Aquaculture

3.1. Fish feeds and feeding systems

In general, carnivorous fish are commonly cultivated in our country. Traditionally, their feed formulations require a high percentage of fish meal, typically around 60-70%. However, recent scientific studies have focused on developing feed alternatives that are entirely plant-based for fish farming. It is evident that the pressure of fishing activities on natural fish stocks makes the sourcing of fishmeal for feeding carnivorous fish a significant bottleneck. Significant quantities of seafood waste and by-products are produced throughout the entire seafood value and supply chain, spanning from the point of extraction from the sea to the eventual consumption by individuals. This pervasive issue has resulted in substantial environmental harm and substantial economic losses. Consequently, urgent attention must be directed towards the development of innovative solutions and alternative approaches to effectively manage seafood discards and alleviate the associated economic and environmental burdens. Leveraging emerging technologies, specifically those aligned with the fourth industrial revolution (Industry 4.0), such as Artificial Intelligence, Big Data, smart sensors, the Internet of Things, and other advanced technologies, holds great potential for mitigating the generation of seafood waste and by-products while concurrently enabling their valorization. Such a strategy could offer a promising avenue to augment the blue economy and promote global food sustainability (Yıldız, 2008; Craig et al., 2017; Hassoun et al., 2023).

Plant based protein and oil sources are also used to lower feed costs. Plant proteins and oils are being used increasingly in aquaculture feeds as a sustainable alternative to fishmeal and fish oil. Fishmeal is a high-quality protein source, but it is also a limited resource. Plant proteins, on the other hand, are abundant and renewable. These proteins can be used to replace fishmeal in whole or in part, depending on the species of fish being raised. Considering the economic benefits and cost ratios, it is essential for the feed given to fish to have limited levels of nitrogen, phosphorus, and mineral substances released into our oceans, seas, and inland

waters. Therefore, the expectation is that feed derived from plant-based raw materials should have a minimal environmental impact. The use of plant proteins in aquaculture feeds has a number of benefits. It can help to reduce the demand for fishmeal, which is a limited resource. It can also help to reduce the environmental impact of aquaculture, as fishmeal production can contribute to overfishing and habitat destruction. Naturally, multiple evaluations and analyses need to be conducted, and the interactions between feed, water, and fish should be monitored to assess the economic benefits on an environmental scale (Kaushik and Hemre, 2008; Yıldız et al., 2018; Zettl et al., 2019; Cavrois-Rogacki et al., 2022).

Furthermore, various processes are employed to increase the processing and biological benefits of feed raw materials, and these processes are continuously being improved. The aim is to achieve maximum benefit with minimum feed usage. In the adjustment of feed rations, the quality characteristics of each raw material are analyzed through biochemical analyses. Consequently, revisions are made in biological, economic, and ecological benefit ratios during each production process. Automation systems are used to standardize the quality of feed formulation, starting from feed raw materials, through the creation of feed components, processing, packaging, storage, and usage (Figure 1). The transition to fully traceable systems has already begun (Munguti et al., 2014, Glencross et al., 2020).

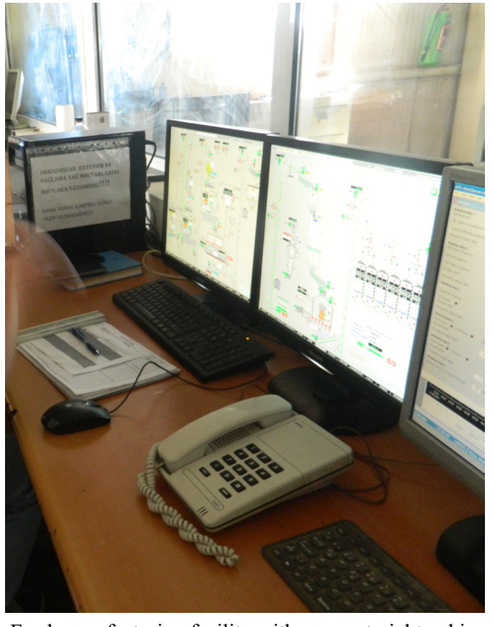

Figure 1. Feed manufacturing facility with raw material tracking software

Digitalization plays a crucial role in this process. In the aquaculture sector, our country possesses the necessary educational infrastructure and qualified workforce to receive support in all aspects. Correct identification of needs and rapid transformation into products can be achieved. As mentioned before, aquaculture production within the agricultural sector relies on fast-growing, fully equipped logistics and operations. The use of the internet, artificial intelligence, software support systems and smart technologies are increasing with each passing day, as they reduce workload and enhance traceability (Daoliang and Chang 2020; Pratiwy et al., 2022).

One of the significant expenses in aquaculture is the feed, which needs to have high economic and biological benefits. It accounts for approximately 70% of the total costs for an aquaculture operation. The goal is to have the fish consume and utilize every feed fed to the fish. Instead of manual feeding, feeding systems are integrated on barge platforms. These automated feeding systems distribute feed to cages at specific times throughout the day (Figure 2). The uptake of valuable feed by fish is monitored using surface and underwater cameras (Figure 3). During this monitoring process, factors such as the amount and size of feed given to the fish, water temperature, oxygen levels, and instantaneous follow-up are considered to optimize the benefits provided during the feeding period (Baki and Yücel, 2017; Zhou et al., 2018).



Figure 2. Off-shore barge system with automated feed distribution



Figure 3. Underwater cameras for the monitoring of feed distribution

The integration of artificial intelligence and smart technologies has revolutionized the fish farming industry, particularly in large-scale cage environments where manual monitoring of feeding systems is impractical. Leveraging sophisticated systems that employ cameras and pressurized feeders, fish farmers now have the means to efficiently deliver feed from silos to the fish. This approach is rooted in the understanding that there exist ideal conditions for fish feeding and growth.

However, effectively monitoring and capturing real-time data on these optimal conditions poses a significant challenge when relying solely on human labor, especially in extensive, large-scale systems. To overcome this hurdle, there is a growing imperative to embrace automation systems and seamlessly integrate the transformative capabilities of Industry 4.0 technologies. By doing so, the industry can guarantee the provision of optimal conditions for fish, fostering accelerated growth rates and improved overall health.

By embracing artificial intelligence and smart technologies, fish farmers can achieve a range of advantages. These systems enable precise monitoring of key parameters such as water quality, temperature, oxygen levels, and fish behavior, providing invaluable insights into the well-being of the aquatic environment. Leveraging this real-time data, farmers can make data-driven decisions to optimize feed delivery and adjust environmental parameters to ensure fish thrive in their habitat.

The benefits of such automated systems extend beyond enhancing fish growth and health. They also lead to improved operational efficiency by minimizing waste and optimizing resource utilization. By eliminating guesswork and enhancing precision, fish farmers can significantly reduce feed costs and mitigate potential environmental impacts. Moreover, the ability to remotely monitor and control aquaculture facilities brings a new level of flexibility and convenience to fish farmers, streamlining operations and promoting sustainable practices.

3.2. Environmental parameters and monitoring

The application of Industry 4.0 in aquaculture has opened up new possibilities for monitoring environmental parameters, leading to improved sustainability and operational efficiency within the industry. By leveraging advanced technologies such as the Internet of Things (IoT), artificial intelligence (AI), and big data analytics, aquaculture practitioners can obtain real-time insights into crucial environmental factors, including water quality, temperature, dissolved oxygen levels, and nutrient concentrations. These applications empower proactive decision-making based on data-driven approaches, optimizing production processes, minimizing environmental impact, and ensuring the long-term viability of aquaculture operations (Antonucci and Costa, 2020; Zhang et al., 2022).

One prominent Industry 4.0 application in aquaculture is the Internet of Things (IoT), which enables the collection of real-time data from a network of interconnected sensors and devices. In the context of monitoring environmental parameters, IoT devices can be deployed to measure and transmit data on water quality, temperature, salinity, dissolved oxygen levels, and other relevant factors. This data, combined with cloud computing capabilities, facilitates comprehensive and continuous monitoring of the aquatic environment, enabling timely interventions and proactive decision-making. Artificial intelligence (AI) techniques also play a crucial role in monitoring environmental parameters in aquaculture. Machine learning algorithms can analyze large volumes of data collected from sensors and historical records, identifying patterns, trends, and anomalies. By leveraging AI algorithms, aquaculture operators can gain insights into the complex interactions between environmental factors and the performance of aquaculture systems. This enables the prediction of potential risks, early detection of anomalies, and the implementation of targeted interventions to maintain optimal environmental conditions (Balakrishnan et al., 2019, Bates et al., 2021).

The utilization of big data analytics in aquaculture provides valuable tools for environmental assessment and decision-making. By integrating data from multiple sources, such as water quality sensors, weather forecasts, and historical records, aquaculture operators can generate

comprehensive and real-time assessments of environmental parameters. This holistic approach facilitates the identification of correlations, trends, and predictive models, enabling effective planning, resource allocation, and risk management strategies.

The integration of Industry 4.0 applications for monitoring environmental parameters in aquaculture offers numerous benefits. Real-time monitoring allows for prompt detection of deviations from desired environmental conditions, enabling immediate corrective actions. Proactive decision-making based on data-driven insights enhances operational efficiency and resource utilization, minimizing waste and optimizing productivity. Moreover, by closely monitoring and controlling environmental parameters, aquaculture practitioners can mitigate potential risks, reduce environmental impact, and ensure the long-term sustainability of their operations. The technical complexity involved in integrating various technologies and systems necessitates expertise and infrastructure investments. Cost implications associated with acquiring and maintaining advanced monitoring technologies pose a challenge, particularly for smaller-scale aquaculture operations (Chayan et al., 2018; Cai et al., 2021).

The future prospects of Industry 4.0 applications in aquaculture involve refining AI algorithms to enhance predictive capabilities and decision support systems. The development of standardized data interfaces and protocols will facilitate interoperability and data sharing among different stakeholders. Integration of advanced monitoring technologies, such as underwater drones and remote sensing, holds promise for expanding the scope and accuracy of environmental parameter monitoring in aquaculture (Figure 4).



Figure 4. Underwater drone system for aquaculture monitoring (Original photo, Onur GÖNÜLAL)

The integration of Industry 4.0 applications in aquaculture for monitoring environmental parameters represents a significant step towards sustainable and efficient operations in the industry. By harnessing IoT, AI, and big data analytics, aquaculture practitioners can make informed decisions based on real-time data, optimizing environmental conditions and minimizing risks (Figure 5). While challenges exist, addressing issues related to data privacy, cybersecurity, technical complexity, and costs will unlock the full potential of Industry 4.0 in aquaculture. With ongoing advancements and future prospects, Industry 4.0 applications offer transformative opportunities for the monitoring and management of environmental parameters, ensuring the long-term viability of aquaculture operations.



Figure 5. Real-time water quality monitoring system

Since aquaculture is an interaction between fish farming and nature, it is necessary to minimize the impact of natural events on fish. Continuous monitoring of instantaneous data such as wind, currents, and temperature, as well as meteorological information, is essential to mitigate such effects. It is known that energy needed for sustainable production can be obtained from sources such as solar power, wind, and currents. The integration of measurement devices into these cage systems enables the utilization of environmentally friendly energy sources (Lafont et al., 2019; Henares et al., 2020).

Real-time monitoring of water quality is crucial in the interaction between feeding systems and living organisms. Even a slight fluctuation of 1 degree in water temperature can be perceived as a stress factor for fish. Similarly, monitoring changes in salinity caused by the

mixing of freshwater and seawater, temperature variations, and fluctuations in oxygen levels are important for feeding fish at the healthiest times (Luna et al., 2019). All these monitoring activities can be conducted simultaneously in line with Industry 4.0 principles (Figure 6). However, in our country, full integration and automation network in this regard have not been fully established, and many aquaculture businesses continue their efforts in various aspects.



Figure 6. Real-time Oxygen monitoring system with built in oxygen support system

Closed recirculating systems have come to the forefront in terms of monitoring system uses. However, the scarcity of qualified personnel and managers in this field limits the feasibility of managing such systems. Generally, producers who prefer open systems naturally have a slightly higher environmental impact compared to closed recirculating systems. Nonetheless, with the importance of efficient and effective water utilization in today's world, various modernization equipment has been developed. Devices for monitoring water quality can be operated personally or connected to automation systems, enabling real-time monitoring. Based on instantaneous monitoring, businesses can model their environmental impacts and future perspectives (De Silva and De Silva, 2016).

3.3. Fish health and welfare

As there is a linear relationship between feeding and water quality, the aim is to ensure that fish have a high welfare level during periods of optimal water quality. In cage systems, artificial intelligence, and imaging models are employed to assess the biological evaluation and growth performance of fish, including measurements of length, weight, feed conversion ratios, and growth rates. In addition to growth performance, the use of this technology has also begun to address potential future issues, such as detecting parasites on fish, monitoring fish behavior and health status, and identifying possible scenarios

In cage systems, apart from feeding, it is necessary to monitor the health and behavior of fish in real-time. During winter months, we often encounter sudden deaths caused by storms, lightning, and thunder, which result in immediate stress for the fish. In such cases, analyzing meteorological data, processing daily, hourly, or even minute-by-minute data, and implementing applications with an Artificial Intelligence module to minimize damage to the system are some of the advantages that Industry 4.0 can bring to aquaculture. When it comes to fish diseases, monitoring the health status of fish in large-scale systems becomes challenging with human labor alone. However, processing visual data based on fish behavior can enable early intervention in the event of potential disease, minimizing fish losses. Real-time, monthly, and yearly data can be processed in automation systems to identify potential risks in the following years and facilitate preparation on a seasonal basis. Another aspect is the administration of vaccines and injections for fish health. By introducing mechanical systems rather than relying on human errors, Industry 4.0 can contribute to minimizing losses.

3.4. Land based and recirculating aquaculture systems

If real-time monitoring of fish health and water quality criteria is achievable, it will provide advantages in enterprises aimed at maximizing production within a unit area. As evident, the most crucial aspect required in Aquaculture is the real-time monitoring of water quality, with instant notifications through alarms. The utilization of artificial intelligence enables the anticipation and notification of potential changes in water quality, facilitating maximum stocking rates and fish welfare within the unit area. Whether it is an open or closed system, monitoring the interaction between fish and water is essential. In our country, particularly in businesses engaged in marine fish farming within earthen ponds, significant losses of fish occur periodically due to intense algal blooms (Figure 7), leading to oxygen depletion issues. Small and medium-sized enterprises are more adversely affected by these matters.



Figure 7. Algal Blooms in Earthen Ponds (Original Photos, Ertan Ercan)

Currently, automation systems have been developed that monitor oxygen and temperature levels in real-time throughout the day, activating pedal aerators (Figure 8) accordingly to maintain maximum oxygen levels. Although these systems are not yet widespread, they will play a crucial role in the future, both in terms of energy efficiency and ensuring the healthy tracking of products. In the production conducted within earthen ponds, there is a reliance on electricity from the moment water is obtained, and investments in solar energy generation have been initiated by these enterprises. Industry 4.0 provides the greatest advantage for enterprises engaged in fish farming within soil ponds by enabling the immediate detection of water changes and timely intervention in potential problem situations, thus minimizing fish losses. In some hatcheries and open systems, liquid oxygen usage is also relevant. The use of pure oxygen has increased costs, but the advantage of utilizing oxygenated water is the ability to increase stocking rates within the unit area and enhance fish feeding. Currently, studies are being conducted to raise the level of oxygenation in water and increase fish welfare through machines capable of producing pure oxygen.



Figure 8. Activated pedal aerators via IoT communication with oxygen sensors (Original photos, Deniz D. Tosun)

In recirculating aquaculture systems (Figure 9), water quality monitoring is recognized as a pivotal aspect. The complex nature of water chemistry, particularly in marine environments with diverse compounds, directly impacts fish health, growth, and welfare. Effective management of water quality necessitates the presence of competent and knowledgeable professionals, such as engineers, within recirculating aquaculture systems. The dynamic changes associated with water quality are influenced by various factors and can have varied effects on fish, contingent upon the specific attributes of the water. Factors such as the abundance of mineral substances and the osmoregulation requirements of marine fish, which rely on water intake to maintain their salt balance, make them highly susceptible to the influence of minerals, compounds, and nutrient salts present in the water.



Figure 9. Recirculating Aquaculture System for hatchery production in Türkiye (Original photo, Deniz D. Tosun)

Presently, recirculating aquaculture systems are primarily employed in fish hatcheries. However, recognizing the imperative for sustainable water utilization and resource management, these systems are increasingly being acknowledged as the future of aquaculture. The monitoring and management of these systems, whether conducted remotely or on-site, typically involve manual interventions by experts, particularly concerning water quality parameters. Nevertheless, during periods of intensified production, the multitude of parameters can be inadvertently overlooked, leading to sudden fluctuations that result in acute or chronic fish mortalities and hindered growth. Hence, it is essential for enterprises to not only monitor dissolved oxygen levels but also to employ online tracking of nutrient salts and dissolved gases (Midilli et al., 2012).

Although fully integrated automation systems are not yet prevalent in our country's aquaculture enterprises, the significance of water quality is progressively gaining recognition due to narrowing profit margins and the necessity to strike a balance between income and expenses. Consequently, water quality considerations are increasingly taking precedence over solely focusing on fish biology. Furthermore, ongoing research is being conducted within recirculating aquaculture systems, encompassing integrated biological water purification mechanisms that leverage the specific characteristics of various organisms. To enable real-time monitoring of water quality parameters, the implementation of Industry 4.0 technologies becomes indispensable in these systems.

3.5. Big Data for enhanced aquaculture management

Industry 4.0 plays a significant role in data archiving within the field of aquaculture. The concept of Industry 4.0 refers to the integration of advanced technologies, such as the Internet of Things (IoT), artificial intelligence (AI), and big data analytics, into industrial processes to optimize efficiency and productivity.

In aquaculture, data archiving is crucial for several reasons. Firstly, it allows for the storage and retrieval of vast amounts of data related to various aspects of fish farming, including water quality parameters, feeding schedules, growth rates, disease outbreaks, and environmental conditions. By leveraging Industry 4.0 technologies, aquaculture facilities can collect, process, and archive real-time data from sensors, monitoring systems, and other IoT devices deployed throughout the farm.

Data archiving enables historical analysis and trend identification. By maintaining a comprehensive database of past data, aquaculture operators and researchers can examine long-term patterns, detect correlations, and identify factors influencing production outcomes. This historical analysis can provide valuable insights for optimizing aquaculture practices, mitigating risks, and improving decision-making processes.

Industry 4.0 facilitates data integration and interoperability. Aquaculture farms often utilize various systems and devices from different manufacturers, each generating data in different formats. With Industry 4.0 principles, these diverse data sources can be seamlessly integrated and standardized, allowing for comprehensive data archiving across multiple parameters and variables. This integration enhances the ability to analyze and correlate different datasets, leading to a more holistic understanding of the aquaculture operation.

Industry 4.0 enables real-time data monitoring and control, contributing to proactive decision-making in aquaculture. By continuously monitoring and archiving data from sensors and IoT devices, operators can promptly detect anomalies, deviations, or critical conditions that may require immediate intervention. Real-time data archiving empowers aquaculture managers to respond quickly to changing circumstances, optimizing production efficiency, and minimizing losses. The availability of comprehensive and well-archived data sets supports research and development efforts in aquaculture. Researchers can access archived data to conduct in-depth analysis, develop predictive models, and identify best practices. This promotes innovation, fosters scientific advancements, and contributes to the sustainable growth of the aquaculture industry.

3.6. Visual data processing

The use of visual data processing in aquaculture, within the scope of Industry 4.0, offers numerous benefits for effective farm management and productivity improvement. Visual data processing involves the analysis and interpretation of images and videos captured from aquaculture facilities using cameras and other imaging devices. This technology, combined with the principles of Industry 4.0, provides advanced capabilities for monitoring, decision-making, and automation in the aquaculture industry.

One of the key applications of visual data processing in aquaculture is fish behavior analysis. By analyzing visual data, aquaculture operators can gain insights into the swimming patterns, feeding behaviors, and overall health of the fish population. This information helps in assessing fish welfare, detecting abnormal behavior, and identifying potential signs of stress or disease. With the aid of machine learning algorithms, visual data processing can automate the recognition and tracking of individual fish, facilitating precise monitoring and enabling timely interventions.

Another important aspect is the use of visual data processing for environmental monitoring. Aquaculture systems require careful management of water quality parameters such as temperature, dissolved oxygen levels, and turbidity. By analyzing visual data, anomalies in water conditions, such as algal blooms or sediment accumulation, can be detected. These insights allow for prompt actions to maintain optimal environmental conditions for the health and growth of the fish. Integration of visual data processing with IoT devices and sensors enables real-time monitoring and automated adjustments to environmental parameters, ensuring efficient and sustainable aquaculture operations.

Visual data processing also supports the identification and classification of aquatic organisms. It can assist in the recognition of different species, including both target fish species and unwanted bycatch. This capability helps in inventory management, ensuring accurate stock counts, and facilitating species-specific monitoring and feeding strategies. Additionally, visual data processing aids in the detection of parasites and diseases by analyzing the appearance and behavior of the fish, allowing for early detection and timely treatment to minimize losses and less use of chemicals.

Visual data processing in aquaculture contributes to farm security and surveillance. Monitoring systems equipped with cameras can provide real-time visual data, enabling remote monitoring of the facility for unauthorized access, equipment malfunctions, or adverse events. Advanced image recognition algorithms can be employed to detect and alert potential security breaches or abnormal situations, ensuring the safety and integrity of the aquaculture operation.

4. Challenges of implementing Industry 4.0 in aquaculture

The implementation of Industry 4.0 in aquaculture can be challenging. There of several factors that need to be considered, including cost, complexity, data security, and regulations.

Cost; The cost of implementing Industry 4.0 technologies can be high, especially for small-scale aquaculture operators. The cost of sensors, robots, and other equipment can be significant, and the cost of training staff to operate these technologies can also be high.

Complexity; The technology can be complex to install and operate, requiring specialized training and expertise. This can be a challenge for small-scale operators who may not have the resources to hire specialized staff.

Data security; The large amounts of data generated by Industry 4.0 technologies can be a security risk, if not properly managed. This data could be used by unauthorized individuals to gain access to aquaculture systems or to steal sensitive information.

Regulations; The aquaculture industry is subject to a variety of regulations, which can make it difficult to adopt new technologies. These regulations may not be designed to accommodate the use of Industry 4.0 technologies, and this can lead to delays and uncertainty in the implementation process.

Despite these challenges, there are of many benefits to implementing Industry 4.0 in aquaculture. These include:

Increased productivity: Industry 4.0 technologies can help to increase productivity by automating tasks and optimizing resource utilization.

Reduced costs: Industry 4.0 technologies can help to reduce costs by automating tasks and optimizing resource utilization.

Improved quality: Industry 4.0 technologies can help improve quality by monitoring fish health and growth rates and optimizing feeding and breeding practices.

New market opportunities: Industry 4.0 technologies can help aquaculture producers' ability access new markets by allowing them to produce high-quality products at a lower cost.

5. Conclusion

The advent of Industry 4.0 has ushered in a new era of technological advancements and opportunities for the aquaculture industry. This chapter has explored the various applications and benefits that Industry 4.0 brings to aquaculture, ranging from automation and data analytics to robotics and artificial intelligence. The integration of these technologies has the potential to revolutionize the way aquaculture operations are managed, leading to increased efficiency, productivity, and sustainability.

One key aspect highlighted in this chapter is the role of automation in aquaculture. From automated feeding systems and water quality monitoring to autonomous underwater vehicles for fish health assessment, automation has proven to be a game-changer in optimizing production processes and minimizing human error. By leveraging sensor technologies and real-time data analysis, aquaculture operators can make informed decisions, leading to improved resource utilization, reduced costs, and enhanced fish welfare.

Furthermore, the use of robotics in aquaculture holds immense promise. Robotic systems can perform a range of tasks, such as cleaning and maintenance of fish tanks, underwater inspections, and even selective harvesting. These advancements not only alleviate the labor-intensive nature of aquaculture but also enable precise and targeted operations, minimizing environmental impact and maximizing profitability.

Another significant contribution of Industry 4.0 to aquaculture is the utilization of big data and artificial intelligence. The integration of sensors, cameras, and intelligent algorithms enables real-time monitoring, predictive analytics, and decision support systems. This empowers aquaculture managers to optimize production parameters, detect disease outbreaks early, and mitigate risks. By harnessing the power of data-driven insights, the industry can achieve higher yields, reduced environmental impact, and enhanced biosecurity measures.

While Industry 4.0 offers great potential for the aquaculture sector, it is important to acknowledge the challenges that come with its implementation. These include initial investment costs, the need for skilled personnel, and addressing concerns regarding data privacy and cybersecurity. Nonetheless, with the right strategies, collaborations, and supportive policies, Industry 4.0 can drive the transformation of aquaculture into a more sustainable, efficient, and profitable industry, ensuring the provision of healthy and abundant seafood for generations to come.

However, despite the numerous advantages and opportunities that Industry 4.0 presents for aquaculture, it is important to note that its widespread adoption in Türkiye's aquaculture industry is still in its early stages. The implementation of advanced technologies and infrastructure requires significant investments and a supportive regulatory framework, which may pose challenges for small-scale aquaculture operators in the country. Additionally, there is a need for increased awareness and training programs to equip aquaculture professionals with the necessary skills to embrace and harness the potential of Industry 4.0. Overcoming these barriers and promoting a culture of innovation and technological integration will be crucial for Türkiye to fully realize the benefits and opportunities that Industry 4.0 can bring to its aquaculture sector. By doing so, Türkiye can position itself as a leader in sustainable aquaculture practices, contributing to economic growth, food security, and environmental stewardship.

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