AQUACULTURE AND FISHERIES: INTERDISCIPLINARY APPROACHES TO SUSTAINABLE DEVELOPMENT

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Authors
Prof. Dr. Akasya TOPÇU
Prof. Dr. Altan LÖK
Prof. Dr. Aysun KOP
Prof. Dr. Berna KILINÇ
Prof. Francesco FAZIO
Prof. Dr. Gürel TÜRKMEN
Prof. Dr. Önder YILDIRIM
Prof. Dr. Şükran ÇAKLI
Assoc. Prof. Dr. Adem Sezai BOZAOĞLU
Assoc. Prof. Dr. Burak Evren İNANAN
Assoc. Prof. Dr. Hatice TEKOĞUL
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Assoc. Prof. Dr. Osman Sabri KESBİÇ
Assoc. Prof. Dr. Şükrü ÖNALAN
Assoc. Prof. Dr. Ümit ACAR
Assist. Prof. Dr. Boran KARATAŞ
Assist. Prof. Dr. Mustafa AKKUŞ
Assist. Prof. Dr. Ömer Alper ERDEM
Res. Assist. Dr. Rifat TEZEL
Dr. Melek YAPICI
PhD Student Bilal ATEŞ
Aybike ÇELİK
Zilan YILMAZ

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PREFACE

Aquaculture and fisheries science are vital for ensuring global food security, preserving biodiversity, and promoting sustainable economic growth. In the face of pressing challenges such as climate change, habitat degradation, and overfishing, the integration of innovative research and practical solutions is critical to maintaining the delicate balance of aquatic ecosystems while meeting the increasing demand for aquatic resources.

This book provides a comprehensive examination of contemporary advancements, challenges, and opportunities in aquaculture and fisheries science. Featuring twelve meticulously curated chapters authored by leading experts, it highlights the importance of harmonizing traditional knowledge with modern sustainability practices. By bridging the gap between foundational research and real-world applications, the book offers valuable insights into how natural and technological innovations can work together to enhance aquatic life and human well-being.

We hope this volume serves as a vital resource for researchers, educators, students, policymakers, and industry professionals. By fostering knowledge exchange and inspiring innovation, we aim to contribute to a future where aquaculture and fisheries are not only more productive but also more sustainable, equitable, and resilient.

EDITORS

CHAPTER 1

THE HISTORY OF ARTIFICIAL REEF RESEARCH AND APPLICATIONS IN TÜRKİYE

Prof. Dr. Altan LÖK[1]

[1] Ege University, Faculty of Fisheries, Department of Fisheries and Processing Technology, İzmir, Türkiye. altan.lok@ege.edu.tr, Orcid ID: 0000-0003-0751-0620

INTRODUCTION

Strategically designed artificial reefs have an important place in achieving fisheries management and restoration goals around the world (Wang et al., 2024). Artificial reefs have been deployed many countries to decrease wave energy and prevent beach erosion (Bruno, 1993), enhance small-scale fisheries and diving tourism, protect habitats, and foster research (Seaman and Jensen, 2000) for years. The most common purposes of artificial reef applications in Turkey can be listed as i) supporting small-scale fishing, ii) protecting biodiversity, iii) developing diving tourism and iv) preventing illegal trawling activities.

Although artificial reef practices and research date back a long time in countries such as Japan, USA and Italy, they are relatively new in Turkey (Lök and Gül., 2005). Although small-scale artificial reef applications by diving centers and local governments date back to the early 1980s, no record of their results has been found (Lök and Tokaç, 2000). The first scientific studies were carried out by the Ege University Faculty of Fisheries on the shores of Hekim Island in the Gulf of Izmir in 1991 (Lök, 1995).

This chapter provides a historical account of the artificial reef applications carried out by various institutions and organizations in Turkey, as well as the historical development of scientific studies carried out by research institutions.

HISTORICAL DEVELOPMENT OF ARTIFICIAL REEF APPLICATIONS

The first known study is the artificial reef application carried out by Ege University Hydrobiology Research Center in 1983 off the coast of Urla in the Gulf of Izmir. Pipe-shaped artificial reef made of concrete material were placed in the vicinity of Taş Island off the coast of Urla. This practice was followed by the deployment of terra cotta, concrete and rubber into the sea as reefs by Beykoz Fisheries Industry Vocational High School and Istanbul diving centers (Cirik & Neşer, 1999). However, there is no detail about these practices. At the end of the 1980s, the Izmir Municipality cleaned the trolleybuses that were no longer in use and placed them in the Izmir Gulf in order to support sport fishing (Fig. 1). Since no preliminary work was done before this project, the trolleybuses were buried in a very short time (Lök and Tokaç, 2000). The deficiencies seen in this project triggered scientific studies and research began in the early 1990s. This topic is discussed in detail in the next section.

Denize gittiler..

ESHOT, 1984 yılında İstanbul Belediyesi'nden 58'i faal 75 tane "Ansaldo" marka 1960 model troleybüs satın aldı. Bu arada elinde bulunan kullanılamayacak kadar eski 15 "Bussing" ve "MAN" troleybüs karoserini de balıklara yuva olması için Körfez'e attı.

Figure 1. Newspaper clipping on the deployment of trolleybuses.

After the results of the first scientific studies were published, especially the coastal local municipalities showed great interest in the subject (Lök et al., 2022).

Çeşme Municipalities located in the west of İzmir province and Ürkmez and Gümüldür Municipalities located in the south have implemented the first artificial reef applications between 1995 and 1998 (Fig. 2).

Figure 2. Underwater photos of the artificial reefs of Çeşme, Ürkmez and Gümüldür from left to right.

Although AR applications started, no legal regulation existed until 1999. A guideline (Project Guide for Artificial Reef Applications) was prepared by. General Directorate of Fisheries and Aquaculture (Lök et al., 2002). From this date onwards, the implementation and permitting of artificial reef projects began to be carried out by this institution. Increased awareness about artificial reefs and streamlining of legal processes have increased demands for their implementation. Artificial reef applications were carried out in four seas located on three sides of Turkey (Table 1). Most implementation projects were carried out on the Aegean Sea coast (Özgül and Lök, 2017).

Table 1. Distribution of artificial reef applications in Turkish seas. Figures in parentheses indicate the total number of reef units.

Turkish Seas	**Project number**	**Deployed material**	
		Designed*	**Scrap****
Black Sea	8	6(308)	2(2)
Sea of Marmara	9	8(3099)	1(1)
Aegean Sea	38	24(10161)	14(23)
Mediterranean Sea	20	8(4720)	12(12)

* Concrete block, terracotta, steel, hdpe

** Aircraft, fighter, warship, ferry

An important turning point in artificial reef applications occurred in 2005. Alanya Municipality planned to deploy an old ship to support diving tourism and received the necessary permissions from the relevant institution. After this application, many projects were carried out to support diving tourism. The deploying of old ships and planes for the purpose of developing diving tourism has found both supporters and protests from environmental organizations. These debates still continue today.

Artificial reef designs and materials used in applications vary depending on the project purpose. In studies aimed at supporting small-scale fisheries and biodiversity, units constructed of concrete are generally preferred. Concrete material is compatible with the marine environment, durable, strong and long-lasting (Fig. 3). It is easy to supply and has the flexibility to be poured in various designs without causing problems (Düzbastılar and Lök, 2004).

Figure 3. Artificial reef units of different designs constructed from concrete materials in Turkey.

In projects aimed at supporting diving tourism, choosing structures that will attract divers and have a story are important criteria for success. The structures that best fit these criteria are old ships and aircraft (Fig. 4). However, it is very important that these old vehicles are thoroughly cleaned and these processes are controlled before they are sunk, in order to avoid creating environmental pollution.

Figure 4. Old ships and aircraft used to support diving tourism in Turkey.

HISTORICAL DEVELOPMENT OF ARTIFICIAL REEF RESEARCH

The first scientific study on artificial reefs was carried out by Ege University Faculty of Fisheries between 1991-1993 within the framework of a doctoral thesis (Lök, 1995). In the study carried out on the coast of Hekim

Island, located in the middle bay of İzmir, 30 artificial reef blocks constructed of reinforced concrete in hollow cubic design were used (Fig.5).

Figure 5. An underwater photograph from the Hekim Island artificial reef area (left) and the press coverage of the study (right).

After this thesis, many theses and research projects were carried out at universities. The research interests focus on mainly i) fish community structures and seasonal changes in artificial reefs, ii) the effects of environmental factors on artificial reefs, iii) species-specific artificial reef designs, iv) floating artificial reefs, and v) socio-economic impacts of artificial reefs. Summary information on these research topics is presented below.

Studies to reveal the fish community structure have generally been carried out in artificial reef areas located in the Aegean Sea. These studies were carried out around both concrete blocks and ship/aircraft wrecks. In fish community determination studies, the main goal is to determine species diversity, individual abundance and biomass of each species in artificial reefs. In addition, detection of daily, monthly and seasonal changes in fish communities, feeding regimes and movements are other important issues. Table 2 presents a summary of the results obtained from studies carried out in some artificial reef areas in the Aegean Sea.

Table 2. Fish community studies and their main results

Study area	Reef age	# of family	# of species	Reference
Hekim Island	8	15	29	Lök & Gül, 2005
Ürkmez	6	12	35	Gül et al.,2006
Monem ship wreck	4	16	40	Gül et al., 2008
Ürkmez/Gümüldür	7	15	39	Lök et al., 2011

Gümüldür	2	14	30	Gül et al., 2011
Dalyanköy	10	13	35	Lök et al., 2011
Edremit Bay	5	20	51	Lök et al., 2018

In most of the studies, Sparidae, Serranidae and Labridae were found to be dominant families. In terms of abundance of individuals, the most dominant species were recorded as two-banded sea bream (*Diplodus vulgaris*), damselfish (*Chromis chromis*) and bogue (*Boops boops*).

Durability of the material and stability of the reef block are the most important design characteristics. Artificial reef blocks must be durable and stable during and after installation (Düzbastılar et al., 2006). While designing the reefs, environmental effects such as wave and current conditions, distribution of bottom material around the reef blocks, and deployment depths are particularly important (Nakamura, 1985). In Turkey, especially the Black Sea and Mediterranean coasts are exposed to high wave energy. Therefore, before deploying artificial reefs in these areas, determining the safe placement depth by conducting hydraulic studies is of great importance in achieving the goals. For example, in hydraulic tests conducted by Düzbastılar (2001) for the Zonguldak coast, it was found that artificial reefs should be deployed at a minimum depth of 25 m (Fig 6).

While it is important to consider environmental factors in artificial reef design, it is also important to consider the biological needs and behaviors of marine organisms. In this context, species-specific artificial reef designs emerge. Although it is not possible to design specifically for every species, it is relatively easier to design for habitat-dependent species.

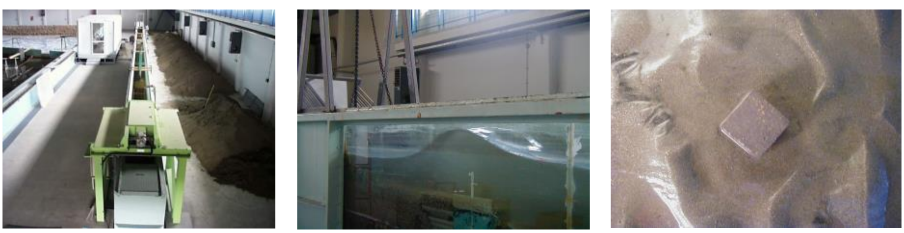

Figure 6. General view of the current channel where hydraulic tests were carried out (left), the wave being given in the channel (center) and the scours formed around the artificial reef block at the end of the test (right).

Ulaş et al., (2011) has conducted studies on developing an artificial reef design specific to octopus (*Octopus vulgaris*). In this study, firstly, observations were made in the marine environment and the characteristics of octopus nests were determined. In the light of the obtained data, an artificial reef was designed, built and placed in the sea (Fig. 7).

Figure 7. Construction of engineered octopus reefs (left) and their use by the octopus after deployment (right).

Some pelagic fish species have been frequently observed by fishermen to gather around floating algae and pieces of wood and form schools. Fishermen who want to take advantage of such behaviors of fish in order to increase their catch efficiency try to attract fish by using bamboo and palm leaves in the past, and large steel buoys filled with foam today. Structures called Fish Aggregating Devices (abbreviated as FADs) in Western literature and Floating Artificial Reefs (Floating Artificial Reefs) in Japanese literature can be defined as devices placed on or just below the water surface to attract pelagic fish species. In his doctoral thesis prepared by Özgül (2010), he conducted studies on the development of a floating artificial reef model that can be used in the Aegean Sea. In the study, two floating artificial reefs constructed from steel were placed at a depth of 50m and 100m (Fig. 8). Data were collected monthly using small-scale fishing gear (pelagic longline, gillnet, surface gillnet, fishing line and pelagic fish pot), underwater visual census and acoustic survey methods. 29 fish and 1 cephalopod species belonging to 18 families were identified in floating artificial reefs. Regarding the small scaled fishing equipment, the number of species gathered with fish hooks is much more compared to the other techniques whereas the pelagic longlines seem the most effective method regarding the biomass.

Figure 8. Technical drawing of the developed floating artificial reef (left), its surface view (center) and a sample of the individuals caught afterwards (right).

The collection and evaluation of socio-economic data is useful to quantify the usage and public benefits of an artificial reef, thus helping justify construction and maintenance costs, and providing information for the successful management of the reef (Milon et al., 2000). Even though the need of evaluating the socio economic effects related to artificial reefs has been highlighted, there is still a general lack of studies (Fabi et al., 2015). Studies on this subject are new and very limited in Turkey, as in the world.

The aim of the study conducted by Tunca et al., (2016) on socio-economic effects was to indicate the socioeconomic characteristics of AR stakeholders, commercial fishermen, recreational fishermen and local residents that are affected by ARs in Altınoluk under the Turkish National Artificial Reef Project.

OVERALL EVALUATION

Artificial reef applications in Turkey started in the late 1980s, accelerated between 1995 and 2015, and came to a halt after 2015. Scientific research also followed this trend. One of the reasons for this is that the planned changes in the legal regulations were never completed and the permitting process was delayed. The legal regulations made in 2001 should be updated as they are not suitable for today's conditions. “The Artificial Reef Scientific and Technical Advice Board” was established by the General Directorate of Fisheries and Aquatic Products in 2024. This committee, consisting of general directorate personnel and scientists, is expected to play important roles in basing artificial reef applications on scientific foundations and bringing them to standards.

REFERENCES

Bruno, M. S. (1993). Laboratory testing of an artificial reef erosion control device. Coastal zone 93. Proc. Symp. Coastal and Ocean Management. *American Society of Civil Engineers* 2: 2147–2154.

Cirik, Ş., & Neşer, M. (1999). A new field of application in marine technology in our country: artificial reefs. National Congress on Shipbuilding and Marine Technology, İstanbul, Turkey (In Turkish)

Düzbastılar, F. O. (2001) A study on structural and technical characteristics of artificial reefs. (Doktora Tezi) Ege Üniversitesi Fen Bilimleri Enstitüsü. 141 sayfa (In Turkish)

Düzbastılar, F. O., Lök, A. (2004) Primary materials in construction of artificial reefs. *E. U. Journal of Fisheries & Aquatic Sciences* 21 (1-2): 181-185 (In Turkish)

Düzbastılar, F. O., Lök, A., Ulaş, A., Metin, C. (2006) Recent developments on artificial reef application in Turkey: Hydroulic experiments. *Bulletin of Marine Science* 78(1):195-202

Fabi, G., Scarcella, G., Spagnolo, A., Bortone, S. A., Charbonel, E., Goutayer, J. J., Haddad, N., Lök, A.Trommelen, M. (2015). Practical Guidelines for the Use of Artificial Reefs in the Mediterranean and the Black Sea. FAO-GFCM, Studies and Reviews, No. 96

Gül, B., Lök, A., Ulaş, A., Düzbastılar, F. O. (2006) The investigation on fish composition at artificial reefs deployed on different substrates off Ürkmez coast. E. U. Journal of Fisheries & Aquatic Sciences 23 (1-3):431-434

Gül, B., Lök, A., Metin, C. (2008) Monem batık gemisindeki balık topluluğunun yapısı. Sualtı Bilim ve Teknolojisi Toplantısı. 8-9 Kasım 2008 İzmir, Bildiriler Kitabı:120-127 (In Turkish)

Gül, B., Lök, A., Özgül, A., Ulaş, A., Düzbastılar, F. O., Metin, C. (2011) Comparison of fish community structure on artificial reefs deployed at different depths on Turkish Agean Sea Cost. *Brazilian Journal of Oceanography* 59:27-32

Lök, A. (1995) Yapay resiflerin uygulanabilirliği üzerine bir araştırma. (Doktora Tezi) Ege Üniversitesi Fen Bilimleri Enstitüsü. 54 sayfa (In Turkish)

Lök, A., Tokaç, A. (2000) Turkey: A new region for artificial habitats. Jensen, A.C., Collins, K.J. and Lockwood, A.P.M. (eds.) Artificial Reefs In European Seas. Kluwer Academic Publications, pp.21-30

Lök, A., Gül, B. (2005) : Evaluation of fish fauna associated with experimental artificial reefs in Hekim Island in Izmir Bay (Aegean Sea, Turkey). *E.U. Journal of Fisheries & Aquatic Sciences* 22 (1-2): 109-114 (In Turkish)

Lök, A., Özgül, A., Ünal, V. (2022) An overview of the first national artificial reef project of Turkey. In Impact of Artificial Reefs on the Environment and Communities. Jorge H. P. Ramos (Editor). IGI Global: 110-125

Milon, J.W., Holland, S.M., Whitmarsh, D.J. (2000) Social and economic evaluation methods. In Seaman, W.Jr., ed. Artificial Reef Evaluation with application to natural marine habitats, pp. 165–194. CRC Press, Boca Raton, Florida.

Nakamura, M. 1985. Evolution of artificial fishing reef concepts in Japan. *Bulletin of Marine Science* 37: 271–278

Özgül, A. (2010) Ege Denizi'nde pelajik balık avcılığı için yüzen yapay resif teknolojisinin geliştirilmesi. (Doktora Tezi) Ege Üniversitesi Fen Bilimleri Enstitüsü. (In Turkish)

Özgül, A., Lök, A. (2017) Türkiye'de yapay resif çalışmaları. 20. Sualtı Bilim ve Teknolojisi Toplantısı 16-17 Kasım 2017 İzmir. Bildiriler Kitabı: 124-136 (In Turkish)

Seaman, W., Jr., Jensen, A.C. (2000). Purposes and practices of artificial reef evaluation. in W. Seaman Jr. (editor) Artificial reef evaluation with application to natural marine habitats. CRC Press, Boca Rat pp. 1–19

Ulaş, A., Lök, A., Düzbastılar, F. O., Özgül, A., Metin, C. (2011) A new artificial reef design for octopus (*Octopus vulgaris* CUVIER, 1797) in the Agean Sea and Preliminary results. *Brazilian Journal of Oceanography* 59:21-25

Tunca, S., Miran, B., Ünal, V. (20016) Non-market economic value of artificial reefs in Edremit Bay (Eastern Mediterranean, Turkey). *E.U. Journal of Fisheries & Aquatic Sciences* 33(2) 97-107 (In Turkish)

Wang, X., Lin, C., Weizhong, W., Zhang, L. (2024) The non-negligible contribution of foundation species to artificial reef construction revealed by Ecopath models. *Journal of Environmental Management* 366, 121887:1-10

CHAPTER 2

FISHERIES MANAGEMENT AND SUSTAINABILITY IN TURKEY: CURRENT SITUATION AND FUTURE PERSPECTIVES

Assist. Prof. Dr. Mustafa AKKUŞ[1]
Assoc. Prof. Dr. Adem Sezai BOZAOĞLU[2]

[1] Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Fishing and Processing Technologies, Zeve Campus, Van, Türkiye. makkus@yyu.edu.tr, Orcid ID: 0000-0002-8900-9495
[2] Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Fishing and Processing Technologies, Zeve Campus, Van 65080, Türkiye. sbozaoglu@yyu.edu.tr, Orcid ID: 0000-0003-4078-5159

INTRODUCTION

Since the beginning of humanity, the most fundamental need has been food. In this regard, seas and inland waters have always been crucial sources of food throughout history. In ancient times, fishing from seas and inland waters was conducted on a small scale due to limited means. However, with technological advancements, such as the development of fish-finding sonars and powerful engines, humans gained the ability to detect fish schools and venture into open seas. In addition to these technological advances, the increasing global population and the consequent rise in food demand have intensified the pressure on fish stocks. The history of fishing dates back to the origins of human civilization. Archaeological evidence shows that humans have been engaged in fishing for approximately 40,000 years. Fishing has long been a vital source of livelihood and food for many societies. For example, during the periods of Ancient Egypt, Greece, and Rome, fishing was an important activity both for trade and for food supply. In the Middle Ages, fishing became a significant economic activity, especially in Europe (Hoffmann, 2017). Fishing is defined as the act of catching aquatic organisms from inland waters or seas using various methods. Fishing activities range from individual fishing with traditional methods to large-scale industrial operations conducted by commercial fleets. Fish stocks worldwide, including those in Turkey, are under serious threat due to pressures stemming from human activities. Overfishing is the primary factor contributing to these pressures. Particularly after the Industrial Revolution, the increase in fishing power has exerted significant pressure on fish stocks. Today, approximately 34.2% of the world's fish stocks are overexploited (FAO, 2020). Recent research in the Black Sea shows that fish stocks face significant threats to sustainability (Oguz et al., 2012). In addition to overfishing, Illegal, Unreported, and Unregulated (IUU) fishing is another major factor negatively affecting fish stocks, especially in developing countries (WWF, 2015). The use of fine-mesh nets and illegal fishing techniques results in the capture of juvenile fish, contributing to the further decline of fish stocks (Akkuş, 2021). Although existing fishing laws in Turkey restrict such practices, the lack of proper enforcement complicates efforts to protect fish stocks (Yerli & Zengin, 2011). Another significant pressure on fish stocks is climate change. Rising ocean temperatures and ocean acidification are causing changes in the habitats of many fish species, leading to habitat loss and even the extinction of some species (IPCC, 2019). Additionally, increasing industrialization and population growth contribute to marine pollution, which severely damages fish stocks. In the Aegean Sea, fishing pressure has increased, leading to a decline in economically valuable species such as grouper and sea bass, while pollution and overfishing in the Mediterranean are similarly threatening fish stocks (Colloca et al., 2017). Plastic waste and chemical pollution pose a threat to the health of fish, reduce water quality, and lower oxygen levels, creating "dead zones" in marine ecosystems (WWF, 2015). Moreover, habitat loss is also contributing to the decline of fish stocks by destroying breeding and feeding areas for fish. Dams and rivers in Turkey disrupt the natural migration routes of fish, particularly affecting their mobility during breeding seasons. This situation has caused a decline in fish species dependent on river systems. For example, fish populations in major rivers like the Kızılırmak and Sakarya have been significantly impacted (Akbulut et al., 2022). Fisheries management is the process of regulating, controlling, and monitoring fishing activities to ensure the sustainable use of fish resources. This management aims to protect fish stocks while also considering the economic interests of communities dependent on fishing for their livelihood. Fisheries management takes into account biological, economic, and social factors, aiming to protect marine ecosystems and ensure that fish stocks are preserved for future generations (Jennings, Kaiser & Reynolds, 2001). The concept of fisheries management began to develop in its modern sense at the beginning of the 20th century. Particularly in the 1950s, it was recognized that fish stocks were declining due to overfishing, leading to the establishment of international agreements and regional fisheries organizations. These organizations implemented regulations such as quotas, fishing seasons, and designated fishing areas to make fishing activities more sustainable (Garcia & Grainger, 2005). The primary goal of fisheries management is to ensure the sustainable use of fish stocks. In line with this goal, preventing overfishing, maintaining balanced exploitation of fish populations, and preventing the degradation of marine ecosystems are the top priorities. Additionally, ensuring the livelihood continuity of communities involved in fishing is another important objective of fisheries management (Caddy & Cochrane, 2001). In this study, the fundamental regulations related to fisheries in Turkey will be discussed, and the extent to which these policies align with sustainable fishing principles will be evaluated. Additionally, considering the emerging problems in aquatic ecosystems, solution proposals for the sustainable management of Turkey's fishery resources will be presented. Ecosystem-based approaches have proven effective in many global fisheries management practices (Garcia & Cochrane, 2005; Hilborn & Ovando, 2014).

THE DEVELOPMENT OF FISHERIES MANAGEMENT IN TURKEY

Turkey, surrounded by seas such as the Mediterranean, Aegean, Marmara, and Black Sea due to its geographical location, has been a country engaged in fishing throughout its history. In addition to being surrounded by seas, its wealth of lakes and rivers has fostered a high level of biodiversity. The roots of fisheries management and policies in Turkey can be traced back to the Ottoman period. During the Ottoman period, regulations were made especially regarding fishing in the Bosphorus. Fishing was managed with these regulations (Doğan, 2011), bans imposed during the breeding season to protect fish populations (Gümüş, 2018), The main purpose of the regulations made to manage fisheries in the Bosphorus and the Black Sea during the Ottoman Empire, when technology was less developed than today, was to ensure the sustainable use of fish stocks (Pesen, 2016; Yurtoğlu, 2017), and strict control of fishing rights in the Istanbul Strait, the Golden Horn, and the Dardanelles, with rules governing the number of boats and types of nets used in the Istanbul Strait (Tunalidir, 2014). These management practices from the Ottoman period laid the foundation for the fisheries management policies applied in the Republic of Turkey today. In the Ottoman period, fishing was conducted by local coastal communities using manpower. Modern fisheries management in Turkey began to develop in the mid-20th century, particularly from the 1950s onward (TÜİK, 2019). The 1950s were a significant period for Turkey because, with the development of industry, industrial fishing activities increased, and the technology used in fishing, such as boats and equipment, advanced, thus putting increasing pressure on fish stocks year by year. With these developments, the need to protect fish stocks and implement sustainable fisheries management became evident, prompting the state to introduce various regulations and laws. One of the earliest official regulations was the Fisheries Law No. 1380, enacted in 1971. This law was the first major piece of legislation aimed at regulating and monitoring fishing activities in Turkey (Kaya and Can, 2022). The Fisheries Law No. 1380 was designed to protect, develop, and ensure the sustainable use of aquatic resources. It covers the fundamental regulations on the fishing, production, control, and trade of aquatic products in both seas and inland waters. The main goal of the law is to protect fish stocks, regulate fishing activities, and ensure the implementation of sustainable fishing practices. This law includes regulations on fishing methods and tools, the determination of fishing seasons and bans, as well as provisions for inspections and penalties related to fishing. Additionally, the law provides the legal framework for aquaculture and the establishment of fishing quotas to protect aquatic ecosystems. Through this law, the goal is to combat issues such as illegal and overfishing and to ensure the sustainable use of aquatic resources. Furthermore, it provides the necessary legal infrastructure for monitoring activities such as aquaculture and coastal fishing. The Fisheries Law No. 1380 is one of the most significant legal regulations contributing to the modernization of Turkey's fishing sector and remains in effect today, playing a crucial role in the protection of water resources.

FUNDAMENTAL ISSUES IN FISHERIES MANAGEMENT

Overfishing and the Decline of Fish Stocks

Overfishing is one of the most significant problems in fisheries management in Turkey. The rapid depletion of fish stocks, especially in regions with heavy fishing activity like the Black Sea and the Sea of Marmara, has serious impacts on ecosystems. Although fishing seasons and quotas for various fish species have been established in Turkey, there are gaps in the enforcement of these quotas, and the problem of overfishing persists. This issue threatens the sustainability of the fishing sector and disrupts the marine ecosystem, putting fish populations at risk. This problem is most apparent in the Black Sea and the Sea of Marmara. In the Black Sea, stocks of economically valuable species such as anchovy, sprat, and bonito have declined, negatively impacting local fishermen and the national economy. In the Sea of Marmara, many fish species, particularly bluefish and horse mackerel, are under threat due to overfishing. The primary causes of overfishing include the use of modern technologies even by small-scale fishing operations and the insufficient enforcement of fishing quotas. Although certain fishing bans, size restrictions, and quotas have been implemented to prevent the depletion of fish stocks in Turkey, stricter monitoring and regulation are needed to make these measures effective. Additionally, it is important to develop management policies based on scientific data and ensure that all fishermen adhere to these policies. In the long term, solving the problem of overfishing depends on the implementation of sustainable fishing practices and the protection of marine ecosystems.

Illegal, Unreported, and Unregulated Fishing (IUU)

Illegal, unreported, and unregulated (IUU) fishing is another significant problem in Turkish fisheries. Despite legal regulations and monitoring mechanisms, these activities are common, particularly among small-scale fishermen (Göktürk and Deniz, 2017). IUU fishing hinders the protection of marine resources and leads to economic losses by allowing illegally obtained products to enter the market. This situation also highlights weaknesses in enforcement and management mechanisms. Globally, IUU fishing undermines sustainability efforts and remains a challenge for fisheries management (Agnew et al., 2009; Miller & Sumaila, 2014).

Enforcement and Implementation Gaps

One of the key challenges in fisheries management is the lack of effective enforcement of regulations. Although there are legal frameworks governing fishing in Turkey, serious deficiencies in monitoring and enforcement persist. The lack of sufficient oversight in the implementation of fishing quotas and seasonal bans creates a significant challenge for fisheries management (Bryndum-Buchholz et al., 2021). Furthermore, the low level of awareness among fishermen and the insufficient deterrence of punitive measures reduce the effectiveness of enforcement mechanisms.

Technological Advances and Increased Fishing Pressure

In recent years, technological advancements in the fishing sector have increased fishing pressure, leading to significant negative effects on fish stocks. Turkey has been heavily affected by this situation, as the uncontrolled use of technological developments threatens marine ecosystems and fish populations. These advancements were intended to improve the efficiency of the fishing sector. For example, sonar technology, radar systems, and advanced net equipment allow fishermen to fish in larger areas and deeper waters. Sonar and radar systems make it easier to detect fish schools, while modern nets enable the capture of larger quantities of fish in a single haul (Brehmer et al., 2016). However, while these developments increase fishing efficiency, they also exacerbate the problem of overfishing. The fact that even small-scale fishermen can access this advanced technology has led to widespread and continuous fishing pressure. Compared to traditional methods, these technologies interfere more with natural habitats, causing fish populations to decline. This situation has especially affected commercially valuable species, leading to their rapid depletion (Ünal & Franquesa, 2019). As a direct result of these technological advances, fishing pressure is increasing, which has destructive effects on marine ecosystems. Increased fishing pressure makes it difficult to manage fish stocks sustainably and puts some species at risk of extinction. In Turkey, intensive fishing activities in the Black Sea, Marmara, and Aegean Seas have caused fish populations to reach critical levels. Species such as anchovy, bluefish, horse mackerel, and bonito have been significantly affected by this pressure. Another important consequence of increased fishing pressure is the negative impact on the overall balance of marine ecosystems. The overfishing of fish populations disrupts ecosystem processes, leading to chain reactions in the food web. Predator species and marine mammals, in particular, lose their habitats and experience population declines due to the decrease in food sources caused by fishing pressure. Additionally, the failure to leave sufficient stock during the breeding periods of fish due to overfishing negatively affects the population dynamics of future generations.

Climate Change

Climate change is one of the most challenging problems to address in fisheries management. Rising sea temperatures, changes in the chemical composition of water, and alterations in fish habitats complicate the management of fishing activities (Zengin & Doygun, 2022). Especially in Turkish waters, changes in fish migration routes and breeding areas due to climate change necessitate updates to fisheries management strategies. One of the most visible effects of climate change is the observed rise in sea temperatures. The increase in sea temperatures in Turkish waters is directly affecting the distribution and migration patterns of many fish species. In particular, the warming of waters in the Aegean and Mediterranean Seas has led to a greater prevalence of tropical fish species in these regions (Bianchi, 2007). This situation is shrinking the habitats of local fish species and increasing competitive pressure. The rise in water temperatures also affects fish reproductive, growth, and feeding behaviors, disrupting population dynamics. For example, in the Black Sea, populations of species like anchovy, which prefer cold water, are showing a decline, while species that prefer warmer waters are migrating to the region. This is altering the economic dynamics of the fishing sector and creating new challenges for local fishermen. Significant changes are also occurring in the migration and distribution patterns of fish species due to the effects of climate change. Some fish species in Turkish waters are changing their migration routes in search of suitable temperatures and food, leading to spatial changes in fishing activities. In the Black Sea and Aegean Sea, it has been observed that some fish species are migrating further north or into deeper waters (Ünal & Franquesa, 2019). These shifts lead to lower catch rates for local fishermen and negatively affect fishing efficiency. These migrations pose significant challenges for local fisheries management because fish species traditionally caught in specific areas are moving to different regions, altering fishing activities and economic expectations. Moreover, the decline of local fish populations as a result of these migrations can disrupt ecosystem balances in the region. Climate change-induced shifts in temperature and ocean conditions have led to large-scale redistributions of fish stocks, impacting local fisheries globally (Brander, 2007; Cheung et al., 2010).

Management Based on Insufficient Data

The management of fisheries based on scientific data is of great importance. However, the insufficient collection of data on fish stocks and the lack of adequate research on sustainable fishing quotas present significant challenges in Turkey. The scarcity of scientific studies makes it difficult to develop and implement effective management policies (Ünal & Franquesa, 2019). Particularly, the lack of long-term data on fish species' population parameters, stock estimation, and future projections hampers sustainable fisheries management.

Social and Economic Pressures

In Turkey, fishing is an important source of livelihood for many small-scale enterprises and local communities. However, economic and social pressures are among the factors threatening the sustainability of fisheries management. Fishermen's concerns about making a living sometimes lead to increased overfishing and illegal fishing activities. These social and economic pressures also create challenges in the implementation of fisheries policies.

SOLUTIONS AND FUTURE PERSPECTIVES

Overfishing is one of the most significant issues leading to the decline of fish stocks in Turkey. To address this problem, fishing quotas must be more strictly enforced, and more effective mechanisms for combating illegal fishing should be implemented. Setting fishing quotas based on scientific data and ensuring their strict enforcement can help protect fish populations. Additionally, educational programs to raise awareness among fishermen and the promotion of sustainable fishing practices are essential. Advanced technologies used in fishing increase the fishing pressure on fish populations. To prevent this, the uncontrolled use of technology should be limited, and regulations regarding the use of sonar and advanced net systems should be implemented. Moreover, the use of technology in monitoring and controlling fish stocks could be an important step toward sustainable fisheries. Climate change is causing changes in sea temperatures and fish migration routes. To manage these effects, fisheries policies should be adapted to the changing climate. In particular, expanding marine protected areas and closely monitoring changes in fish population distribution dynamics will be critical for long-term sustainability. Illegal, unreported, and unregulated (IUU) fishing is another significant problem that increases pressure on fish stocks. To address this issue, effective monitoring and surveillance mechanisms should be established, and penalties should be made more deterrent. Additionally, registering small-scale fishermen and raising awareness among local communities about the fight against illegal fishing can contribute to solving the problem (Ünal & Franquesa, 2019).In the coming years, Turkey will need to adopt more sustainable policies in fisheries management. As the effects of climate change become more pronounced, the decline in fish populations may accelerate. Therefore, it will be important to develop adaptable management strategies and implement ecosystem-based fisheries practices to protect Turkey's marine resources. Furthermore, more comprehensive policies to combat marine pollution should be implemented, and the health of marine ecosystems must be preserved. International best practices in fisheries management stress the importance of ecosystem-based approaches to ensure the longevity of fish stocks and marine biodiversity (Garcia & Cochrane, 2005).

REFERENCES

Agnew, D. J., Pearce, J., Pramod, G., Peatman, T., Watson, R., Beddington, J. R., & Pitcher, T. J. (2009). Estimating the worldwide extent of illegal fishing. *PloS one*, *4*(2), e4570. Miller, D.

Akbulut, N. E., Bayarı, S., Akbulut, A., Özyurt, N. N., & Sahin, Y. (2022). Rivers of Turkey. In Rivers of Europe (pp. 853-882). Elsevier.

Akkuş, M. (2021). Van Lake Fishery Management and Pearl Mullet (Alburnus tarichi Guldenstaedtii, 1814) Conservation Studies. Voice of Nature, (8), 47-59. (in Turkish)

Bianchi, C. N. (2007). Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia, 580, 7-21.

Brander, K. M. (2007). Global fish production and climate change. *Proceedings of the national academy of sciences*, *104*(50), 19709-19714.

Brehmer, P., Lafont, T., Georgakarakos, S., Josse, E., Gerlotto, F., & Collet, C. (2006). Omnidirectional multibeam sonar monitoring: applications in fisheries science. FISH and Fisheries, 7(3), 165-179.

Bryndum-Buchholz, A., Tittensor, D. P., & Lotze, H. K. (2021). The status of climate change adaptation in fisheries management: Policy, legislation and implementation. Fish and Fisheries, 22(6), 1248-1273.

B: Biological Sciences, 360(1453), 21-46. https://doi.org/10.1098/rstb.2004.1580

Caddy, J. F., & Cochrane, K. L. (2005). A review of fisheries management past and present and some future perspectives for the third millennium. *Ocean & Coastal Management*, *44*(9-10), 653-682. https://doi.org/10.1016/S0964-5691(01)00072-7

Cheung, W. W., Lam, V. W., Sarmiento, J. L., Kearney, K., Watson, R. E. G., Zeller, D., & Pauly, D. (2010). Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. *Global change biology*, *16*(1), 24-35.

Christensen, V., & Maclean, J. (Eds.). (2011). *Ecosystem approaches to fisheries: a global perspective*. Cambridge University Press.

Colloca, F., Scarcella, G., & Libralato, S. (2017). Recent trends and impacts of fisheries exploitation on Mediterranean stocks and ecosystems. Frontiers in Marine Science, 4, 244.

Doğan, F. (2011). Fisheries on the Bosphorus in the Ottoman Empire. History School, X(39-57).(in Turkish)

FAO. (2020). *The state of world fisheries and aquaculture 2020*. Food and Agriculture Organization of the United Nations.

Garcia, S. M., & Grainger, R. J. R. (2005). Gloom and doom? The future of marine capture fisheries. *Philosophical Transactions of the Royal Society*

Göktürk, D., & Deniz, T. (2017). Illegal, Unreported, and unregulated fishing in Turkey: Effects on Marine Ecosystem and sustainable fisheries. Researches on Science and Art in 21st Century Turkey, Arapgırlıoglu H., Atık A., Elliott R., Turgeon E., Eds, 2, 2629-2638.

Gümüş, Ş.Ö. 2018. The Fishing and Fishing Income in The Kilia Fishgarths In 1763. *The Journal of Academic Social Science, 82 (36-74).*

Hoffmann, R. C. (2017). Economic development and aquatic ecosystems in medieval Europe. In Internal Colonization in Medieval Europe (pp. 123-161). Routledge.

Hilborn, R., & Ovando, D. (2014). Reflections on the success of traditional fisheries management. *ICES journal of Marine Science*, *71*(5), 1040-1046.

Hilborn, R., & Ovando, D. (2014). Reflections on the success of traditional fisheries management. *Marine Policy*, *44*, 9-14. https://doi.org/10.1016/j.marpol.2013.01.017

IPCC, (2019). *Special Report on the Ocean and Cryosphere in a Changing Climate.*

Jennings, S., Kaiser, M., & Reynolds, J. D. (2009). *Marine fisheries ecology*. John Wiley & Sons.

Kaya, H. B., & Can, M. F. (2022). Characterization of Fisheries Violations From the Türkiye's Mediterranean Coasts Within the Scope of Fisheries Law No. 1380. Acta Natura & Scientia, 3(2).

Miller, D. D., & Sumaila, U. R. (2014). Flag use behavior and IUU activity within the international fishing fleet: Refining definitions and identifying areas of concern. *Marine Policy, 44,* 204-211.

Oguz, T., Akoglu, E., & Salihoglu, B. (2012). Current state of overfishing and its regional differences in the Black Sea. Ocean & coastal management, 58, 47-56.

Pesen, B. (2016). Fishing in The Black Sea During The Ottoman Period (1500-1700). Cumhuriyet Üniversity, Institute of Social Sciences, Master Thesis, Sivas, Türkiye. 8p. (in Turkish)

TUİK, (2019). Turkey Fisheries Statistics (in Turkish). Turkish Statistical Institute Publications.

Tunalidir, F. (2014). The evaluation of Ottoman fishery in the 19th century according to the archival resources. Yeditepe Univeristy, Constantinople, Türkiye., Master's thesis, 47p.

Yerli, S. V., & Zengin, M. (2011). Çıldır Gölü Balıkçılık Yönetimi Üzerine Bir Değerlendirme. 1. Çıldır Gölü Çalıştayı Bildirileri, 21-22 Haziran 2011, Ardahan.

Yurtoğlu, N. (2017). History School (1923-1960). Journal of Historical Studies, 32(1), 233-263. (in Turkish)

WWF. (2015). Living blue planet report: Species, habitats and human well-being (in Turkish). World Wide Fund for Nature.

CHAPTER 3

LAKE BALIK FISHERY

Assoc. Prof. Dr. Adem Sezai BOZAOĞLU [1]
Assist. Prof. Dr. Mustafa AKKUŞ [2]

[1] Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Fishing and Processing Technologies, Zeve Campus, Van 65080, Türkiye. sbozaoglu@yyu.edu.tr, Orcid ID: 0000-0003-4078-5159
[2] Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Fishing and Processing Technologies, Zeve Campus, Van, Türkiye. makkus@yyu.edu.tr, Orcid ID: 0000-0002-8900-9495

INTRODUCTION

With the rapid increase in the human population, the need for food has also increased accordingly. Population growth has put pressure on agricultural production, water resources, and food supply. This situation has made issues such as food security, the sustainable use of agricultural land, water resource management, and climate change more important. Therefore, innovations in agricultural technologies, sustainable food production systems, and the efficient use of resources are becoming increasingly crucial. The quantity of fish harvested from aquatic ecosystems, which are rich in protein, plays an important role in meeting this demand. However, with the advancement of technology, the development of fishing tools used in fisheries has increased the fishing pressure on fish stocks. The increase in this pressure has negatively affected many fish stocks. Research suggests that if overfishing on fish stocks continues, 90% of the world's fish stocks will be completely depleted by 2050. (Hilborn, 2021). Although seas are at the forefront in fishing, lakes and rivers in regions far from the sea constitute important fishing resources. Lake Balık is a freshwater lake located within the borders of Ağrı Province in the Fırat Basin, covering an area of 34 km² (Figure 1.).

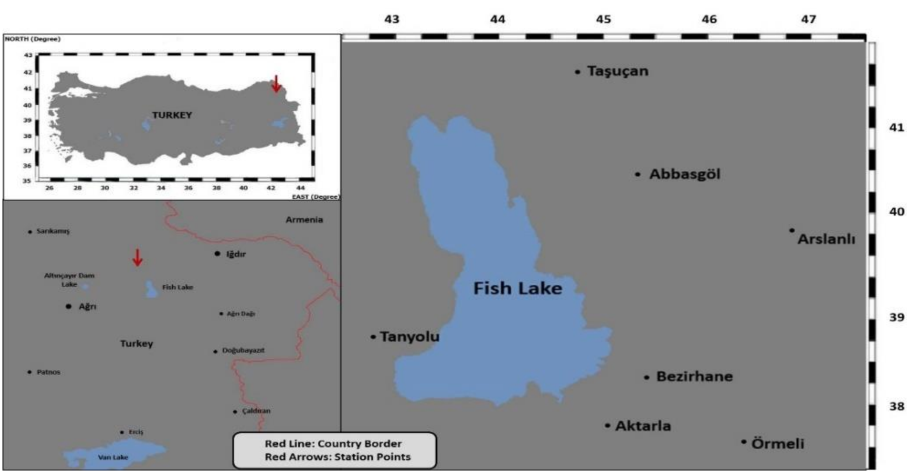

Figure 1. Lake Balık

Due to its elevation of 2,241 meters above sea level, it holds the distinction of being Turkey's highest natural lake (Karabulut, 2018). Lake Balık is home to three species belonging to two families as native species: *Cyprinus* *carpio* Linnaeus, 1758), *Salmo trutta* (Linnaeus, 1758) and *Capoeta capoeta* (Guldenstaedt, 1772). (Öztürk, 2014).

In Turkey, 3,806 tons of the 33,532 tons of inland water catch consist of capoeta and carp, which account for approximately 10% of the total catch (TUİK, 2023). These species are the target species in Lake Balık. In Lake Balık, the only fishing gear used are gillnets and trammel nets. These nets are the most important passive fishing gear used in coastal fisheries in Turkey.

The primary biological goal of fisheries management is to regulate the amount of fish that can be harvested from a stock while ensuring its continuous renewal as a sustainable resource. In this context, sustainability refers to the ability of fishers to exploit the resource without impairing its production capacity (Kolding, J., & van Zwieten, P. 2014). In particular, small-scale fisheries are common in small lakes, such as Lake Balık. In small-scale fisheries, all fishers are local residents who practice traditional fishing methods. Additionally, within small-scale fisheries, some individuals engage in fishing as a secondary occupation or a hobby, working part-time in this activity (Ünal, 2003).

The success of fisheries management largely depends on the collection of reliable data and its accurate processing using appropriate methods (Ünal, 2001). A significant portion of these data consists of biological data, information related to fishing gear and equipment, as well as demographic and socioeconomic data. The number of fishers and fishing techniques in a region, the diversity and capacity of fishing gear, the species caught, and the quantities harvested are just as necessary and important as stock information about the target species. Continuous collection and monitoring of such data are essential (Tokaç *et al.*, 2007). Moreover, determining effective fishing effort and understanding the types of fishing pressures exerted by different techniques on fish stocks are not only crucial for the protection and sustainable use of fishery resources but also for preserving the income and livelihoods of fishers. Therefore, it is necessary to produce fisheries maps for each region, complete inventory studies, and conduct research not only on fisheries but also on the fishers themselves (Ünal, 2003).

İn this context, in this section;

1. Technical plan of the gill nets and trammel nets used in Lake Balık
2. Operational information about fishing

3. Characteristics of fishing boats using these nets
4. Determination of the fish caught
5. Problems encountered by fishermen are identified and solution suggestions are presented.

TECHNICAL PLANS OF GILLNETS AND TRAMMEL NETS USED IN LAKE BALIK

It has been determined that two different types of gillnets and trammel nets are used in Lake Balık. Multifilament gillnets with a trammel structure are used for the fishing of carp and capoeta fish. For trout fishing, simple monofilament gillnets are employed. All the specifications of these nets are illustrated in the accompanying figure.

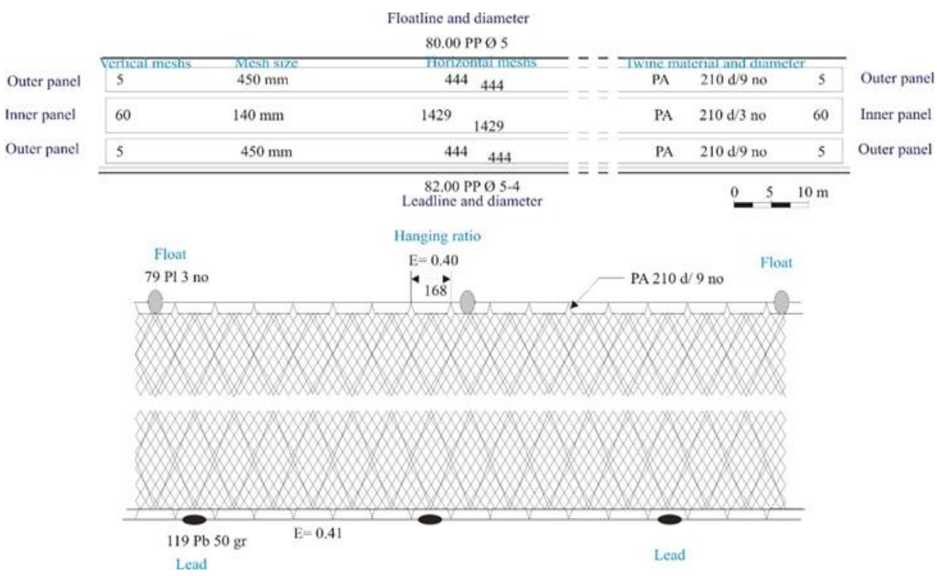

Figure 2. Technical plan of trammel net used in carp and capeota fishing.

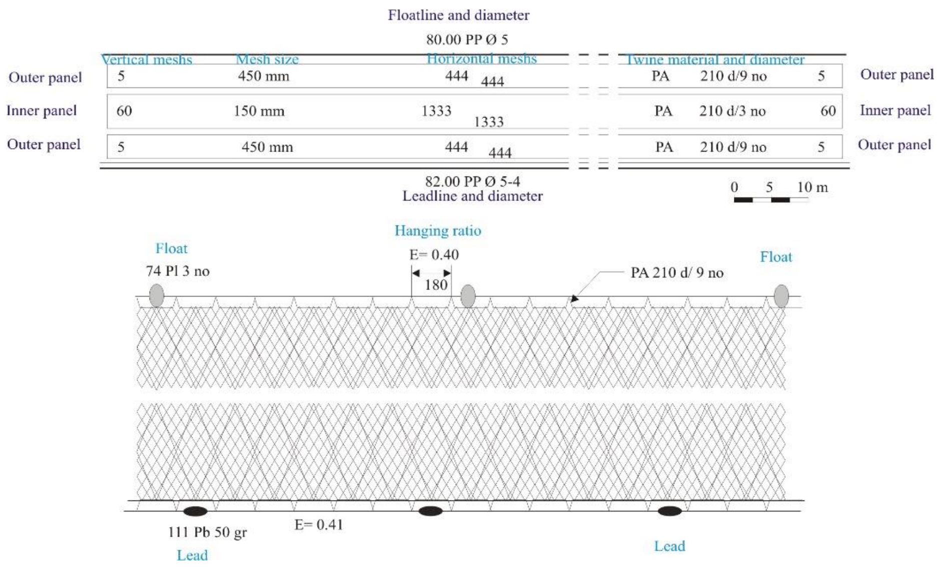

Figure 3. Technical plan of trammel net used in carp and capeota fishing.

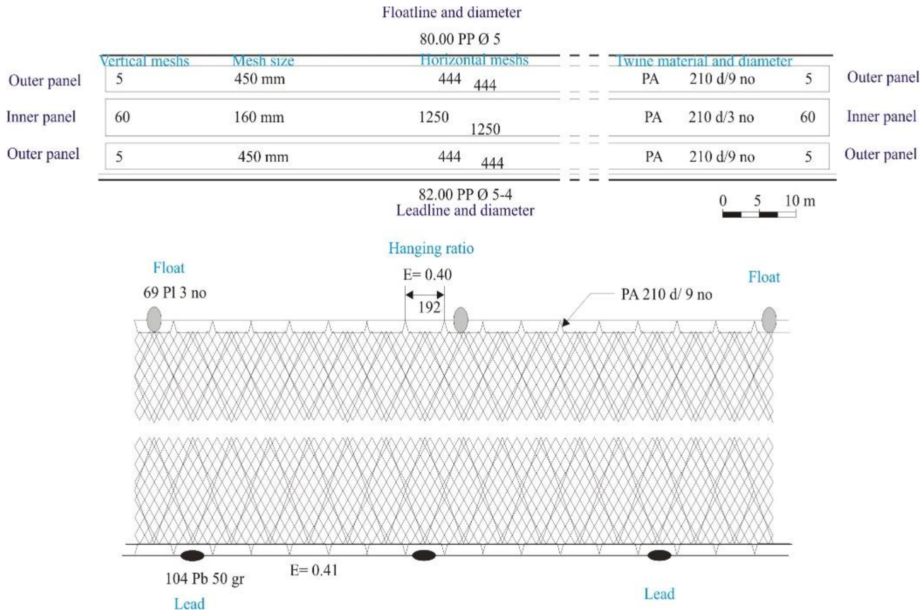

Figure 4. Technical plan of trammel net used in carp and capeota fishing.

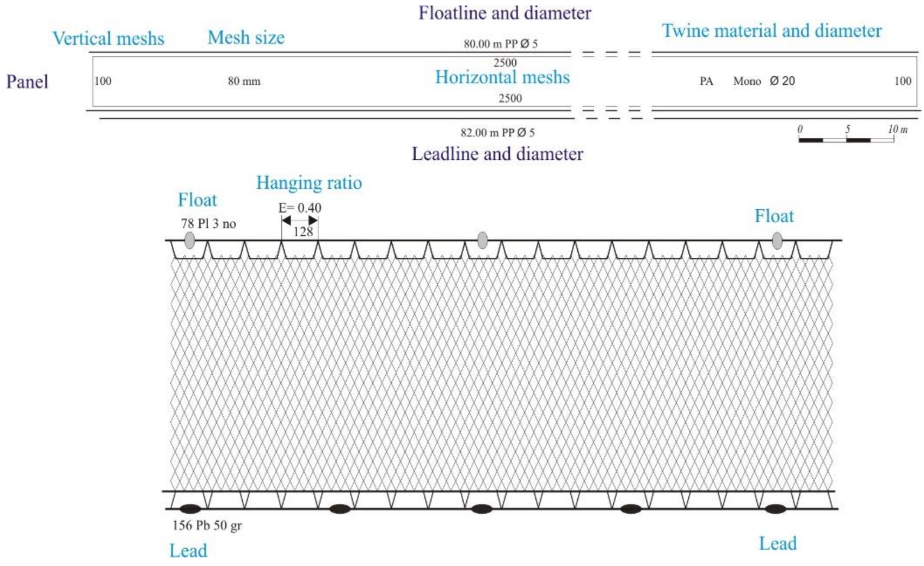

Figure 5. Technical plan of plain monofilament gillnet used in trout fishing

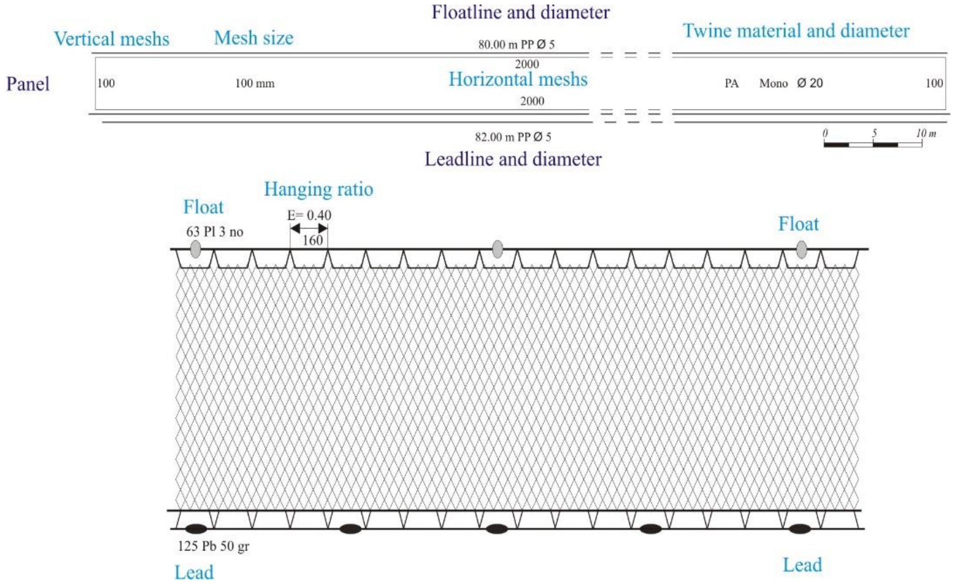

Figure 6. Technical plan of plain monofilament gillnet used in trout fishing

OPERATIONAL INFORMATİON ON FISHING ACTIVITIES

In gillnet and trammel nets fishing, the nets are typically set before sunset and retrieved at sunrise. However, in Lake Balık, to reduce fuel costs and save on labor, the fishers often leave the nets in the water after collecting the fish in the morning. As a result, the nets remain in the water for approximately 24 hours. Since none of the fishing boats are equipped with net drums, the nets are deployed and retrieved manually.

THE FEATURES OF FISHING BOATS

Until 2020, boats on Lake Balık were generally made from sheet metal, but in recent years, they are predominantly made from wooden materials (Figure 7). According to a study conducted by Bozoğlu 2020, the average age of the boats is reported to be 11,14±5,03 years, with an average length of 5,54±0,34 meters, an average engine age of 11,61±3,97 years, and an average engine power of 9,50±1,80 horsepower (Figure 8). None of the boats are equipped with a windlass. Boats with similar features are still used today.

Figure 7. Fishing boat

Figure 8. Fishing boat engine

AMAUNT OF CAUGHT FISH

Three species of fish are caught in Lake Balık: carp, capoeta, and trout. Fishing activities are not possible between December and May due to the lake being frozen during this period. A study conducted in 2020 reported that a total of 6,326 kg of fish is caught annually in the lake (Bozaoğlu, 2020).

PROBLEMS FACED BY FISHERMEN

In recent years, the biggest issue encountered at Lake Balık has been the decline in water levels, primarily due to drought, global warming, and the use of lake water for drinking and agricultural irrigation (Figure 9).

Figure 9. Water level in Lake Balık

The reduction in water levels has negativelyimpacted aquatic species, especially fish, by reducing their access to key habitats and spawning areas. Natural trout in the lake are particularly impacted during their spawning period, when they migrate to freshwater sources flowing into the lake to lay their eggs. The decrease in water levels disrupts this process, leading to a decline in natural trout populations.

Another issue is the release of freshwater crayfish, whose origin in the lake is unknown (Figure 10). This situation negatively impacts the native species in the lake. This invasive species not only competes with native species for food but also poses a threat to their eggs, further endangering their populations. Additionally, the potential for disease transmission presents a serious risk to the native species.

Figure 10. Freshwater crayfish

RECOMMENDATIONS

The lake basin should be protected and improved in accordance with the principles outlined in the Ministry of Forestry and Water Affairs' "Regulation on the Protection of Drinking-Use Water Basins."

The State Hydraulic Works (DSI) should implement additional measures to prevent water leakages in Lake Balık, building upon their previous efforts, and should develop investment plans accordingly.

Inspections should be increased, and more deterrent penalties should be enforced (Figure 11.).

Figure 11. Controls and protection activities

Awareness raising meetings regarding the sustainability of fisheries should be organized (Figure 12.).

Figure 12. Meetings

CONCLUSION

Lake Balık has recently gained popularity for amateur and sporty fishing activities. This development has made the lake a popular destination for nature enthusiasts and fishing enthusiasts alike. In addition to its natural beauty, these activities attract visitors to the region, contributing both economically and to the area's tourism potential. Lake Balık offers an ideal setting for those looking to enjoy nature in a peaceful and clean environment, while fishing activities bring economic vitality to the region.

In the management of fish resources in Lake Balık, alongside the implementation of a sustainable fisheries policy, effective and proper management strategies are needed to ensure the continued existence of fishing activities. To protect fish stocks and ensure sustainable fishing in Lake Balık, it is essential to monitor and continuously update data on fishing gear specifications, boat information, fishing operational data, as well as ecological, biological, and socioeconomic information. Moreover, the identification of fishing-related problems and efforts to address these issues should also be pursued.

According to the notification numbered 6/1 regarding the regulation of commercial fishing, published by the Ministry of Agriculture and Forestry in the Official Gazette on 11.08.2024, fishing in Lake Balık has been banned. There is currently no information on the duration of this newly implemented ban or whether the lake will be reopened for fishing in the future. In this context, when discussions about resuming fishing activities in the lake arise in the future, the studies conducted here will serve as an important resource during the decision-making process.

REFERENCES

Bozaoğlu, A. S. (2020). General Status of Lake Ağrı Balık Fishery. *Journal of Anatolian Environmental and Animal Sciences*, *5*(4), 750-758.

Hilborn, R. (2021). Increasing fisheries harvest with MPAs: Leaving South and Southeast Asia behind. *Proceedings of the National Academy of Sciences*, 118 (17), e2026410118

Karabulut, K. 2018. Socio-Economic Profile of Ağrı Province. AİÇÜ Publications. Publication No: 19. Ankara. Türkiye.

Kolding, J., & van Zwieten, P. (2014). Sustainable fishing of inland waters. *Journal of Limnology*. 73(s1): 128-144.

Öztürk, O. (2014). *Ağrı Fish Lake City Living in Determining The Executıon of Species of Fish and Search of Some Biological Characteristics of Species*. Balıkesir University, Institute of Science. Master Thesis, Balikesir, Türkiye. 102p.

Tokaç, A., Ünal, V., Tosunoğlu, Z., Akyol, O., Özbilgin, H., & Gökçe, G. (2007). Structural Analysis of Aegean Sea Fisheries, Ege University Scientific Research Projects. 2002/SÜF/006, 161 p. Bornova, Türkiye.

Ünal, V. (2003). Socio-economic analysis of part time small-scale fishery, Foça (Aegean Sea), *Ege Journal of Fisheries and Aquatic Sciences*, 20(1-2), 165-172.

Ünal, V., Akyol, O., & Hoşsucu, H. (2001). Requirements for bio-economic data in fisheries management. *Ege Journal of Fisheries and Aquatic Sciences*, 18(1-2), 243-253. http://doi:10.12714/egejfas.2001.18.1.5000157215

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CHAPTER 4

CONSERVING AQUATIC REALMS: THE RISK OF SYNTHETIC PROGESTINS

Assoc. Prof. Dr. İlknur MERİÇ TURGUT [1]
Dr. Melek YAPICI [2]
Prof. Dr. Akasya TOPÇU [3]

[1] Ankara University Faculty of Agriculture, Department of Fisheries and Aquaculture Engineering, Ankara, Türkiye, meric@agri.ankara.edu.tr, Orcid ID: 0000-0001-7404-5680
[2] Ankara University Faculty of Agriculture, Department of Fisheries and Aquaculture Engineering, Ankara, Türkiye, myapici@ankara.edu.tr, Orcid ID: 0000-0002-7200-855X
[3] Ankara University Faculty of Agriculture, Department of Fisheries and Aquaculture Engineering, Ankara, Türkiye, atopcu@ankara.edu.tr, Orcid ID: 0000-0002-5229-4181

INTRODUCTION

OVERVIEW OF STEROID HORMONES

Steroid hormones, known to be among the most potent endocrine-disrupting chemicals (EDCs) affecting ecosystems, enter the environment through hormonal medications used by over 100 million women worldwide (Hoffmann and Kloas 2012) and their effects raise concerns in off-target realms more particularly aquatic organisms in last decades (Länge et al., 2001; Pawlowski et al., 2004; Sumpter and Johnson, 2005; Jobling et al., 2006; Caldwell et al., 2008; Tyler and Jobling, 2008; Christen et al., 2010; Besse and Garric, 2009). The use of these hormonal drugs, preferred in fertility and birth control methods, has been increasing globally each year with the development of sexual freedom culture (Stanczyk et al., 2013). The market for these drugs, which was $42.1 billion in 2015, is expected to reach $64 billion by 2025 (Kaur et al., 2021).

Like other EDCs, hormonal drugs are excreted through urine and feces as hydroxylated and methylated metabolites that can conjugate with glucuronides and sulfates (Lai et al., 2000; Junior et al., 2010; Brito et al., 2010), mixing with domestic wastewater. Since these drugs cannot be completely removed by sewage treatment plants (Zheng et al., 2008), they lead to the contamination of surface and groundwater. Wastewater treatment plants are the primary pathways and also point sources of these drugs, in the mean while the concentration of them in aquatic biota, based on both different exposure scenarios, the types of steroid and effluent discharges.

Steroid hormones, which enter the ecological environment and exhibit environmental effects, show their physiological effects by binding to steroid hormone receptors, causing changes in gene transcription and cell function (Anonymous, 2023). Upon reaching the target cell, they dissociate and enter the cell by diffusion. Some initiate new mRNA formation from DNA in the chromatin without binding to the receptor in the cytoplasm (Anonymous, 2017).

Steroid hormones synthesized from cholesterol compounds in tissues such as the ovary, testis, and adrenal cortex are first converted to pregnenolone in the cell and then to progesterone, a fat-soluble compound with 21 carbons (Anonymous, 2017). Progesterone is an intermediate in the synthesis of adrenal cortex steroid hormones. The pathway of pregnenolone conversion to progesterone involves its conversion to progesterone by a dehydrogenase effect or to 17-hydroxypregnenolone by a specific 17-hydroxylase effect. Progesterone and 17-hydroxypregnenolone are converted to various active hormones by specific oxygenases and dehydrogenases (Anonymous, 2017). Progesterone is transported in the blood by binding to globulin. Approximately 75% of progesterone and its metabolites are excreted with feces after being transported to the intestines via bile. The main metabolite of progesterone, pregnanediol, is excreted with urine. In target cells, it binds to a specific binding protein in the cytosol, and the resulting hormone-receptor complex is transported to the nucleus, interacting with chromatin to affect RNA synthesis and thus exhibit hormonal effects (Anonymous, 2017). Progesterone, along with estrogen hormone, is effective in the development of secondary sexual characteristics.

DEFINITION OF PROGESTINS

Chemical substances that exhibit progesterone hormone effects are called "gestagens." Although they are used with names such as progestogen, gestagen, or progestin, the most commonly used term is "progestin." Since natural progesterone hormone is rapidly inactivated in the organism, various synthetic progestins have been developed. In the human therapy context, progestogens are used in pharmaceutical formulations, helping the treatment of breast and ovarian cancer, osteoporosis, and as components of the contraceptive pill, being the substrate of the progestin-only contraceptive or a part of the combined pill.

Progesterone and synthetic progestins primarily exert their effects through receptors such as the progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR) (Fent, 2015).

Synthetic progestins can have various hormonal activities, including estrogenic, antiandrogenic, and androgenic. They are also part of many oral contraceptives (a mixture of synthetic estrogen and a progestin) and hormone therapy drugs (Hoffmann and Kloas 2012; Rocha and Rocha 2022). Synthetic progestins (gestagens) and their metabolites also exhibit synergistic effects with xenoestrogens, thereby triggering the formation of free radicals. In this context, oxidative stress occurs as a result of the imbalance between free radicals and/or reactive oxygen species (ROS) and the antioxidant system.

IMPACTS ON AQUATIC ECOSYSTEMS

Aquatic ecosystems are more than the final repositories of residues of contaminants that are found everywhere. They are used to previously dispose of most residues that have no other direct final outlet, thus concentrating them and forming a pluri-polluted mixture that is subsequently diffused everywhere and also affects human health. This is particularly significant in the case of water-soluble drugs. Domestic, zoonotic, farmed, and wild animal populations are all medicated. The corresponding active ingredients as a whole follow the procedures for wastewater from sewage, but high quantities of inert ingredients are also in play.

Fish, amphibians, and aquatic reptiles are used as indicators to show the effects of exposure to natural and synthetic steroid hormones and other emerging chemical substances in the aquatic environment (Bergman et al., 2013; Damstra et al., 2002; Kloas et al., 2009; Richardson et al., 2005). Research has focused on the effects of estrogenic, androgenic, and thyroidogenic compounds and some antagonists of these compounds on the aquatic realm. The results of gestagen exposure studies in fish show changes in reproduction, development, and behavior (Orlando and Ellestad, 2014). The adverse effects of progesterone and progestins on fish reproduction have been identified by Zeilinger et al., 2009; Paulos et al., 2010; Runnalls et al., 2013 screening a diversed group of bony fish such as rainbow trout (Oncorhynchus mykiss), zebrafish (Danio rerio), three-spined stickleback (Gasterosteus aculeatus), medaka (Oryzias latipes), and fathead minnow (Pimephales promelas). While some studies have examined the bioaccumulation capabilities of certain progestins in different fish, most published studies indicate that fish are exposed to one or more gestagen concentrations (Orlando and Ellestad, 2014). Acoording to the findings, they have been shown to cause changes in hormone levels (Runnalls et al., 2013), transcriptional effects in adult fish (Zucchi et al., 2013, 2014) and embryos (Zucchi et al., 2012), changes in sex development (Liang et al., 2015), and the induced development of male secondary sexual characteristics in female fish (Zeilinger et al., 2009; Runnalls et al., 2013). Despite their extensive use alongside synthetic estrogenic steroids, the ecotoxicological effects of progestins have not been sufficiently examined (Fent, 2015).

The environmental impacts of gestagens used by humans have been investigated (Besse and Garric 2009). Although limited data on these molecules prevented a conclusion on environmental risk, it was possible to evaluate the danger and biological effects of gestagens and their metabolites. The results can be summarized as follows:

-Synthetic progestins are found in waste samples and possibly surface waters, especially in the form of metabolites, at concentrations ranging from ng/L to even 100 ng/L,

-The main compounds with progestogenic activity and some active metabolites can affect spawning behavior in fish,

-Metabolites of progestins derived from nortestosterone can act as estrogenic compounds and thus have additional effects with other xenoestrogens like ethinylestradiol, triggering free radical formation,

-Some progestins, such as chlormadinone acetate, cyproterone acetate, and their metabolites, are anti-androgenic and pose a risk to aquatic species.

OUTCOMES OF PROGESTIN-SPECIFIC STUDIES

A variety of responses of progestins among examined aquatic species might be perceived through bio-assays and other trials and we try to compile a few of them belove.

Pietsch et al., (2009) was observed that medroxyprogesterone acetate (MPA) could affect the natural immune system in fish by significantly reducing nitric oxide formation by head and body kidney cells. Both levonorgestrel (LNG) and drospirenone (DRO) have been reported to inhibit reproduction in adult fathead minnows (Pimephales promelas).

In Japanese medaka fish, it was found that norethindrone (NET) caused a significant decrease in reproduction at concentrations greater than 25 ng/L (Paulos et al., 2010). Morphological changes (e.g., spots on the fins of female fish) also indicated that NET exposure could have a strong androgenic effect on fish.

The effects of applying progesterone hormone (P4) at a high concentration of 375 ng/L on reproduction and embryonic development in a species of carp, Pimephales promelas, were examined. Fish were exposed to 0, 10, 100, and 1000 ng/L P4 concentrations for 21 days. The experiment concluded that P4 caused a decrease in egg number and fertilization success and significantly reduced the gonadosomatic index and vitellogenin gene expression in females (DeQuattro et al., 2012).

A variety of progestins as progesterone, didrogesterone, drospirenone, and all progesterone-derived progestins were evaluated on the gene expression of brain aromatase (cyp19a1b) in glial cells of zebrafish. The study found that these progestins did not affect GFP expression. However, it was determined that 19-nortestosterone-derived progestins revealed estrogenic activity by promoting cyp19a1b expression in radial glial cells (Cano-Nicolau et al., 2016).

A study conducted by DeCourten and Brander (2017), the effects of multi-generational exposure to EDCs on reproduction and development in the inland silverside (Menidia beryllina) were investigated. Fish exposed to 1 ng/L of ethinylestradiol (EE2) for 21 days were significantly affected, and an increase in mortality rates and developmental deformities was observed in the next generation.

To examine the effects of synthetic progestin norgestrel (NGT) on reproduction by measuring egg production, histology, and transcriptional expression profiles along the hypothalamic-pituitary-gonadal (HPG) axis in adult zebrafish and fish were exposed to 6, 29, and 69 ng/L NGT for 21 days. It was found that exposure to 69 ng/L NGT led to specific transcriptional changes. This explained that NGT exhibited strong progestogenic and androgenic activities in zebrafish (Liang et al., 2018).

Hou et al., (2018), investigated the effects of norethindrone (NET), an endocrine-disrupting chemical, on sex differentiation and the underlying mechanisms in zebrafish (Danio rerio). Juvenile zebrafish (20 days post-fertilization) were exposed to 5, 50, 500, and 1000 ng/L NET for 45 days. The study examined the sex ratio of populations, gonadal histology, sex differentiation, and transcriptional profiles of regulatory genes involved in steroidogenesis. The results demonstrated that exposure to NET accelerated sexual maturity in males and delayed ovarian maturation in female zebrafish.

The effects of etonogestrel on reproductive behavior, fertility, gonadal histology, and secondary sexual characteristics in male and female Endler guppies (Poecilia wingei) were evaluated. Fish were exposed to two different concentrations of etonogestrel (3.2 ng/L and 320 ng/L) for 34 days. It was found that the mating frequency of fish exposed to etonogestrel was significantly lower compared to control groups. All females exposed to the chemical were found to be infertile. Additionally, less developed oocytes were observed in females exposed to etonogestrel. As a result, low etonogestrel concentration (3.2 ng/L) led to a decrease in mating activity without affecting reproductive success in males but completely inhibited reproduction in females (Steinbach et al., 2019).

The short-term effects of dydrogesterone (DDG) on adult zebrafish (Danio rerio) were assessed and fish were exposed to three different doses of DDG (32 ng/L, 305 ng/L, and 2490 ng/L) for 14 days. Real-time quantitative PCR analysis showed that DDG significantly increased the transcripts of most genes involved in the gonadotropin-releasing hormone (GnRH) pathway in the female brain. In males, a decrease in these gene transcripts was observed. The transcription of cyp19a1a in the ovary increased 2.3-fold at 2490 ng/L DDG, and the transcription of hsd17b2 in the testis increased 2-fold at 305 ng/L DDG and 2.4-fold at 2490 ng/L DDG. Histopathological analysis revealed that exposure to 2490 ng/L DDG significantly increased the percentage of atretic follicles in the ovary. The study concluded that DDG had potential endocrine-disrupting effects and affected ovarian development in zebrafish (Shi et al., 2019).

The effects of norgestrel (NGT) on fin morphology and liver transcriptome in adult female mosquito fish (Gambusia affinis) were evaluated by Hou et al. (2019). Fish were exposed to 377 ng/L NGT for 42 days, and compared to the control group, the genes showing the most significant expression changes were found to be related to fin development, androgen biosynthesis, lipid, and fatty acid metabolism.

Chen et al., (2021) conducted a trial to determine whether gestodene (GES), a commonly used progestin, could alter the expression of genes related to sex hormone synthesis and cause changes in morphological characteristics, courtship behavior, and oocyte development. Mosquito fish (Gambusia affinis) exposed to different concentrations of GES (2.96, 32.9, and 354 ng/L) for 40 days showed that GES, especially at 354 ng/L, affected masculinization in female fish and caused a decrease in body weight relative to length.

The potential toxic effects of norethisterone (NET) on swimming behavior, hormone levels, oxidative-antioxidant enzyme activities, gene transcription patterns, and gonadal differentiation were reported in Japanese medaka (Oryzias melastigma) larvae exposed to 1.31 ng/L NET for 10 days. It was concluded that NET in the marine environment posed potential risks to fish health and could thus destroy marine resources (Dong et al., 2023).

To provide insight into the toxicity mechanism of NGT, a bivalvia, Mactra veneriformis, was used and it was proven that NGT applied at doses of 0, 10, and 1000 ng/L for 21 days disrupted steroid hormone metabolism and damaged the antioxidant defense system. These effects showed that NGT could cause adverse effects on oysters by altering their transcripts and metabolites (Zhao et al., 2023).

The 21-day effects of LNG on fathead minnows (Pimephales promelas) were examined in a study conducted by Zeilinger et al., (2009). Fish exposed to 0.8, 3.3, and 29.6 ng/L LNG showed no effect on male sexual characteristics at the lowest concentration of 0.8 ng/L, but higher concentrations of 3.3 and 29.6 ng/L doses affected masculinization in females.

Kroupova et al., (2014), examined the effects of LNG on the endocrine system of a freshwater fish species, Rutilus rutilus. The experiment was planned for 28 days, and through the period, fish were exposed to four different doses of LNG (3, 31, 312, and 3124 ng/L). It was observed that males exposed to LNG at concentrations higher than 31 ng/L showed a decrease in 11-ketotestosterone (11-KT) levels, a major androgen produced in the gonads. Males exposed to 31 and 312 ng/L LNG produced a high amount of spermatogonia. The 3124 ng/L LNG dose caused an increase in estrogen receptors in both female and male fish. The results of this study showed that LNG caused reproductive system disorders in fish by affecting sex hormone levels.

So as to investigate the effects of LNG on sex differentiation in zebrafish (Danio rerio), embryos were exposed to five different environmentally relevant concentrations (0, 1, 10, 33, and 100 ng/L) of LNG, and their development was followed until sexual maturity. Histological examination 63 days after fertilization showed 100% masculinization in fish exposed to 10, 33, and 100 ng/L. This study showed that environmentally relevant LNG concentrations caused sex differentiation and altered gonadal development in zebrafish (Hua et al., 2015).

Juvenile zebrafish were exposed to three different LNG concentrations (5.5, 79, and 834 ng/L) during sex differentiation in a study conducted by Svensson et al., (2016). As a result, 100% masculinization was observed even at the lowest concentration.

The mechanisms of LNG exposure in Eastern mosquito fish (Gambusia holbrooki) were evaluated by Brockmeier et al., (2016). Hepatic microarray analysis was performed on male and female fish exposed to progestins and anti-progestagens, and the ability of LNG to modulate anal fin growth during exposure to progesterone and androgen receptor antagonists were determined. Gene expression analyses were conducted on male and female fish exposed to agonist LNG, antagonist mifepristone, or a mixture of the two chemicals for 48 hours. Microarray analysis showed that mifepristone did not act as an anti-progestagen in liver tissues and that LNG had strong effects on embryo development and lipid transport processes. Additionally, LNG was found to trigger the formation of male secondary sexual characteristics in females, and the presence of anti-androgen flutamide prevented the elongation of the anal fin in the presence of androgen or LNG.

The evaluation of the effects regarding to multiple stress factors such as temperature and LNG on the condition and reproduction of zebrafish (Danio rerio), both temperature (primarily setting the ambient temperature to 27°C, against warming (+3°C)) and LNG levels (10 ng/L and 1000 ng/L) were adjusted, and a multifactorial experiment was applied for 21 days. While the increase in temperature caused a decrease in the gonadosomatic index (GSI) of females, no effect of LNG was observed. However, the number of eggs was adversely affected by both temperature and LNG. Fish exposed to 1000 ng/L LNG (at both temperatures of 27°C and 30°C) did not engage in reproductive activities, and reproductive disorders were observed in fish exposed to the lowest progestin dose (10 ng/L) at higher temperatures. Accordingly, the degree of ovarian maturation was found to be at the lowest level at the high dose (1000 ng/L) LNG concentration. This study suggests that in a future scenario of global warming and exposure to synthetic hormones, the reproduction of fish species like zebrafish could be at risk, potentially affecting the structure and functioning of related aquatic ecosystems (Cardoso et al., 2017).

Another bio-factor related research by Cardoso et al., (2019), the effects of climate change and pharmaceutical pollution were investigated and adult zebrafish were exposed to different temperatures (27°C and 30°C) and different doses of LNG (10 ng/L and 1000 ng/L) for 21 days. The results showed that multiple stress factors such as temperature and progestins led to an increase in the coefficient of variation of the number distribution of hepatocyte volume (CVN(y)) for zebrafish exposed to LNG. This study indicated that LNG should be seen as a warning sign for its potential effects on other aquatic organisms.

A study to demonstrate the high ecological risk of LNG, newly hatched minnow fish larvae (Gobiocypris rarus) were exposed to 1 ng/L and 10 ng/L LNG for 6 months. The sex ratio of the fish was not affected by the exposure, but histological evaluation revealed disrupted gonadal development. The 1 ng/L LNG dose significantly promoted estradiol in females and testosterone plasma concentrations in both sexes. DNA methylation levels were significantly reduced in the testis tissue of fish exposed to 10 ng/L LNG (Hua et al., 2022).

The bioaccumulation of LNG and its effects on the antioxidant defense mechanism in zebra mussels (Dreissena polymorpha) were represented by Contardo-Jara et al., (2011) and it was found that LNG caused protein damage and increased SOD gene expression, indicating oxidative stress.

The effect of LNG on the mating behavior of male African clawed frogs (Xenopus laevis) was investigated and male frogs were exposed to three different concentrations of LNG (10^{-7} M, 10^{-8} M, and 10^{-10} M). It was observed that all concentrations of LNG increased sexual stimulation in frogs. However, according to Hoffman and Kloas (2012), LNG had negative effects on the reproductive behavior of females, and exposure to LNG could reduce the reproductive success of these organisms.

Lorenz et al., (2016) were investigated the effects of different doses of LNG (0.01 and 10 nM) applied for 72 hours on thyroid hormone on African clawed frogs (Xenopus laevis). In the juvenile frogs, there were not any notably changes which were detected in the brain-pituitary tissue, whereas thyroidal gene expression was directly affected by LNG.

The impacts of different doses of LNG (10 ng/L and 100 ng/L) applied for 8 days on the pathology of the anal fin and reproductive behavior of mosquito fish were investigated by Frankel et al., (2016). When fish were exposed to 100 ng/L LNG, an increase in sexual behavior in males and elongation of the anal fin were observed. Moreover, masculinization was observed in females too.

The endocrine-disrupting effect of two different doses of LNG (10 nM and 100 nM) applied for 24 hours was found to cause higher sensitivity in the gonads of female frogs compared to males. LNG also severely damaged the thyroid system of African clawed frog (Xenopus laevis) larvae. Zikova et al., (2017) were concluded that LNG not only affects the reproductive system but also causes disorders in the thyroid system, negatively affecting the development of the organism.

The freshwater snail species Lymnaea stagnalis was exposed to a mixture of four progestagens (progesterone, LNG, drospirenone, and gestodene) at 10 ng/L for 3 weeks. As a result of the exposure, egg production decreased in the first week, and low-quality eggs were observed (Zrinyi et al., 2017).

In another bivalvia, Ruditapes decussatus, the effects of 1000 ng/L LNG alone or in combination with increasing water temperatures (20, 24, and 28°C) on biochemical parameters and gonad histology were investigated for 28 days. The research showed that clams treated with LNG alone exhibited impaired defense against oxidative stress, decreased lysosomal membrane stability, and reduced gonad oocyte numbers. Furthermore, exposure to water temperature stress alone or in combination with LNG at 20, 24, and 28°C increased SOD antioxidant activity, which was ineffective in preventing lipoperoxidation, altering the physiological processes of the organism (Mannai et al., 2022).

Yapici and Meriç (2023) reported that LNG exposure leads to changes in liver and muscle tissue of Danio rerio in terms of antioxidant parameters as SOD, CAT, GPX and lipid peroxidation indicator, MDA, and they concluded that a type of progestin LNG could be acting as an xenobiotic in the aquatic environment for non-target species.

CONCLUSION AND FINAL THOUGHTS

Pharmaceuticals excreted by humans or animals undergo microbial degradation or biotransformation in wastewater treatment plants and in receiving surface waters. The base compounds and their main secondary metabolites are likely to still be biologically active with potential endocrine disrupting effects. When they enter the aquatic environment in low concentrations (i.e. in the range of ng-μg/L) or low doses, progestins are expected to occur in biota since their physico-chemical properties or bioaccumulation are similar to those of natural steroids. Synthetically manufactured progestins are not easily biodegradable. Hence, in the absence of regular application, they tend to accumulate and are transferred from one environmental compartment to another through ingestion and respiration, resulting in varying degrees of hazard for aquatic organisms.

Due to the low environmental concentration of progestins in an aquatic ecosystem, the detection of trace amounts of these compounds is a great challenge. In current laboratory detection methods, the preconcentration, separation, and detection of endogenous progestins from aquatic organisms such as algae, fish, and aquatic plants is essential but usually complex, time-consuming, and costly.

To remediate risks and mitigate potential future problems, academia, pharmaceutical industries, and animal health and environmental protection agencies should develop a collective discussion that enables the establishment of risk-based strategies to manage the continuously increasing levels of unique progestins found by nearly all industrial societies. Regulatory agencies should be called to enforce the prescription, the discharge, and the further management of pharmaceuticals in sewage effluent that impacts on water sources. Regulations that obligate measurements at detection limits lower than present values are expected to be enforced, allowing for a more accurate risk assessment process.

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REFERENCES

Anonymous.2017.http://cdn.istanbul.edu.tr/FileHandler2.ashx?f=endokrin-sistem-fizyolojisi.pdf, Access: 18.08.2020.

Anonymous.2023.https://tr.wikipedia.org/wiki/%C3%87evre_kirlili%C4%9Fi ,Access:15.05.2023.

Bergman, A., Heindel, J. J., Jobling, S., Kidd, K. A., Zoeller, R.T. 2013. State of the Science of Endocrine Disrupting Chemicals 2012. Inter-Organization Programme for the Sound Management of Chemicals, United Nations Environment Programme and the World Health Organization, Geneva, Switzerland, 2013, p.289.

Besse, J., Garric, J. 2009. Progestagens for human use, exposure and hazard assessment for the aquatic environment. Environmental Pollution, 157, 3485-3494.

Brito, D. M., Porto, R. M., Vidal, M. T. C., Ojeda, R. S., Perez, A. R. 2010. Excretion study of clomiphene in human urine, evaluation of endogenous steroids profile after multiple oral doses. J. Braz. Chem. Soc., 21, (12), 2220–2225.

Brockmeier, E. K., Scott, P. D., Denslow, N. D., Leusch, F. D. L. 2016. Transcriptomic and physiological changes in eastern mosquitofish (Gambusia holbrooki) after exposure to progestins and anti-progestagens. Aquat Toxicol., 179, 817.

Caldwell, D. J., Mastrocco, F., Hutchinson, T. H., Lange R., Heijerick, D., Janssen, C., Anderson, P.D., Sumpter, J. P. 2008. Derivation of an aquatic predicted no-effect concetration for the synthetic hormone, 17 alpha-ethinyl estradiol. Environ. Sci. Technol., 42, 7046-7054.

Cano-Nicolau, J., Garoche, C., Hinfray, N., Pellegrini, E., Boujrad, N., Pakdel, F., Kah, O., Brion, F. 2016. Several synthetic progestins disrupt the glial cell specific-brain aromatase expression in developing zebra fish. Toxicology and Applied Pharmacology, 305, 12–21.

Cardoso, P.G., Rodrigues, D., Madureira, T.V., Oliveira, N., Rocha, M.J., Rocha, E. 2017. Warming modulates the effects of the endocrine disruptor progestin levonorgestrel on the zebrafish fitness, ovary maturation kinetics and reproduction success. Environmental Pollution, 229, 300-311.

Cardoso, P.G., Resende-de-Oliveira, R., Rocha, E. 2019. Combined effects of increased temperature and levonorgestrel exposure on zebrafish female liver, using stereology and immunohistochemistry against catalase, CYP1A, HSP90 and vitellogenin. Environmental Pollution, 252, 1059-1067.

Chen S., Lin C., Tan, J., Wang, Y., a, Wang, X., Wang, X., Liu, L., Li, J., Hou, L., Liu, J., Leung, J.Y.S. 2021. Reproductive potential of mosquitofish is reduced by the masculinizing effect of a synthetic progesterone, gestodene: Evidence from morphology, courtship behaviour, ovary histology, sex hormones and gene expressions. Science of the Total Environment, 769, 144570.

Christen, V., Hickmann, S., Rechenberg, B., Fent, K. 2010. Highly active human pharmaceuticals in aquatic systems: a concept for their identification based on their mode of action. Aquat. Toxicol., 96, 167-181.

Contardo-Jara, V., Lorenz, C., Pflugmacher, S., Nützmann, G,. Kloas, W., Wlegand, C. 2011. Molecular effects and bioaccumulation of levonorgestrel in the non-target organism Dreissena polymorpha. Environmental Pollution, 159, 38-44.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R., Van Der Kraak, G. J. 2002. Global Assessment of the State of the Science of Endocrine Disruptors. World Health Organization, p.180.

DeCourten, M., Brander, S. M. 2017. Combined effects of increased temperature and endocrine disrupting pollutants on sex determination, survival, and development across generations. Scientific Reports, 7, 9310.

DeQuattro, Z. A., Peissig, E. J., Antkiewicz, D. S., Lundgren, E. J., Hedman, C. J., Hemming, J. D. C., Barry, T. P. 2012. Effects of Progesterone on Reproduction and Embryonic Development in the Fathead Minnow (Pimephales Promelas). Environmental Toxicology and Chemistry, 31 (4), 851–856.

Dong Z., Xueyou Li, X., Chen Y., Zhang, N., Wang, Z., Liang Y., Yusong Guo, Y. 2023. Short-term exposure to norethisterone affected swimming behavior and antioxidant enzyme activity of medaka larvae, and led to masculinization in the adult population. Chemosphere, 310, 136844.

Fent, K. 2015. Progestins as endocrine disrupters in aquatic ecosystems: concentrations, effects and risk assessment. Environ. Int. 84, 115-130.

Frankel, T. E., Meyer, M. T., Orlando, E. F. 2016. Aqueous exposure to the progestin, levonorgestrel, alters anal fin development and reproductive behavior in the eastern mosquitofish (Gambusia holbrooki). General and Comparative Endocrinology, 234, 161–169.

Hoffmann, F., Kloas, W. 2012. The synthetic progesteron, levonorgestel, but not natural progesterone, affects male mate calling behavior of Xenopus laevis. General and Comparative Endocrinology, 176, 385-390.

Hou, L. Chen, H., Tian, C., Shi, W, Liang, Y., Wu, R., Fang, X., Zhang, C., Liang, Y., Xie, L. 2018. The progestin norethindrone affects sex differentiation and alters transcriptional profiles of genes along the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal axes in juvenile zebrafish Dario rerio. Aquatic Toxicology, 201, 31–39.

Hou, L., Chen, S., Liu, C., Guo, J., Chen, Z., Zhu, Q., Zhang, W., Xu, G., Liang, Y., Wu, R., Fang, X., Zhang, C., Xing, K. 2019. Transcriptomic and physiological changes in western mosquitofish (Gambusia affinis) after exposure to norgestrel. Ecotoxicology and Environmental Safety, 171, 579–586.

Hua, J., Han, J., Guo, Y., Zhou, B. 2015. The progestin levonorgestrel affects sex differentiation in zebrafish at environmentally relevant concentrations. Aquat Toxicol., 166: 19.

Hua, J., Zhu, B., Guo, W., Wang, X., Guo, Y., Yang, L., Han, J., Zhou, B. 2022. Endocrine disrupting effects induced by levonorgestrel linked to altered DNA methylation in rare minnow (Gobiocypris rarus). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 257, 109332.

Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C.R., van Aerle, R., Santos, E., Brighty, G. 2006. Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. Environ. Health Perspect, 114, 32-39.

Junior, E.A., Duarte, L.F., Pirasol Vanunci, M.L., Teixeira, M.L. 2010. Bioequivalence of Two Oral Contraceptive Drugs Containing

Ethinylestradiol and Gestodene in Healthy Female Volunteers. J. Bioequival. Bioavail., 2 (6), 125–130.

Kaur, H., Kumar Bansal, G, Althobaiti, F., Aldhahrani, A., Usmani, S., Bala, M. 2021. Prevalence of reproductive drugs usage in humans and animals: A pilot study in Patiala city of India. Saudi Journal of Biological Sciences, 28, 3727–3734.

Kloas, W., Urbatzka, R., Opitz, R., Würtz, S., Behrends, T., Hermelink, B. 2009. Endocrine disruption in aquatic vertebrates. Ann. N. Y. Acad. Sci., 1163, 187-200.

Kroupova, H. K., Trubiroha, A., Lorenz, C., Contardo-Jara, V., Lutz, I., Grabic, R., Kocour, M., Kloas, W. 2014. The Progestin Levonorgestrel Disrupts Gonadotropin Expression and Sex Steroid Levels in Pubertal Roach (Rutilus rutilus). Aquatic Toxicology, 154, 154-162.

Lai, K.M., Johnson, K.L., Scrimshaw, M.D., Lester, J.N. 2000. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ. Sci. Technol., 34, 3890–3894.

Länge, R., Hutchinson, T.H., Croudace, C.P., Siegmund, F., Schweinfurth, H., Hampe, P., Panter, G.H., Sumpter, J.P., 2001. Effects of the synthetic estrogen 17aethinylestradiol on the life-cycle of the feathead minnow (Pimephales promelas). Environ. Toxicol. Chem., 20, 1216–1227.

Liang, Y.Q., Huang, G.-Y., Ying, G.-G., Liu, S.-S., Jiang, Y.-X., Liu, S., Peng, F.-J., 2015. A time-course transcriptional kinetics of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary- adrenal axes in zebrafish eleutheroembryos after exposure to norgestrel. Environ. Toxicol. Chem., 34, 112-119.

Liang, Y., Huang, G., Lin, Z., Li, J., Yang, J., Zhong, L., Ying, G. 2018. Reproductive effects of synthetic progestin norgestrel in zebrafish (Danio rerio). Chemosphere, 190, 17-24.

Lorenz, C., Opitz, R., Trubiroha, A., Lutz, I., Zikova, A., Kloas, W. 2016. The synthetic gestagen levonorgestrel directly affects gene-expressionin thyroid and pituitary glands of Xenopus laevis tadpoles. Aquatic Toxicology, 177, 63–73.

Mannai, A., Hmida, L., Bouraoui, Z., Guerbej, H., Gharred, T., Jebali, J. 2022. Does thermal stress modulate the biochemical and physiological responses of Ruditapes decussatus exposed to the progestin levonorgestrel. Environ Sci Pollut Res., 29 (56), 1-18.

Orlando, E. F., Ellestad, L. E. 2014. Sources, concentrations and exposure effects of environmental gestagens on fish and other aquatic wildlife, with an emphasis on reproduction. General and Comperative Endocrinology, 203, 241-249.

Paulos, P., Runnalls, T. J., Nallani, G., Point, T., Scott, A. P., Sumpter, J. P., Huggett, D. B. 2010. Reproductive Responses in Fathead Minnow and Japanese Medaka Following Exposure to a Synthetic Progestin, Norethindrone. Aquatic Toxicology, 99, 256-262.

Pawlowski, S., van Aerle, R., Tyler, C.R., Braunbeck, T. 2004. Effects of 17α-ethinyestradiol in a fathead minnow (Pimephales promelas) gonadal recrudescence assay. Ecotoxicol. Environ. Saf., 57, 330-345.

Pietsch, C., Neumann, N., Knopf, K., Wuert, Z.S., Kloas, W. 2009. Progestogens cause immunosuppression of stimulated carp (Cyrinus carpio L) leukocytes in vitro. Comp Biochem Physiol., C ,150: 16–24.

Richardson, B.J., Larn, P. K. S., Martin, M., 2005. Emerging chemicals of concern: Pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. Marine Pollution Bulletin 50, 913–920.

Rocha, M. J., Rocha, E. 2022. Synthetic Progestins in Waste and Surface Waters: Concentrations, Impacts and Ecological Risk. Toxics, 10 (4), 163.

Runnalls, T.J., Beresford, N., Losty, E., Scott, A.P., Sumpter, J.P. 2013. Several synthetic progestins with different potencies adverselly affect reproduction of fish. Environ. Sci. Technol., 47, 2077-2084.

Shi, W., Hu, L., Huang, G., Liu, Y., Zhang, J., Xie, L., Ying, G. 2019. Dydrogesterone affects the transcription of genes in GnRH and steroidogenesis pathways and increases the frequency of atretic follicles in zebrafish (Danio rerio). Chemosphere, 216, 725-732.

Stanczyk F.Z., Archer D.F., Bhavnani B.R. 2013. Ethinyl estradiol and 17b-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. Contraception, 87, 706–727.

Steinbach, C., Císař, P., Šauer, P., Klicnarová, J., Schmidt – Posthaus, H., Golovko, O., Kroupová, H.K. 2019. Synthetic progestin etonogestrel negatively affects mating behavior and reproduction in Endler's guppies (Poecilia wingei). Science of the Total Environment, 663, 206–215.

Sumpter, J.P., Johnson, A.C. 2005. Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. Environ. Sci. Technol., 39, 4321-4332.

Svensson, J., Mustafa, A., Fick, J., Schmitz, M., Brunström, B. 2016. Developmental exposure to progestins causes male bias andprecocious puberty in zebrafish (Danio rerio). Aquatic Toxicology, 177, 316–323.

Tyler, C.R., Jobling, S. 2008. Roach, sex, and gender-bending chemicals: the feminization of wild fish in English rivers. Bioscience, 58 (11), 1051-1059.

Yapici, M and Meriç Turgut, İ. 2023. Farklı doz ve sürelerde levonorgestrel uygulamasının zebra balıklarında (Danio rerio, Hamilton, 1822) antioksidatif metabolizma üzerine etkileri. Ankara University, Graduate School of Natural and Applied Science, Ankara. PhD thesis, 80 p.

Zeilinger, J., Steger-Hartmann, T., Maser, E., Goller, S., Vonk, R., Lange, R. 2009. Effects of synthetic gestagens on fish reproduction. Environmental Toxicology and Chemistry, 2663-2670.

Zhao, X., Wang, Q., Li, X., Xu, H., Ren, C., Yang, Y., Xu, S., Wei, G., Duan, Y., Tan, Z., Fang, Y. 2023. Norgestrel causes digestive gland injury in the clam Mactra veneriformis: An integrated histological, transcriptomics, and metabolomics study. Science of the Total Environment, 871, 162110.

Zheng, W., Yates, S. R., Bradford, S. A., 2008. Analysis of steroid hormones in a typical dairy waste disposal system. Environ. Sci. Technol. 42, 530–535.

Zikova, A., Lorenz, C., Hoffmann, F., Kleiner, W., Lutz, I., Stöck, M., Kloas, W. 2017. Endocrine disruption by environmental gestagens in amphibians e A short review supported by new in vitro data using gonads of Xenopus laevis. Chemosphere, 181, 74-82.

Zrinyi, Z., Maasza, G., Zhangb, L., Vertesb, A., Lovasa, S., Kissc, T., Elekesc, K., Pirgera, Z. 2017. Effect of progesterone and its synthetic analogs on reproduction and embryonic development of a freshwater invertebrate model. Aquatic Toxicology, 190, 94–103.

Zucchi, S., Castiglioni, S., Fent, K. 2012. Progestins and antiprogestins affect gene expression in early development in zebrafish (Danio rerio) at environmental concentrations. Environ. Sci. Technol. 46 (9), 5183-5192.

Zucchi, S., Castiglioni, S., Fent, K. 2013. Progesterone alters global transcription profiles at environmental concentrations in brain and ovary of female zebrafish (Danio rerio). Environ. Sci. Technol. 47, 12548-12556.

Zucchi, S., Mirbahai, L., Castiglioni, S., Fent, K. 2014.Transcriptional and physiological responses induced by binary mixtures of drospirenone and progesterone in zebrafish (Danio rerio). Environ. Sci. Technol. 48 (6), 3523-3531.

CHAPTER 5

ALGAE-BASED PROTEIN SPORTS NUTRITION DRINKS AND SEAWEED-ENRICHED BEVERAGES

Prof. Dr. Berna KILINÇ[1]
Assoc. Prof. Dr. Hatice TEKOĞUL[2]

[1] Ege University Fisheries Faculty Fish Processing Technology Department, 35100 Bornova-Izmir, Türkiye. berna.kilinc@ege.edu.tr, Orcid ID: 0000-0002-4663-5082
[2] Ege University Fisheries Faculty Agriculture Department, 35100 Bornova-Izmir, Türkiye Izmir, Türkiye. hatice.tekogul@ege.edu.tr, Orcid ID: 0000-0002-9755-8339

INTRODUCTION

Sports drinks, called isotonic and liquid substitute drinks, are generally considered drinks formulated to rapidly replace fluids, electrolytes, and carbohydrate fuel for working muscles (Guo et al., 2025). The industry of sports nutrition drinks began in the 1960s as a response to the needs of athletes for hydration and recovery after exercise. New ingredient technologies connected to research in sports nutrition have facilitated the introduction of multiple beverage offerings to the market, thereby creating a whole new industry dedicated to meeting the nutritional needs of athletes. Protein supplementation in the form of drinks began as low-cost by-products of fat and cheese production processes. It was initially made available as raw materials for commercial soy and dairy protein products aimed at bodybuilders. By 2015, the combined hard drink mixes and sports drinks categories were worth more than seven billion dollars in the United States. Initially, a niche market was where products were consumed primarily by athletes and sports enthusiasts; in the late 2000s, the categories saw mainstream appeal as world-class multinational companies launched their mass-marketed nutritious drinks (Galaz, 2019). Research showed that sports-related food and beverage products created positive emotions, excitement, and self-image among adults and children. Despite this, the authors reported that self-regulatory pledges made by food companies to limit exposure to unhealthy products to children have not improved the nutritional quality of foods marketed to children. For this reason, the authors also stated that more sports organizations and athletes should stop promoting unhealthy food products and drinks and work with health professionals to promote healthy eating habits among young people (Bragg et al., 2018).

One of the main macronutrients that is necessary for human nutrition is protein. Due to the high cost of protein sources, particularly those derived from animals, extensive research has been done to find substitutes. One of the newer protein sources is seaweed, often known as macroalgae (Thiviya et al., 2022). Algae can be ingested fresh, fermented, dried, or frozen. They can also be crushed into various sizes of flakes, granules, or powders (Mouritsen et al., 2019). Additionally, they are an attractive food source to address food security and nutrition concerns due to their healthfulness, sustainability, minimal environmental footprint, and low production costs. They are increasingly used in many food systems to reap the nutritional benefits for human health (Kumar et al., 2023). Since vegetables and fruits are inadequate providers of vital fatty acids and vitamin B12 (cobalamin), seaweeds can serve as a vegetarian substitute. Functional food items and supplements made from seaweed have a lot of potential health advantages and may even be able to alleviate malnutrition (Imchen, 2021). Although seaweeds have been utilized as food from ancient times, mostly in Asian countries, they are mostly used in Western countries as gelling agents and colloids for the food, pharmaceutical, and cosmetic industries (Penalver et al., 2020). They are also rich in many bioactive compounds such as vitamins, proteins, fibers, minerals, essential amino acids, fatty acids, and pigments, giving them exceptional antihypertensive, antidiabetic, antioxidant, anti-inflammatory, antitumor, antiviral, and antimicrobial properties. They could contribute to future global security in functional foods and nutraceuticals, and be an important compound in the pharmaceutical and biotechnology industries for drug development, among other uses (Salido et al., 2024). Functional food is a type of food that provides additional or enhanced benefits over and above its basic nutritional value (Lim et al., 2021). Seaweeds are an important resource used in the production of many functional foods (Kılınç and Kılınç, 2022). For example, red seaweed stands out as a beautiful effect because it can contain protein concentrations of up to 47%, the highest among terrestrial plants and other algal populations. Besides, these proteins offer a rich source of essential amino acids, making them excellent candidates for human food formulation (Jimenez-Gonzalez et al., 2023). The seaweeds such as Ulva fasciata, Ulva lactuca, Chaetomorpha linum, and Gracilaria edulis also serve as a good source of functional ingredients and can be used in the development of functional food products such as biscuits, chocolates, bread, cookies, snacks, soups, tea, and beverages (Qin, 2018; Bajad et al., 2024; Salgadoet al., 2024). Besides, algae-based products, including cookies, pasta, bread, and beverages, have been gaining popularity in Europe, accounting for 1.34% of new food and drink released in 2017 (Nova et al., 2020). On the importance of the subject, the production of seaweed-enriched drinks was not only produced for athletes but also for people of all ages was carried out using different types of seaweeds. In addition to this, studies conducted on algae-based sports nutrition drinks and seaweed-enriched beverages, other seaweed-supplemented beverages as well as other properties of algae that can be used for beverages compiled in this study.

CULTIVATION OF SEAWEEDS, LIVING CONDITIONS AND HARVESTING

Algae, which are very important food to meet the increasing food demand, are produced in large quantities in some parts of the world. Especially in Asia, 99.5% of the world's seaweed production is produced (Chopin, 2018). Algae, which develop by using mineral substances in the water in the marine environments they live in, are very good biological filters. They both clean the water and thus increase the quality of the water and grow their biomass. In the meantime, they synthesize mineral substances and have a wide variety of bioactive molecules. As controlled production increases, their use can also increase. World seaweed production is one of the fastest-growing industries in the world, with production facilities in 132 countries worldwide, with a farm area of 48 million km2. Production is concentrated in approximately 33 to 45 countries (Froehlich et al., 2019).

Production in macroalgae farms reached 35.8 million tons in 2019, accounting for 97% of global seaweed production, while the amount of seaweed obtained from natural sources remained at 1.1 million tons (FAO, 2021). 10.7% of this production is cultured Gracilaria macroalgae. Gracilaria verrucosa has a highly branched thallus of 5-30 cm in length. The optimum temperature for Gracilaria production is 16 °C, while the optimum temperature range is between 4°C and 37°C. The optimum salinity range is between 0-15% and 0-50% as well as the optimum salinity range is between 0-20% and 0.35% (Düsedau et al., 2023) (Picture 1).

Ulva lactuca var. rigida C. Agardh has a lettuce-like structure and a not-so-thin thallus (Figure 1). The increasing use of healthy snacks and food supplements has increased the expected demand for seaweed products. For large-scale ulva production, salinity between 15% and 30% and temperatures between 10°C and 35°C have been used. The optimum growth of eutrophic water-loving Ulva lactuca was found at 0.15% salinity and 10°C (Malta et al., 1999; Chin et al., 2023) (Picture 2).

Codium fragile is a living green alga with spongy, thick, rounded finger-like branched leaves. Asia. Distributed worldwide including North America, South America, Greenland, Europe, Africa, Australia and New Zealand. Originating in Asia. It grows by attaching to hard surfaces in a wide variety of substrates. Codium fragile is tolerant to a wide range of temperatures from -2°C to 30°C and salinities from 12 PSU to 42 PSU. (Malinowski and Ramus 1973; Hanisak 1979)(Picture 3).

Enteromorpha are simple or branched species with a hollow, long-branched tubular thallus, a single-celled layer resembling a gut (Starmach, 1972). The leaves are usually unbranched. The leaves are usually rounded-tipped, 10-30 cm long and 6-18 mm in diameter. The optimum temperature of Enteromorpha intestinalis is 20 to 25° C and optimum salinity is 25 to 30 PSU. Light has a significant effect on initial growth. Optimum growth occurs in waters where salinity varies between 15 and 30 PSU (Bermejo, R., et al., 2022) (Picture 4).

Algae are cultured in open-air tanks with an initial density of 1 kg fresh weight per m2. In macroalgae production, 10 mL/lt F/2 Culture Medium is applied to seawater weekly. Algae are propagated and harvested vegetatively. The pictures of the seaweeds used in the study are given below.

Picture 1. Gracilaria verrucosa (Hudson) Papenfuss (Original)

Picture 2. *Ulva rigida C.Agardh, 1823 (Original)*

Picture 3. Codium fragile subsp. fragile (Suringar) Hariot, 1889 (Original)

Picture 4. Enteromorpha intestinalis (Linnaeus) Nees, 1820 (Original)

THE PROPERTIES OF SEAWEEDS TO BE USED FOR SPORTS NUTRITION DRINKS AND SEAWEED-ENRICHED BEVERAGES

Seaweed, a diverse group of marine macroalgae, has emerged as a rich source of bioactive compounds with numerous health-promoting properties. Among them, phenolic compounds have received significant attention due to their various therapeutic applications (Sadeghi et al., 2024). The bioactive compounds (proteins, carbohydrates, enzymes, omega-3 polyunsaturated fatty acids, vitamins, essential amino acids, essential minerals, trace elements, carotenoids, and polyphenols) of seaweeds have been associated with various biological activities such as antimicrobial, antioxidant, antiviral, hypocholesterolemic, anti-inflammatory, immunomodulatory, anticancer, anticoagulant, antidiabetic, and prebiotic. Due to their richness in highly valuable compounds and their balanced composition, seaweeds have become a promising and innovative source of new functional ingredients that can be used in the development of new food products as well as nutraceuticals, thereby improving human health, well-being, and quality of life, and also contributes to the prevention and treatment of diseases (Matos et al., 2024). Algae biomass and extracts are utilized as dietary supplements and food additives for flavor, color, preservative, emulsifier, and antioxidant properties (Mendes et al., 2022). Edible seaweed is also a valuable source of natural pigments such as carotenoids, phycobiliproteins, and chlorophylls). They are functional components and are well-recognized for beneficial therapeutic properties. They are used as coloring agents providing techno-functional, nutritional, and sensory functions in foods (Manzoor et al.,2024). Carotenoids including lutein, β-carotene, lycopene, and astaxanthin are commonly utilized as nutritional supplements, food fortifiers, and natural colorants in beverages. A growing number of consumers prefer natural products over synthetic ones due to allergies and health concerns. (Bogacz-Radomska, 2020). The use of marine-derived pigments has also witnessed a significant increase over the past two decades due to their environmentally safe and health-promoting properties. Pigments found in seaweeds play crucial roles in a variety of industries, including cosmetics, textiles, biomedical and food applications. Seaweeds, including red algae, brown algae, and green algae, serve as excellent natural sources for a variety of food colors, contributing both sensory and health-promoting properties to food products. The variability in the type, amount, biochemical compositions, and biological properties of seaweed pigments requires careful consideration in the selection of appropriate extraction protocols (Thangaraj and Sundaramanickam, 2025). Polysaccharide derivates, including alginate, carrageenans, and agar, are commonly employed as thickening, emulsifying, stabilizing, and gelling agents in the beverage and food industry (Abe et al., 2018, Mendes et al., 2022). Seaweed contains a variety of soluble fiber types (hydrocolloids). The source of (Agar is from red seaweeds: Gelidium, Glacilaria, Pterocladia), (Carrageenans is from red seaweeds: Gigartina, Hypnea, Eucheuma, Chondrus), (Alginate is from brown seaweeds: Laminaria, Ascophyllum, Macrocystis), (Fucoidan is from brown seaweeds: Nemacystus decipiens, Laminaria religiosa), (Laminarin is from brown seaweeds: Laminaria japonica, Saccharina latissima), (Porphyran is from red seaweeds: (Porphyra spp.), (Ulvan is from green seaweeds: Ulva lactuca, Enteromorpha spp.) (Rajapakse and Kim, 2011).

PRODUCTION OF DIFFERENT FORMULATED ALGAE-BASED PROTEIN NUTRITION DRINKS

Picture 5. Different Formulated Algae-Based Protein Nutrition Drinks (Original)

Picture 6. Drink enriched with *U. rigida* (Original)

Picture7. Drink enriched with *G. verrucosa* (Original)

Picture 8.Drink enriched with *E. intestinalis* (Original)

Picture 9. Drink enriched with *C. fragile* (Original)

Picture 10. Drink enriched with *U. rigida* (Original).

Picture 11. Drink enriched with *G. verrucosa* (Original).

Picture 12. Drink enriched with *E. intestinalis* (Original).

Picture 13. Drink enriched with *C. fragile* (Original).

Picture 14. Drink enriched with *U. rigida* (Original).

Picture 15. Drink enriched with *G. verrucosa* (Original).

Picture 16. Drink enriched with *E. intestinalis* (Original)

Picture 17. Drink enriched with *C. fragile* (Original)

Picture 18. Drink enriched with *U. rigida* (Original)

Picture 19. Drink enriched with *G. verrucosa* (Original)

Picture 20. Drink enriched with *E.* intestinalis (Original)

Picture 21. Drink enriched with *C. fragile* (Original)

STUDIES CONDUCTED ON ALGAE-BASED SPORTS NUTRITION DRINKS AND SEAWEED-ENRICHED BEVERAGES

Rising incomes, urbanization and an aging population are leading to changes in consumption patterns, leading to an increase in demand for proteins. From a sustainability perspective, there is a consensus that animal protein production has a disproportionate impact on the environment, especially in intensive systems that require a significant amount of feed crops. Macroalgae have emerged as a promising raw material for the transition to a blue bioeconomy (Jimenez-Gonzalez et al., 2023). In other words, the rapid growth of the world's population drives up food demand. Animal protein consumption is expected to increase by 2050. However, rising consumption of animal protein increases climate crisis worries because it might result in a larger carbon and water footprint, as well as increased land use. Therefore, a sustainable sports nutrition paradigm is evolving for athletes who consume a lot of animal protein. It is anticipated that interest in and research into alternative protein sources for sustainable sports nutrition will rise in the future as a result of growing awareness of the potential impacts of animal protein production on the climate crisis (Turnagöl et al., 2023). Currently, the need for an alternative protein-rich plant-based source is increasing to meet the daily requirements of vegetarian/vegan athletes. In addition to being a potential source of protein, algae containing all the essential amino acids can be consumed by vegetarian/vegan athletes (Bleakley and Hayes, 2017). Plant-based drinks can be considered not as an alternative to milk, but as a different product with its own nutritional and functional assets. Their inclusion in a diversified balanced diet can provide interesting functional components, such as soluble fiber or unsaturated fatty acids (mainly soybeans and almond drinks), which can help improve the health status of the population (Perez-Rodriguez et al., 2023). Seaweed has important amino acids, vitamins, minerals, and antioxidants. Its protein level ranges from 11% to 32% by dry weight, making it suitable for a wide range of dietary preferences, including vegetarian and vegan diets (Pereira et al., 2024). Protein quality in marine macroalgae edible seaweeds is significantly higher than in other aquatic plants and typical plant-based protein sources. Seaweeds have been shown to boost health and nutrition, making them a promising alternative source of full plant protein. Seaweeds are suitable for consumers who are concerned about their health because of their low calorific value and high concentration of vital amino acids (Raja et al., 2022). All amino acids, but particularly glycine, alanine, arginine, proline, glutamic, and aspartic acids, can be found in seaweed protein. Essential amino acids (EAAs) make up over half of all amino acids in algae, and their protein profiles are similar to those of egg protein (Cerna et al., 2011). They have a high protein content and a good amino acid profile that is on par with other traditional protein sources. Bioactive substances found in seaweed protein include lectins, peptides, free amino acids, and phycobiliproteins, which include phycocyanin and phycoerythrin, among others. The antihypertensive, antidiabetic, antioxidant, anti-inflammatory, antitumoral, antiviral, antibacterial, and numerous other advantageous functional qualities of seaweed proteins have been demonstrated. Seaweed proteins may thus be a naturally occurring substitute source for the creation of functional foods (Thiviya et al., 2022). For example, the four selected seaweeds (Gracilaria edulis, Ulva lactuca, Ulva fasciata and Chaetomorpha linum serve as a good source of functional ingredients and can be used in the development of functional food products such as cookies, snacks, biscuits, bread, soups, tea, and beverages (Bajad et al., 2024). Additionally, increasing environmental concerns and the desire for health-conscious food choices are increasing consumers' demand for sustainable and ethical food sources. For example, Ulva algae (Ulva genus, Phylum Chlorophyta) is emerging as a promising candidate due to its rich nutrient profile, which includes carbohydrates, lipids, proteins, and antioxidants. Therefore, Ulva species is an easily available natural resource that has the potential to address global malnutrition without compromising the health of the food ecosystem (Khan et al., 2024).

Fermentation has been used as a means to increase the value and longevity of food resources even before the beginning of agriculture. It has been applied to animal and plant-based terrestrial foods (Reboleira et al., 2024). In one study, two types of brown seaweed (Alaria esculenta and Saccharina latissima) were studied as fermentation substrates for beverages. This study showed the potential of using brown seaweed species as a fermentation substrate to develop new, fermented beverages (Healy et al., 2023). Spirulina platensis, a microalgae known for its exceptional nutritional value, especially its bioactive compounds and protein content, holds the promise of inclusion in functional food products. Ricotta cheese whey is a by-product of the production of ricotta cheese, which is difficult to use in industries due to its low pH and less favorable processing qualities. Fermented whey-based drinks containing a mixture of spirulina-lemon and mint juice increased the concentration of vitamins, minerals, antioxidants and total phenolic compounds in the final product. The probiotic bacteria count in all fermented beverage samples exceeded 7 log CFU/ml throughout storage, indicating that the fermented beverage retained its probiotic properties. The addition of 0.5% Spirulina platensis significantly improved the structural properties and sensory acceptance of the final product (Elkot et al., 2024). In another study, a traditional Korean alcoholic drink was produced by adding 20% raw brittle Codium fragile to the rice and oat base (1: 1 ratio) (COM). The results showed that COM was a Makgeolli with improved antioxidant function against yeast and lactic acid bacteria and an improved flavor, suggesting the possibility of commercialization (Park et al., 2024). The other study highlighted the complexities of anthocyanin degradation and its effects on the color, aroma, and antioxidant properties of these enriched with phenolic extract from jaboticaba peel or blueberry pulp sports drinks (Rigolon et al., 2024). In the other study, it was also stated as follows. The technology and formulation of a protein fruit and berry drink with spirulina, which contained both proteins and a vitamin and mineral complex increased the drink's nutritional and biological values (Gubanenko et al., 2018). Another study aimed to develop isotonic beverages mixed with herbal and spice extracts, based on grape juice as a source of carbohydrates, and pigments and rich in antioxidants, without chemical additives, to improve the taste of beverages. Sensory evaluation showed that the combination of grape juice with herbal and spice extracts supported the use of natural resources to enrich sports drinks with antioxidants and improve their sensory profile (Bendaali et al., 2024). Hydropuntia eucheumatoides (HE) seaweed is indicated as abundant in tropical oceans and is a popular food source along the coast. For this purpose, the study aimed to create seaweed milk from it, and the result showed that it was a potential benefit as a food supplement as well as a good source of antioxidants with a pleasant sensory profile. The product was reported to have outstanding sensory details and seemed to be safe for consumption (Thanh et al., 2014). The inclusion of seaweed extract or seaweed powder in food products that are part of people's daily diet, rather than the consumption of seaweed as a whole, will benefit consumers in terms of general acceptability and not going through their cultural diet. Therefore, it has been concluded that value-added products have the potential to meet both the sensory and nutritional requirements of consumers (Raja et al., 2022). For example, according to nutritional data from Spanish products, seaweed-based snacks were substantially lower in energy, fat, and salt than their seaweed-free equivalents. Conversely, spirulina-enriched ready-to-drink beverages were noticeably more salted and energetic than those without algae. Accordingly, choosing items that satisfy customer demands and/or expectations requires careful consideration of the nutrition label. This study emphasized the significance of labeling laws to give customers comprehensive product information, as only 8% of goods disclosed the types of algae and the degree of inclusion (Boukid and Castellari, 2021). Two species of seaweed (Alaria esculenta and Saccharina latissima) were studied in another study as fermentation raw materials for the development of a drink. It was highlighted that this study was the potential for optimization of the seaweed fermentation process through strain selection, optimization of fermentation conditions (temperature, time, pH), and pretreatment of seaweed biomass. Besides, glutamic acid was reported to be responsible for the umami flavor. Furthermore, Saccharomyces cerevisiae was found in larger quantities which meant that fermentation using S.cerevisiae cultures can improve the umami flavor profile of a seaweed-fermented beverage product (Healy et al., 2023).

When it comes to non-essential amino acids, the amounts of the three groups, green, brown, and red seaweeds are comparable. Given that its protein content exceeds 47%, red seaweed appears to be a suitable source. The search for a novel and affordable source of protein is bolstered by the problem of protein deficiency. Due to their relatively high nitrogen component content, algae may be able to play a significant role in the aforementioned difficulty. The industry may utilize algae as a source of very nutritious ingredients (Cerna et al., 2011). Hence, seaweeds are incredibly beneficial algae for both nutrition and medicine and can be further explored in innovative food products to meet the growing global food need (Fasogbon et al., 2024).

CONCLUSION

Healthy eating is becoming more popular as people place a greater value on their health. Algae are very valuable in terms of human health due to the nutrients they contain and the useful components they have. They can be consumed in different ways in the world, especially in Asian and European countries, and they also increase their nutritional value by taking part in the formulations of various foods. Nowadays, Despite the increasing popularity of algae especially in European countries, its production and consumption in Türkiye are quite low. In recent years, sales of products such as sushi and onigiri have been started in grocery stores in Türkiye, despite this consumption is still quite low. Besides, pills made from seaweed are offered for sale as food supplements. Therefore, protein sports nutrition drinks were created in various formulations including different algae species to promote the consumption of people of all ages drinks containing algae in the study. People who play sports are not only expected to consume these seaweed-enriched drinks with a good source of protein, but also people of all ages particularly children and the elderly, can consume these drinks made from seaweed healthfully. In the coming days, it is expected that algae consumption will increase further worldwide due to decreasing food resources. By ensuring that consumable algae species are cultivated and processed in a way that is free from contamination, as well as by ensuring that algae are used in the formulations of various food products and are encouraged for human consumption, the production of healthy foods should be realized by giving algae cultivation the attention it deserves. It is estimated that the algae-containing protein sports nutrition drinks formulated in the study will be commercially produced and offered for sale in grocery stores in similar formulations.

REFERENCES

Abe, J.I., Kotla, S., Yu, J., Baker, A.B., Patil, N.P., Le, V., Sligar, A.D., Mei, L., Chavarria, D., Yang, E.Y. (2018). Algal Polysaccharides as Therapeutic Agents for Atherosclerosis. *Front. Cardiovasc. Med*., 5, 153. https://doi.org/10.3389/fcvm.2018.00153.

Bajad, R.B., Kamble, M.G., Gawai, S.R., Syed, I., Roy, M. (2024). Analysis of physicochemical, functional, and antioxidant properties of four different Indian seaweed species. *Future Foods*, 10, 100435. https://doi.org/10.1016/j.fufo.2024.100435

Bendaali, Y., Escott, C., Vaquero, C., Gonzalez, C., Morata, A. (2024). Physicochemical, antioxidant activity, and sensory properties of grape juice-herbs extract based isotonic beverages. *International Journal of Gastronomy and Food Science,* 37, 100986. https://doi.org/10.1016/j.ijgfs.2024.100986

Bermejo, R., Golden, N., Schrofner, E., Knöller, K., Fenton, O., Serrao, E., & Morrison, L. (2022). Biomass and nutrient dynamics of major green tides in Ireland: Implications for biomonitoring. *Marine Pollution Bulletin*, 175, 113318.

Bleakley, S., Hayes, M. (2017). Algal Proteins: Extraction, Application, and Challenges Concerning Production. Foods, 6(5): 33, https://doi.org/10.3390/foods6050033

Bragg, M.A., Roberto, C.A., Harris, J.L., Brownell, K.D., Elbel, B. (2018). Marketing Food and Beverages to Youth Through Sports. *Journal of Adolescent Health*, 62(1), 5-13. https://doi.org/10.1016/j.jadohealth.2017.06.016

Bogacz-Radomska, L., Harasym, J., Piwowar, A. (2020). Commercialization aspects of carotenoids. In Carotenoids: Properties, Processing and Applications; Elsevier: Amsterdam, The Netherlands, 327–357.

Boukid, F., Castellari, M. (2021). Food and Beverages Containing Algae and Derived Ingredients Launched in the Market from 2015 to 2019: A Front-of-Pack Labeling Perspective with a Special Focus on Spain. Foods, 10(1): 173; https://doi.org/10.3390/foods10010173

Cerna, M. (2011). Seaweed Proteins and Amino Acids as Nutraceuticals. *Advances in Food and Nutrition Research*, 64, 297-312. http://dx.doi.org/10.1016/B978-0-12-387669-0.00024-7

Chopin, T. (2018). Seaweeds: a multi-purposed bioresource well-suited for Integrated Sequential BioRefinery (ISBR) processing. *Int Aquafeed*, 21(11), 14–15.

Chin, Y.Y., Chang, K.A., Ng, W.M., Eng, Z.P., Chew, L.Y., Neo, Y.P., Yan, S.W., Wong, C.L., Kong, K.W., Ismail, A. (2023). A comparative evaluation of nutritional composition and antioxidant properties of six Malaysian edible seaweeds. *Food Chemistry Advances*, 3, 100426. https://doi.org/10. http://www.fao.org/fishery/nems/41320/en 1016/j.focha.2023.100426

Düsedau, L., Ren, Y., Hou, M., Wahl, M., Hu, Z. M., Wang, G., & Weinberger, F. (2023). Elevated Temperature-Induced Epimicrobiome Shifts in an Invasive Seaweed Gracilaria vermiculophylla. Microorganisms, 11(3), 599.

Elkot, W.F., Elmahdy, A., El-Sawah, T.H., Alhag, S.K., Al-Shahari, E.A., Al-Farga, A., Ismail, H.A. (2024). Development and characterization of a novel flavored functional fermented whey-based sports beverage fortified with Spirulina platensis.*International Journal of Biological Macromolecules*, 258, Part 2, 128899. https://doi.org/10.1016/j.ijbiomac.2023.128999

Fasogbon, B.M., Ademuyiwa, O., Ogundipe, O.O. (2024). Therapeutic potential and roles of dietary seaweeds in food: A systematic review. *World Development Sustainability*, 4, 100141. https://doi.org/10.1016/j.wds.2024.100141

FAO (2021) The global status of seaweed production, trade and utilization, Vol. 124, *Globefish Research Programme* (2021)

Froehlich, H. E., Afflerbach, J. C., Frazier, M., & Halpern, B. S. (2019). Blue growth potential to mitigate climate change through seaweed offsetting. *Current Biology*, 29(18), 3087-3093.

Galaz, G.A. (2019). Chapter 19-An Overview on the History of Sports Nutrition Beverages. Nutrition and Enhanced Sports Performance (Second Edition). Muscle Building, Endurance, and Strength, 231-237. https://doi.org/10.1016/B978-0-12-813922-6.00019-9

Gubanenko, G., Naimushina, L., Zykova, I. (2018). Spirulina as a protein ingredient in a sports nutrition drink. Conference: Proceedings of the 4th International Conference on Innovations in Sports, Tourism and Instructional Science (ICISTIS 2019). http://dx.doi.org/10.2991/icistis-19.2019.42

Guo, M., Liang, S., Guo, F. (2025). Chapter 9-Sports drinks. Functional Foods (Second Edition) Principles and Technology. Woodhead Publishing Series in Food Science, Technology and Nutrition, 307-325. https://doi.org/10.1016/B978-0-443-19100-8.00010-5

Hanisak, M. D. (1979). Growth patterns of Codium fragile ssp. tomentosoides in response to temperature, irradiance, salinity, and nitrogen source. *Marine Biology*, 50, 319-332.

Healy, L.E., Zhu, X., Kakagianni, M., Poojary, M.M., Sullivan, C., Tiwari, U., Curtin, J., Sun, D.W. Tiwari, B.K. (2023). Fermentation of brown seaweeds Alaria esculenta and Saccharina latissima for new product development using Lactiplantbacillus plantarum, Saccharomyces cerevisiae and kombucha SCOBY. *Algal Research*, 76, 103322. https://doi.org/10.1016/j.algal.2023.103322

Imchen, T. (2021). Nutritional value of marine seaweeds and their potential to serve as nutraceutical supplement. *Phycologia*, 60(6), 534-546.

Jimenez- Gonzalez, C., Agrasar, A.M.T., Mallo, F., Rua, M.L., Fucinos, C. (2023). Red seaweed proteins: Valuable marine-origin compounds with encouraging applications. *Algal Research*, 75, 103262. https://doi.org/10.1016/j.algal.2023.103262

Lim, S.J., Chang, L.S., Fazry, S., Mustapha, W.A.W.M., Babji, A.S. (2021). Functional food & ingredients from seaweed, edible bird's nest and tropical fruits: A translational research. LWT, 151, 112164. https://doi.org/10.1016/j.lwt.2021.112164

Khan, N., Sudhakar, K., Mamat, R. (2024). Eco-friendly nutrient from ocean: Exploring Ulva seaweed potential as a sustainable food source. *Journal of Agriculture and Food Research*, 17, 101239. https://doi.org/10.1016/j.jafr.2024.101239

Kılınç, İ., Kılınç, B. (2022). Chapter 5 “Seaweed Value -Added Functional Food Products.” In Book: International Research in Agriculture, Forestry and Aquaculture Sciences. Editor: Akar, T., pp. 85-107.

Serüven Publishing, Türkiye, December 2022, ISBN: 978-625-6399-25-9, 233p.

Kumar, A., Devi, H.M., Kishore, P., Ravishankar, C.N. (2023). Exploitation of Seaweed Functionality for the Development of Food Products. *Food and Bioprocess Technology*, 16(10):1-31. http://dx.doi.org/10.1007/s11947-023-03023-2

Malta, E. J., Draisma, S. G., & Kamermans, P. (1999). Free-floating Ulva in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. *European journal of phycology*, 34(5), 443-454.

Manzoor, M.F., Afraz, M.T., Yılmaz, B.B., Adil, M., Arshad, N., Goksen, G., Ali, M., Zeng, X.A. (2024). Recent progress in natural seaweed pigments: Green extraction, health-promoting activities, techno-functional properties and role in intelligent food packaging.*Journal of Agriculture and Food Research*, 15, 100991. https://doi.org/10.1016/j.jafr.2024.100991

Matos, J., Cardosa, C., Serralheiro, M.L., Bandarra, N.M., Afonso, C. (2024). Seaweed bioactive potential as nutraceuticals and functional ingredients: A review. J*ournal of Food Composition and Analysis*, 133, 106453. https://doi.org/10.1016/j.jfca.2024.106453

Malinowski, K. C., & Ramus, J. (1973). Growth Of The Green Alga Codıum Fragıle In A Connectıcut Estuary 1. *Journal Of Phycology*, 9(1), 102-110.

Mendes, M.C., Navalho, S., Ferreira, A., Paulino, C., Figueiredo, D., Silva, D., Gao, F., Gama, F., Bombo, G., Jacinto, R., Aveiro, S.S., Schulze, P.S.C., Gonçalves, Pereire, H., Gouveia, L., Patarra, R.F., Abreu, M.H., Silva, J.L., Navalho, J., Varela, J.C.S., Speranza, L.G. (2022). Algae as Food in Europe: An Overview of Species Diversity and Their Application. Foods, 11,1871. https://doi.org/10.3390/foods11131871

Mouritsen, O.G., Rhatigan, P., Pérez-Lloréns, J.L. (2019). The rise of seaweed gastronomy: *Phycogastronomy*. *Bot. Mar*. 2019, 62, 195–209. https://doi.org/10.1515/bot-2018-0041.

Nova, P., Martins, A.P., Teixeira, C., Abreu, H., Silva, J.G., Silva, A.M., Freitas, A.C., Gomes, A.M. Foods with microalgae and seaweeds fostering consumers health: A review on scientific and market innovations. *J. Appl. Phycol*. 2020, 32, 1789–1802. https://doi.org/10.1007/s10811-020-02129-w.

Park, E.H., Jeon, E.B., Roy, P.K., Park, S.H., Park, S.Y. (2024). Quality characteristics and antioxidant activity of the Korean traditional rice-oat wine "Makgeolli" supplemented with green seaweed Codium fragile, *Heliyon*, 10(20), e39348. https://doi.org/10.1016/j.heliyon.2024.e39348

Penalver, R., Lorenzo, J.M., Ros, G., Amarowicz, R., Pateiro, M., Nieto, G. (2020). Seaweeds as a Functional Ingredient for a Healthy Diet. Mar. Drugs, 18(6):301. https://doi.org/10.3390/md18060301

Pereira, L., Cotas, J., Gonçalves, A.M. (2024). Seaweed Proteins: A Step towards Sustainability?. Nutrients, 16(8), 1123. https://doi.org/10.3390/nu16081123

Perez-Rodriguez, M.L., Serrano-Carretero, Garcia-Herrera, P., Camara-Hurtado, M., Sanzhez-Mata, M.C. (2023). Plant-based beverages as milk alternatives? Nutritional and functional approach through food labelling. *Food Research Internationa*l, 173, Part 1, 113244. https://doi.org/10.1016/j.foodres.2023.113244

Qin, Y. (2018). 6- Applications of Bioactive Seaweed Substances in Functional Food Products. Bioactive Seaweeds for Food Applications. Natural Ingredients for Healty Diets. 111-134. https://doi.org/10.1016/B978-0-12-813312-5.00006-6

Raja, K., Kadirvel, V., Subramaniyan, T. (2022). Seaweeds, an aquatic plant-based protein for sustainable nutrition-A review. Future Foods, 5, 100142. https://doi.org/10.1016/j.fufo.2022.100142

Rajapakse, N., Kim, S.K. (2011). Chapter 2-Nutritional and Digestive Health Benefits of Seaweed, 17-28.

Reboleira, J., Silva, S.F.J., Chatzifragkou, A., Niranjan, K., Lemos, M.F.L. (2024). Chapter 15 -Seaweed fermentation for the development of novel food products. Applications of Seaweeds in Food and Nutrition, 235-249. https://doi.org/10.1016/B978-0-323-91803-9.00019-6

Rigolon, T.C.B., Borges, L.L.R., Nascimento, A.L.A.A., Fernandes, J.G., Marins, J.C.B., Martins, E., Campelo, P.H., Stringheta, P.C. (2024). Study of the stability of hydroelectrolytic sports beverages enriched with phenolic extract from jaboticaba peel or blueberry pulp. Food and Humanity, 2, 100214. https://doi.org/10.1016/j.foohum.2023.100214

Sadeghi, A., Rajabiyan, A., Nabizade, N., Nezhad, N.M. Zarei-Ahmady, A. (2024). Seaweed-derived phenolic compounds as diverse bioactive molecules: A review on identification, application, extraction and purification strategies. *International Journal of Biological Macromolecules*, 266, Part 1, 131147. https://doi.org/10.1016/j.ijbiomac.2024.131147

Salgado, A., Moreira- Leite, B., Mata, P., Afonso, A., Infante, P. (2024). Chocolates enriched with seaweed: Emotional data and its combination with sensory data for products profiling and consumer segmentation. *International Journal of Gastronomy and Food Science*, 37, 100955. https://doi.org/10.1016/j.ijgfs.2024.100955

Salido, M., Soto, M., Seoane, S. (2024). Seaweed: Nutritional and gastronomic perspective. A review. *Algal Research*, 77, 103357. https://doi.org/10.1016/j.algal.2023.103357

Thanh, T.V., Tran, H., Khoa, T.A., Thuy, D.T. (2024). Seaweed Milk Food Supplement Produced from Hydropuntia eucheumatoides (Harvey). Natural Product Communications, 19(10), http://dx.doi.org/10.1177/1934578X241274992

Thangaraj, M., Sundaramanickam, A. (2025). Chapter 10- Pigments from seaweeds: green extraction technologies, characterization, and industrial applications. Marine Molecules from Algae and Cyanobacteria. Extraction Purification, Toxicology and Applications, 169-183. https://doi.org/10.1016/B978-0-443-21674-9.00012-X

Thiviya, P., Gamage, A., Gama-Arachchige, N.S., Merah, O., Madhujith, T. (2022). Seaweeds as a Source of Functional Proteins. *Phycology*, 2(2), 216-243. https://doi.org/10.3390/phycology2020012

Turnagol HH, Aktitiz S, Balturk SI, Yakisikli I, Erbas Z. Alternative protein sources in sustainable sports nutrition. *Turkish Journal of Sports Medicine*. 2023 58(1):47-54. https://doi.org/10.47447/tjsm.0694

CHAPTER 6

MEASURED AND EVALUATED OF GAPING ON PINK SALMON (*Onchorhynchus gorbuscha*) USING MACHINE VISION ANALYSIS SYSTEM

Assist. Prof. Dr. Ömer Alper ERDEM[1]
Prof. Dr. Şükran ÇAKLI [2]

[1] Ege University, Faculty of Fisheries, Department of Fishing Technology and Seafood Processing Technology, İzmir, TURKİYE. omer.alper.erdem@ege.edu.tr, Orcid ID: 0000-0002-1745-1751
[2] Ege University, Faculty of Fisheries, Department of Fishing Technology and Seafood Processing Technology, İzmir, TURKİYE. sukran.cakli@ege.edu.tr, Orcid ID: 0000-0002-2419-9064

INTRODUCTION

Once fish deteriorates in freshness and quality, no procedure or technology can restore it. Handling fish during capturing and harvesting frequently causes severe damage. How the fish is handled (gently or forcefully) affects harm. Physical damage is the major cause of net-caught salmon quality reduction. Primary defects separate, discolour, and soften skin. Net damage causes scale losses. Fish morphology is affected by extension network traces, however superficial modifications are fixable (Doyle, 1995).

Fresh fish must satisfy modern customers' taste, smell, touch, and sight. Fracture affects vision and freshness. Fish laceration after death causes splits, rips, and breaks (Bremmer, 1999). Many definitions of division are alike. This includes visible tears or fissures between muscle blocks (Love and Robertson, 1968), muscle layer separation due to weakened connective tissue (Doyle, 1995), raw fillet detachment from the myocommata, a thin membrane that separates the blocks (Whittle and Howgate, 2000), and small tube rupture. Definitions list common meat gaping traits. Fish connective tissue fragility divides muscle layers following cutting, generating tiny fractures or bigger splits.

Multiple sources explain the split. Doyle (1995) reported bending, tail tugging during gill net catch, and moving large fish reduce fish nutrition. These ingredients toughen fish at high temperatures (Love and Haq, 1970). Rough handling of whole fish, exposure to high temperatures during rigour, flesh gaping in salmon smoked at elevated temperatures early in processing, the increased likelihood of gaping in fillets from thawed whole fish compared to fresh whole fish, and summer-harvested salmon's superior feeding efficiency affected variation. According to Love (1973), inappropriate handling, lengthy hold times before freezing, and high-temperature cleaning, washing, and shipping can harm fish flesh. Boat-handled fish broke more flesh than bigger fish under equal biological settings. Fish feed heavily in July, which affects muscle chemistry and splits flesh after spawning.

Tissue examination before a tear is complicated. Vacuum-sealed smoked fillets have shiny liquid spots and a decreased appearance. Skin removal is difficult, and smoked fillet slices commonly break. This fish is inappropriate for various culinary purposes, thus people won't buy it at a premium. The salmon processing industry needs to understand flesh gaping in salmon fillets and establish a categorisation system for produced salmonids with tissue anomalies (Andersen et al., 1994).

Love (1973) and Lavéty (1984) found similar fragmentation reductions. The procedures include immediate icing and cooling of the fish after capture, careful handling, prompt freezing post-mortem and before rigging if freezing is planned, maintaining thawing temperatures below 18°C (Love, 1973) or 20°C (Lavéty, 1984), regulating temperature increases during initial smoking, ensuring complete thawing before filleting, and avoiding bending the fish while it is firm. (Love, 1973).

In a 1973 technical note, Love responded to the question, "When does fish gaping occur?": "Fillets from fish stored on ice, especially when the fish is considerably spoilt, demonstrate gaping." Challenges faced at sea are especially crucial on vessels with freezers, since thawed fish fillets may suffer considerable gaping. When the fillets contain bones, the connective tissue undergoes less strain. The mechanical force applied by the ice cream efficiently divides the fillets into separate halves. If the connective tissue of a severed fillet is relaxed, this situation does not occur. As a result, when fish are frozen whole for later thawing and filleting, they may sometimes have fissures in the flesh. However, they do not invariably fail. By exerting due diligence during the preparation and processing phases, the occurrence of meat gaping may be significantly reduced.

Machine vision collects and analyses photographs from real-world activities or monitors equipment and processes. Computer vision analyses and processes pictures to classify objects and characteristics. Computerised picture technology began in the 1960s. Computer graphics advanced conceptually and practically after the 1970s boom. Automatic production, monitoring, autonomous vehicles, and robotic aid are part of modern medical diagnostics. Computer vision applications are widely used for objective, quick, non-intrusive, and automated quality control and monitoring due to advances in image analysis and processing. This improves processed food quality evaluation. The findings suggest that computer-generated pictures may replace human vision in quantitative data and automation (Brosnan & Sun, 2004).

Machine vision is used more in food quality assurance. Autonomous systems evaluate raw or processed food quality. Over the past 15 years, computer technology and software have improved computer vision. Computers use pictures for surveillance and vision-guided robots. Machine vision is flexible and affordable. It improves accuracy and output. Food processors use computer-based imaging systems for online and real-time quality control. (Gunasekaran, 1996).

Computer imaging systems capture UV, VIS, or NIR reflection, transmittance, and fluorescence pictures of agricultural goods. Cameras, computers with image capture panels, and lighting systems make up basic computer vision systems. Signalling the computer, taking photos, storing them, and analysing them require software (Chen et al., 2002).

Advances in computers and algorithms for faster online processing need machine vision in automated food processing systems. Its versatility and non-destructiveness make it food industry-friendly. X-ray, 3D, and colour vision will help the food business understand this technology. (Brosnan & Sun, 2004).

This work aims to utilise developing computer imaging technologies for the identification of pink salmon (*Onchorhynchus gorbusca*) tissue damage using subjective approaches (visual observation). This adaption investigates the objective and quantitative detection of meat gaping. The objective assessment of its division can provide the fish processing business novel options, including reduced costs, less labour, and rapid, precise quality control.

1. MATERIAL AND METHOD

1.1. Fish Samples

In October 2007, 15 pink salmon fillets (*Onchorhynchus gorbusca*) were obtained from a commercial fishing enterprise in Kodiak, Alaska. The item has been maintained at a temperature of -20°C at the cold storage facility of the Fisheries Industrial Technology Centre (FITC), which is affiliated with the School of Fisheries and Ocean Sciences (SFOS) at the University of Alaska Fairbanks. From 29 May to 2 June 2009, a total of 15 fillets were thawed, with three fillets thawed daily, and photographs were taken over this period. The fillets were classified into three tiers based on the extent of meat gaping, which is a characteristic of pink salmon fillets (PSF). These groups demonstrate varying degrees of meat gaping, from extreme to acceptable or typical, with some exhibiting no gaping at all.

1.2. Machine Vision System and Its Components

This study employs a system comprising two light reflectors, two supporting arms for the reflectors, two fluorescent lamps rated at 1600 lumens each measuring 25 centimetres in length, a reflection plate (Rosco Polarising #7300 Filter), and two windows positioned with the reflection plate in front of the light reflectors (Figure 1).

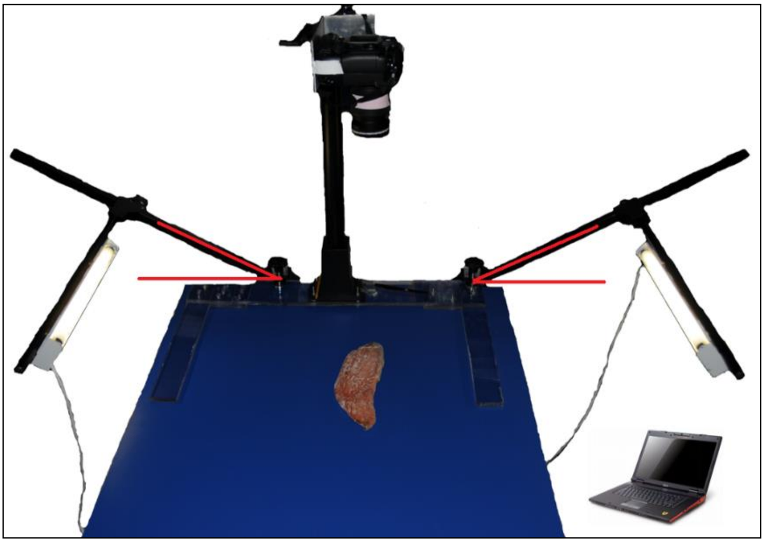


Figure 1. Machine vision system

The photo shoot utilised a Nikon D200 SLR digital camera, paired with a Nikon AF-S 18-200 mm F/3.5-5.6G ED-IF AF DX VR lens, a HOYA HMC Filter Multi-Coated Cir-Polarizing filter, and a hand-lifted stand for camera support. The lift has a height of 110 centimetres, with the right side ranging from 28.5 centimetres to 110 centimetres.

The elevator, light reflectors, and support arms are provided as a unified system known as the Bogen-Manfrotto Maxi Repro Stand Lite Assembly, which includes four reflector heads.

In the photo shoot, the calibration colour values for image analysis—L* (lightness), a* (± redness/blueness), b* (± yellowness/greenness)—were applied using a fixed colour standard (L*: 52.14, a*: 38.68, b*: 22.35) that maintained a constant position. The colour standard has been acquired from the GretagMacbeth® ColorChecker product.

A flat blue laminate of "Formica" brand, measuring 114×90 centimetres, serves as the background for the placement of the fillets. The laminate was acquired from a local furniture supplier. An adhesive has been utilised to affix a board of identical dimensions atop the laminate piece, ensuring it remains flat. The light stands and the camera stand are secured to the board using clamps. The item is positioned on a stationary fish cutting table situated within the pilot facility of the Fisheries Industry Technology Centre (Figure 3.1).

A Precision laptop equipped with an M65 Intel® Core™2 CPU T7200 operating at 2.00 GHz and 2.00 GB of RAM has been utilised. Corel PHOTO-PAINT X3 (Version 13.0.0.739; ©2005 Corel Corporation, CA) was utilised to prepare the captured images for analysis. The LensEye Program (Version 9.7.7; Engineering and CyberSolutions, Gainesville, FL) was utilised for image analysis.

1.3. Experimental Design

In order to inhibit polarisation, specifically reflection, a film has been positioned in front of both light sources. A camera filter has been attached to the lens in order to reduce reflections. The objective of utilising reflection at this juncture is to mitigate the phenomenon of excessive light reflection and thereby reduce the brightness observed on the fillet. Images were captured under two conditions: with and without reflection. The images with applied polarisation were captured using the aforementioned system. The effect of the polarisation film has been nullified by rotating the camera filter by ±90°. Furthermore, this method has been employed for images lacking polarisation. The study employed three distinct light angles. In this context, the term "angle of light" refers to the angle formed between the supporting arm of the light source and the background on which the fillets are positioned. The angles employed are 30°, 45°, and 60°, respectively. The light system employs the application of polarisation and the utilisation of three distinct light angles (30°, 45°, and 60°), which result in the formation of six distinct groups.

The colour standard employed during the photographic sessions has remained in a fixed position throughout. The colour standard has been consistently located in the upper right quadrant of the images. Two groups have been established on the basis of the degree of similarity between the colour of the head or tail of the fillets and that of the colour standard (fillet position).

Upon bisection of the image, the extremity or tail of the fillet remains within the section containing the colour standard. Consequently, two groups have been established, one at the front and the other at the rear.

The fillets are positioned on the ground at three distinct angles. The following section outlines the completed process. Two light sources are positioned on either side of the system. However, the aforementioned light sources are not discernible on the sides of the image; they are positioned at the top and bottom regions. The light source on the left is situated in the upper section of the image, while the light source on the right is located in the lower section. The fillet angle is determined by considering the angle formed between the light source in the upper region and the fillet axis. This method has established and utilised three distinct fillet angles: 60°, 90°, and 120°.

The Nikon D200 SLR camera has been elevated to a height of 78 cm using a handheld lift stand. The zoom settings of the objective have been modified to 33 millimetres. The camera's exposure, aperture, and sensitivity settings have been modified on a single occasion. The remaining features have remained unaltered. The aperture is set to f/16 and the sensitivity is configured to ISO 640 for the "Manual" option. The shooting speeds have been modified with the objective of optimising the capture of light in the images. The manipulation of capture speed is contingent upon the application of polarisation and the angle of the incident light, as these factors influence the intensity of the light. Images utilising polarisation exhibit reduced exposure times, whereas those lacking polarisation require increased exposure times. In images characterised by low light angles, the shutter speed is reduced, whereas in images with high light angles, the shutter speed is elevated. The images were captured using a computer for each scenario, with the settings configured in accordance with the requisite specifications. The images were downloaded to the computer in the JPEG format via a wireless connection. The dimensions of each photograph are 3872 pixels in length and 2592 pixels in width. Images that do not conform to the specified dimensions are deemed to be incorrect. Images deemed to be incorrect have been excluded from the subsequent analysis. The aforementioned images have been resized to 600x402 pixels for use with the LensEye software (Engineering and CyberSolutions, 4008 N.W. 122 Street Gainesville, FL 32606, USA), which performs fillet edge analysis. Subsequently, the file extensions were altered to BMP (Figure 2). During the course of the photo shoot, the calibration colour values for image analysis (L*, a*, and b*) were established using a fixed colour standard (L*: 52.14, a*: 38.68, b*: 22.35) (GretagMacbeth® ColorChecker,) (Figure 2b) that maintained a consistent position.

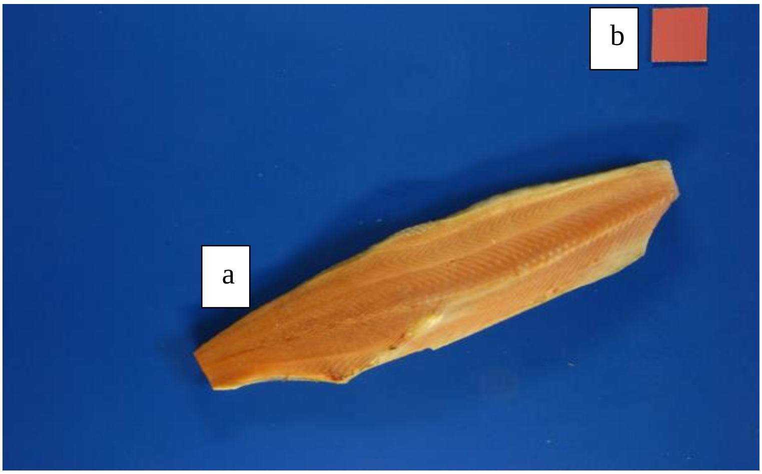


Figure 2. The file altered to BMP (a) pink salmon fillet (b) colour standard

The LensEye (versions of Program 9.7.7 and Gaping Program 1.6.0) software employs image analysis techniques to identify and quantify colour standards and fillets in visual stimuli (Figure 3). The results of the image analysis present the mean L*, a*, and b* colour values for the fillet, together with the standard deviations. Fissure formation is contingent upon the L* value falling below a specified threshold. The software has calculated a L* value for each fillet, delineating areas below this value with green lines and indicating the percentage of these identified areas relative to the fillet area.

Figure 3. The image analysis result obtained from the LensEye software provides insight into the underlying vision.

1.4. Statistical Analysis

All analyses were performed in triplicate. Statistical analysis utilised Microsoft Excel 2007 software for the calculation of means and standard deviations. Statistical evaluation was performed using One-way ANOVA with SPSS Standard Version 11.5, following Tukey and Duncan tests. The statistical evaluation is displayed in tables utilising letters.

2. RESULTS AND DISCUSSION

A total of 36 images, each representing a different group, were captured of 15 pink salmon fillets (PSF) using a machine vision system to ascertain the extent of fillet gaping. The machine vision system has been employed for the purpose of controlling fillets, with the result that fillet gaping has been entirely eliminated. PSF number 5 is devoid of a fillet gaping and exhibits red spots, designated as bruising, which are observable from the centre of the fillet towards the tail, particularly along the backbone line. The LensEye program has indicated these observations (Figure 4). In this scenario, although the fillet gaping area may expand to 2% of the total fillet surface in certain photos (Table 1), the region identified by the algorithms does not indicate fillet gaping.

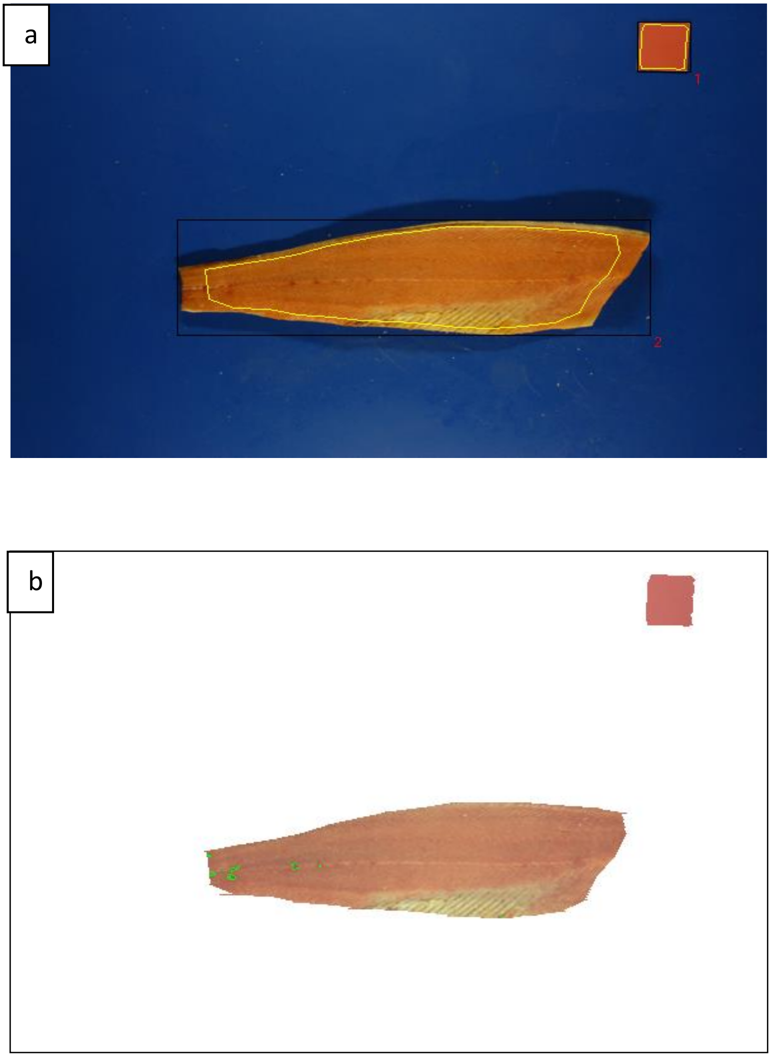


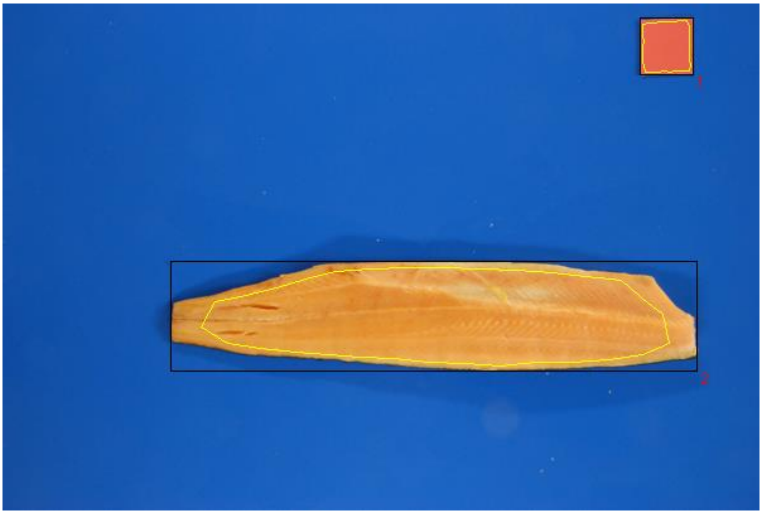
Figure 4. PSF number 5 (a) image before analysis of machine vision system, (b) image of PSF number 5 analysed by LensEye program

Table 1. The image groups are associated with PSF number 5 and the fillet gaping ratios as detected by the machine vision.

	Reflection	Light angle	Fillet position	Filet angle	%L Değeri
1	No polarisation	30°	Head	60°	0,91±0,01
2	No polarisation	30°	Head	90°	0,92±0,00

3	No polarisation	30°	Head	120°	0,33±0,02
4	No polarisation	30°	Tail	60°	2,00±0,06
5	No polarisation	30°	Tail	90°	0,47±0,01
6	No polarisation	30°	Tail	120°	0,50±0,03
7	No polarisation	45°	Head	60°	0,26±0,10
8	No polarisation	45°	Head	90°	0,41±0,00
9	No polarisation	45°	Head	120°	0,14±0,01
10	No polarisation	45°	Tail	60°	1,90±0,07
11	No polarisation	45°	Tail	90°	0,44±0,12
12	No polarisation	45°	Tail	120°	0,19±0,01
13	No polarisation	60°	Head	60°	0,22±0,00
14	No polarisation	60°	Head	90°	0,23±0,00
15	No polarisation	60°	Head	120°	0,12±0,00
16	No polarisation	60°	Tail	60°	0,49±0,02
17	No polarisation	60°	Tail	90°	0,27±0,01
18	No polarisation	60°	Tail	120°	0,19±0,02
19	Polarisation	30°	Head	60°	0,63±0,11
20	Polarisation	30°	Head	90°	0,50±0,02
21	Polarisation	30°	Head	120°	0,17±0,00
22	Polarisation	30°	Tail	60°	1,12±0,26
23	Polarisation	30°	Tail	90°	0,29±0,01
24	Polarisation	30°	Tail	120°	0,28±0,01
25	Polarisation	45°	Head	60°	0,17±0,01
26	Polarisation	45°	Head	90°	0,24±0,00
27	Polarisation	45°	Head	120°	0,17±0,00
28	Polarisation	45°	Tail	60°	0,64±0,00
29	Polarisation	45°	Tail	90°	0,24±0,00
30	Polarisation	45°	Tail	120°	0,21±0,00
31	Polarisation	60°	Head	60°	0,17±0,01
32	Polarisation	60°	Head	90°	0,17±0,01
33	Polarisation	60°	Head	120°	0,19±0,0
34	Polarisation	60°	Tail	60°	0,25±0,01
35	Polarisation	60°	Tail	90°	0,24±0,01
36	Polarisation	60°	Tail	120°	0,20±0,00

The PSF number 6 is indicative of a fillet gaping level that is in accordance with the stipulated requirements. The fillet gapings that were identified in the tail region have been successfully located by the machine vision program through its automated analysis (Figure 5). The LensEye software has identified only the meat splits, with no defects reported aside from fillet gaping. The image groups numbered 4 and 22, which satisfy the criteria of light angle, fillet position, and fillet angle factors outlined in Table 2, yield the two highest outcomes. The fillet gaping observed at the tail section has resulted in alterations to the surrounding area, leading to an increase in the incidence of fillet gaping, which is dependent on the L* value.



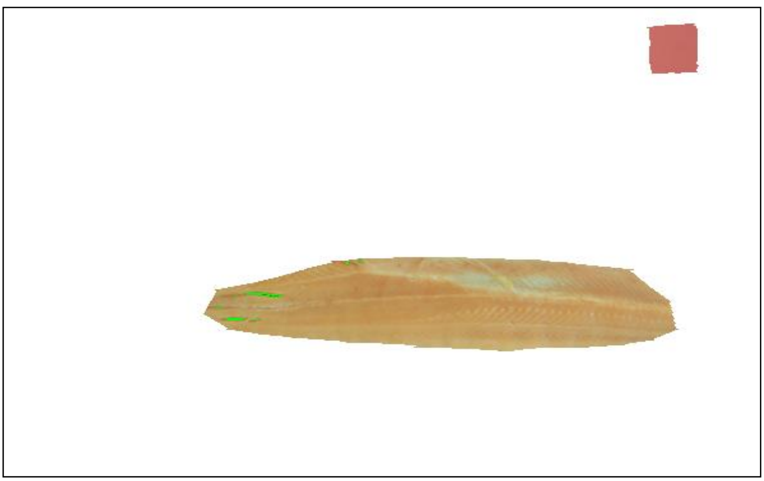


Figure 5. PSF number 6 (a) image before analysis of machine vision system, (b) image of PSF number 6 analysed by LensEye program

Table 2. The image groups are associated with PSF number 6 and the fillet gaping ratios as detected by the machine vision.

	Reflection	Light angle	Fillet position	Filet angle	%L Değeri
1	No polarisation	30°	Head	60°	0,64±0,05
2	No polarisation	30°	Head	90°	0,48±0,03

3	No polarisation	30°	Head	120°	0,62±0,01
4	No polarisation	30°	Tail	60°	1,68±0,04
5	No polarisation	30°	Tail	90°	0,38±0,00
6	No polarisation	30°	Tail	120°	0,25±0,00
7	No polarisation	45°	Head	60°	0,49±0,0
8	No polarisation	45°	Head	90°	0,47±0,01
9	No polarisation	45°	Head	120°	0,42±0,00
10	No polarisation	45°	Tail	60°	0,68±0,03
11	No polarisation	45°	Tail	90°	0,30±0,0
12	No polarisation	45°	Tail	120°	0,30±0,0
13	No polarisation	60°	Head	60°	0,36±0,00
14	No polarisation	60°	Head	90°	0,36±0,00
15	No polarisation	60°	Head	120°	0,37±0,02
16	No polarisation	60°	Tail	60°	0,35±0,00
17	No polarisation	60°	Tail	90°	0,32±0,00
18	No polarisation	60°	Tail	120°	0,35±0,02
19	Polarisation	30°	Head	60°	1,05±0,16
20	Polarisation	30°	Head	90°	0,68±0,11
21	Polarisation	30°	Head	120°	0,93±0,02
22	Polarisation	30°	Tail	60°	2,97±0,04
23	Polarisation	30°	Tail	90°	0,70±0,00
24	Polarisation	30°	Tail	120°	0,65±0,02
25	Polarisation	45°	Head	60°	0,70±0,02
26	Polarisation	45°	Head	90°	0,69±0,00
27	Polarisation	45°	Head	120°	0,59±0,01
28	Polarisation	45°	Tail	60°	1,53±0,01
29	Polarisation	45°	Tail	90°	0,45±0,00
30	Polarisation	45°	Tail	120°	0,46±0,00
31	Polarisation	60°	Head	60°	0,68±0,03
32	Polarisation	60°	Head	90°	0,72±0,01
33	Polarisation	60°	Head	120°	0,55±0,01
34	Polarisation	60°	Tail	60°	1,01±0,07
35	Polarisation	60°	Tail	90°	0,56±0,00
36	Polarisation	60°	Tail	120°	0,48±0,00

PSF number 11 displays a notable degree of fillet gaping, particularly in the dorsal region, where it extends longitudinally to a considerable extent, accompanied by a distinct separation of the underlying tissue (Figure 6). The machine vision software's markers for detecting the fillet gap on the 11th PSF showed that the 1st image group had the highest ratio at 9.48%, while the 33rd image group had the lowest ratio at 3.40%.

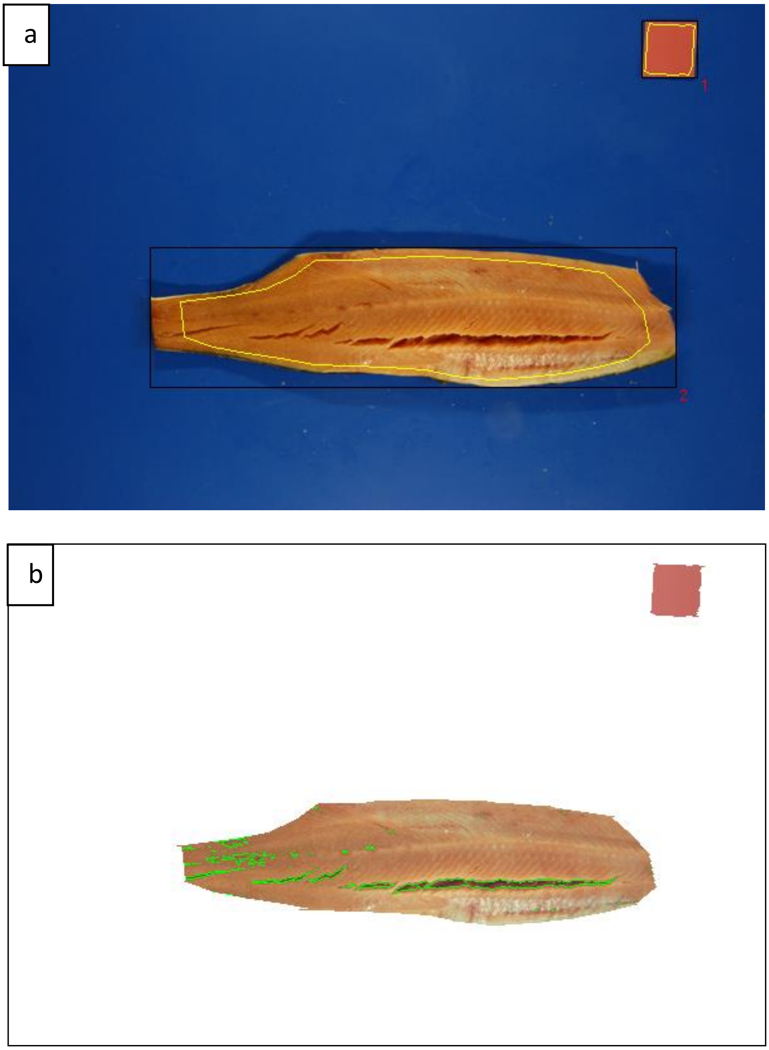


Figure 6. PSF number 11 (a) image before analysis of machine vision system, (b) image of PSF number 11 analysed by LensEye program

Table 3. The image groups are associated with PSF number 11 and the fillet gaping ratios as detected by the machine vision.

	Reflection	Light angle	Fillet position	Filet angle	%L Değeri
1	No polarisation	30°	Head	60°	9,48±0,04
2	No polarisation	30°	Head	90°	9,21±0,03
3	No polarisation	30°	Head	120°	5,57±0,08
4	No polarisation	30°	Tail	60°	8,21±0,05

5	No polarisation	30°	Tail	90°	7,19±0,04
6	No polarisation	30°	Tail	120°	5,89±0,01
7	No polarisation	45°	Head	60°	6,36±0,02
8	No polarisation	45°	Head	90°	6,49±0,05
9	No polarisation	45°	Head	120°	4,82±0,02
10	No polarisation	45°	Tail	60°	7,39±0,06
11	No polarisation	45°	Tail	90°	6,10±0,04
12	No polarisation	45°	Tail	120°	5,39±0,01
13	No polarisation	60°	Head	60°	4,08±0,04
14	No polarisation	60°	Head	90°	4,12±0,06
15	No polarisation	60°	Head	120°	3,95±0,01
16	No polarisation	60°	Tail	60°	6,25±0,03
17	No polarisation	60°	Tail	90°	7,17±0,42
18	No polarisation	60°	Tail	120°	4,53±0,00
19	Polarisation	30°	Head	60°	7,82±0,09
20	Polarisation	30°	Head	90°	7,16±0,05
21	Polarisation	30°	Head	120°	5,05±0,03
22	Polarisation	30°	Tail	60°	7,58±0,08
23	Polarisation	30°	Tail	90°	6,54±0,01
24	Polarisation	30°	Tail	120°	5,52±0,05
25	Polarisation	45°	Head	60°	5,16±0,03
26	Polarisation	45°	Head	90°	5,25±0,02
27	Polarisation	45°	Head	120°	4,27±0,01
28	Polarisation	45°	Tail	60°	6,01±0,06
29	Polarisation	45°	Tail	90°	5,22±0,03
30	Polarisation	45°	Tail	120°	4,96±0,19
31	Polarisation	60°	Head	60°	3,64±0,04
32	Polarisation	60°	Head	90°	3,71±0,04
33	Polarisation	60°	Head	120°	3,40±0,05
34	Polarisation	60°	Tail	60°	5,25±0,12
35	Polarisation	60°	Tail	90°	5,25±0,00
36	Polarisation	60°	Tail	120°	4,35±0,05

The observed discrepancies in fillet outcomes within the Excessive fillet gaping group at the same site are attributed not only to the surface area affected by the muscle split but also to the position, length, breadth, and depth of the fillet gaping. The machine vision program yielded ratios that were found to be in closer approximation to reality than the actual ratios in fillet surface cracking measurements. This was determined to be due to the irregular and undulating nature of the fillet surfaces and the occurrence of dark red, burgundy, or purplish spots resembling blood pooling in the muscle.

In a study conducted by Mathiassen et al. (2007), two melanin point detectors were developed for use in salmon processing plants. The sensors employed were of two categories, derived from Linear Discriminant Analysis (LDA) of the R-channel and RGB. Each feature comprised a single channel, and the identical melanin spot identification technique was employed for both feature images. The initial stage involved adjusting the feature picture to the threshold y0, while the subsequent step delineated sensor efficacy across a broad spectrum of detection rates and the quantity of erroneously identified melanin spots. Subsequently, the images underwent further processing via a median line filter, with the objective of eliminating minor noise and enhancing the identification of melanin spots. The residual patches were classified as melanin spots. However, some melanin patches were incorrectly identified due to inadequate illumination, the presence of blood and fragments of fins in the vicinity of the abdominal organ. The melanin spot detector may prove advantageous despite the presence of poor detection and false alert rates.

The study by Misimi et al. (2008) aimed to differentiate between the superior and ordinary quality classifications of Atlantic salmon through the utilisation of computer-based image and model recognition methodologies. The images of the salmon were captured with a digital colour camera and saved in bitmap file format within the RGB colour space. A classifier based on the Linear Discriminant Analysis (LDA) method was selected for the purpose of classification. The research demonstrated that the geometric distinctions between standard and superior degree cases were inconsequential, and the classifier was unable to assess and discriminate between them. Additionally, other fish that were incorrectly categorised as superior were consistently identified by human inspectors due to their elongated size and diminished condition factor. The research concluded that the most effective approach was to process and segment fish photos under regulated illumination settings. It is important to consider the potential financial implications of misclassification; as premium salmon may be incorrectly categorised as either ordinary or superior. The algorithm may facilitate flexibility in adjusting decision boundaries, and computerised quality grading may offer a more consistent and objective evaluation than hand grading. It is anticipated that the melanin spot detector will prove beneficial in salmon processing facilities where there is a need to achieve optimal detection and false alarm rates.

In a study conducted by Kohler et al. (2002), the categorisation of salted fish fillets was investigated through the use of coloured imagery. The categorisation process is based on subjective quality standards, with customers generally preferring a luminous and pristine fillet. In Norway, the salted fish sector employs manual categorisation, with the general appearance of the fish fillet being the primary criterion for classification. Three methodologies were investigated: black and white level histogram analysis, colour histogram analysis, and colour change histogram analysis. The study employed a total of 12 salted fish fillets and reached the conclusion that brightness and redness are autonomous markers of quality rating. However, the traditional red channel values did not correspond with an effective quality marker for categorising the fillets. The proposed approaches were found to be flawed, although the misclassification of one instance in each method did not present a significant practical issue. The conventional categorisation system, which relies on visual assessment and subjective evaluation, has proven to be effective in practice.

3. CONCLUSION

The machine vision system has identified splits on the pink salmon fillet and has also detected defects, including blood settling on the surface of the fillet, as previously noted. The objective of this study is to examine the methodology employed for the detection of fillet gaping as a defect on the fillet surface. However, instances of erroneous markings have been observed. In addition to the aforementioned flaws, instances of incorrect markings have been observed as a consequence of the fillet surface structure. It is possible that small, shallow linear lacerations may occasionally go unnoted. The study demonstrates that the structure of fillet gaping is discernible not only on the surface, including melanin spots and blood settling. A crack is characterised by specific surface features and a defined depth. The shadows created at this depth have been employed, and a method based on the L* value has been established. The reflection of light became a concern during the experimental setup, necessitating the placement of polarisation films in front of the light sources. Future research will focus on the development of systems that can be tailored to meet the specific needs of industry. The variability of the fillet's position on the production line requires an analysis of fillet gaping that considers both the fillet's location and its angles. A specialised version of the LensEye software was employed for this purpose. The resulting analysis yields values expressed as the ratio of the area designated as "fillet gaping" by the software to the total surface area of the fillet.

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REFERENCES

Andersen, U.B., Strømsnes, A.N., Steinsholt, K., & Thomassen, M.S. (1994). Fillet gaping in farmed Atlantic salmon (*Salmo salar*). *Norwegian Journal of Agricultural Sciences*, 8, 165-179.

Bremmer, H.A. (1999). Gaping in fish flesh. In K. Sato, M. Sakaguchi & H. A. Bremmer (Eds.), *Extracellular matrix of fish and shellfish* (pp. 81-94). Research Signpost.

Brosnan, T., & Sun, D.W. (2004). Improving quality inspection of food products by computer vision- a review. *Journal of Food Engineering*, 61, 3-16. https://doi.org/10.1016/S0260-8774(03)00183-3

Chen, Y.R., Chao, K., & Kim, M.S. (2002). Machine vision technology for agricultural applications. *Computers and Electronics in Agriculture*, 36, 173-191. https://doi.org/10.1016/S0168-1699(02)00100-X

Gunasekaran, S. (1996). Computer vision technology for food quality assurance. *Trends in Food Science & Technology*, 7, 245-256. https://doi.org/10.1016/0924-2244(96)10028-5

Kohler, A., Skaga, A., Hjelme, G., & Skarpeid, H.J. (2002). Sorting salted cod fillets by computer vision: a pilot study. *Computers and Electronics in Agriculture*, 36, 3-16. https://doi.org/10.1016/S0168-1699(02)00068-6

Lavéty, J. (1984). Gaping in farmed salmon and trout. Ministry of Agriculture, Fisheries and Food Torry Research Station, Torry Advisory Note No.90, Aberdeen, UK.

Love, R.M. (1973). Gaping of fillets. Ministry of Agriculture, Fisheries and Food Torry Research Station, Torry Advisory Note No.61, Aberdeen, UK.

Love, R.M. & Haq, M.A. (1970). The connective tissues of fish III. The effect of pH on gaping in cod entering rigor mortis at different temperatures. *International Journal of Food Science & Technology*, 5, 241-248. https://doi.org/10.1111/j.1365-2621.1970.tb01563.x

Love, R.M. & Robertson, I. (1968). The connective tissues of fish I. The influence of biological condition in cod on gaping in frozen-thawed muscle. *International Journal of Food Science & Technology*, 3, 215-221. https://doi.org/10.1111/j.1365-2621.1968.tb01459.x

Mathiassen, J.R., Misimi, E. & Skavhaug, A. (2007). A Simple computer vision method for automatic detection of melanin spots in Atlantic salmon fillets. *11th International Machine Vision and Image Processing Conference* (*IMVIP 2007*) (pp. 192-197) National University of Ireland (NUI) Maynooth, County Kildera, Ireland, 5th-7th September. https://doi.org/10.1109/IMVIP.2007.28

Misimi, E., Erikson, U., Digre, H., Skavhaug, A. & Mathiassen, J.R. (2008). Computer vision-based evaluation of pre-and postrigor changes in size and shape of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) fillets during rigor mortis and ice storage: effects of perimortem handling stress. *Journal of Food Science*, 73(2), E57-E68. https://doi.org/10.1111/j.1750-3841.2007.00626.x

Whittle, K.J. & Howgate, P. (2000). Glossary of fish technology terms. *Fisheries Industries Division of the Food and Agriculture Organization of the United Nations*, Rome, Italy.

CHAPTER 7

MARINE FISH PRODUCTION IN EARTHEN PONDS

Res. Assist. Dr. Rifat TEZEL[1]

[1] Muğla Sıtkı Koçman University, Faculty of Fisheries, Department of Aquaculture, Muğla, Türkiye, rifattezel@mu.edu.tr, Orcid ID: 0000-0002-0870-7049

INTRODUCTION

The aquaculture sector has been the fastest growing animal food production sector in the recent past. Since the aquaculture is a relatively new sector compared to other agricultural sectors, it has the potential to continue to grow and develop in the future (Guillen et al., 2019). Türkiye has important water resources suitable for aquaculture. In Türkiye, commercial rainbow trout farming was first started in the 1970s (SBD, 2014). In order to reduce production costs, to register the sector and to increase the competitiveness of the sector at international level, the aquaculture sector has been included in the governmental incentive since 2003 (SBD, 2014). Governmental incentive given to the aquaculture sector, technological developments, experiences gained and the production of new information have brought success. As a result of the steps taken to ensure the development of the sector, the amount of aquaculture production increased from 3,075 tons in 1986, when aquaculture was just started, to 556,287 tons in 2023 (Table 1).

Table 1. Aquaculture Production in Türkiye (tons) (TURKSTAT, 2024)

Years	*Sea bass*	*Sea bream*	*Rainbow trout*	*Others*	*Total*
2023	160,802	154,011	222,486	18,988	556,287
2022	156,602	152,469	191,103	14,631	514,805
2021	155,151	133,476	167,286	15,773	471,686
2020	148,907	109,749	146,594	16,161	421,411
2019	137,419	99,730	125,745	10,453	373,356
2018	116,915	76,680	114,497	6,445	314,537
2017	99,971	61,090	109,657	5,784	276,502
2016	80,847	58,254	107,013	7,281	253,395
2015	75,164	51,844	108,038	5,288	240,334
2014	74,653	41,873	113,593	5,014	235,133
2013	67,913	35,701	128,059	1,721	233,393

In Türkiye, marine fish farming is carried out in net cages in the sea and in earthen ponds on land. Freshwater fish farming is carried out in concrete ponds on land and in net cages in lakes and dams. In recent years, rainbow trout fattened in fresh waters have been transported to the Black Sea and they are raised to produce Turkish salmon. In Türkiye, rainbow trout, sea bass and sea bream are mostly produced species in aquaculture. In 2023; 222,486 tons of rainbow trout, 160,802 tons of sea bass and 154,011 tons of sea bream were produced in aquaculture (TURKSTAT, 2024).

According to General Directorate of Fisheries and Aquaculture official records in 2024, there are a total of 2,385 aquaculture enterprises in Türkiye, including 1,831 freshwater enterprises and 554 marine enterprises (GDFA, 2024). The project capacity of aquaculture enterprises operating in the seas is 536,321 tons, while the project capacity of aquaculture enterprises operating in freshwaters is 277,311 tons. When the total project capacity and real production values are analyzed, it is seen that these enterprises produce 68.37% of the total project capacity. In the Twelfth Development Plan, it is stated that aquaculture production and exports will be increased through new investments to be made by protecting natural resources and biodiversity, new aquaculture regions will be planned, capacity utilization rates and production efficiency of aquaculture facilities will be increased (SBD, 2023). In the light of these data, it is understood that the increase in fish production will continue in the future.

CURRENT SITUATION OF EARTHEN POND AQUACULTURE

Aquaculture can be defined as the production of saleable seafood as cost-effectively as possible under controlled conditions. This usually requires the highest possible stocking density in the system, the use of high quality feeds and active water quality management (Ebeling et al., 2006). However, today, increasing environmental sensitivities are shifting consumer interest towards the products of small-scale production enterprises. The lower environmental risk of semi-intensive aquaculture and consumers' demands for food safety and the welfare of produced species have brought products produced in semi-intensive systems back to the forefront (Banas et al., 2008; Bosma and Verdegem, 2011; Serpa et al., 2013). For this reason, marine fish production in earthen ponds, which currently operates in Muğla Province of Türkiye and constitutes a regional value, is very important (Figure 1).

Figure 1. The Region That the Earthen Pond Enterprises Established

The sustainability of semi-intensive aquaculture systems depends on management practices that increase production efficiency and reduce environmental impacts (Serpa et al., 2013). Today, environmental impact of aquaculture can be assessed as the environmental impact on biodiversity, the water quality of effluents, the amount of water consumed, the impact on soil and surface waters, the amount of land area used, and the amount of natural fish caught for aquaculture production (Bosma and Verdegem, 2011).

Marine fish production in earthen ponds started in Milas District of Muğla Province in the 1980s. Earthen pond aquaculture is carried out in fields that used to be used as agricultural land and became inefficient due to salinization of the soil. The aquacultere activity that started in these inefficient fields has become an economic outlet for the people of the region. The fact that the enterprises producing fish in earthen ponds are generally small family businesses is an indicator of this. According to Muğla Provincial Directorate of Agriculture and Forestry official records, the number of active enterprises producing fish in earthen ponds and the change in the production capacities of these enterprises over the years are given in table 2 (MPDAF, 2022).

Table 2. Number of Active Earthen Pond Enterprises and Production Capacities

Years	*Number of Active Enterprises*	*Production Capacity of Active Enterprises (tons/year)*
2011	145	7,381
2012	148	7,449.5
2013	165	8,866.5
2014	163	8,943.5
2015	155	8,931.5
2016	149	8,729
2017	138	8,590
2018	106	7,100
2019	90	6,000
2020	90	6,000
2021	88	5,942

Statistical data shows a significant decrease in the number of enterprises in production over the years. Today, production in the region is only carried out in currently licensed enterprises, but no new enterprises are allowed to be established (Figure 2).

Tezel and Güllü (2017) found that the problems experienced in the management of fish marketing and production processes in earthen pond enterprises threaten the sustainability of the enterprises. Due to the negativities experienced, some of the enterprises interrupt their production while others sell their businesses. In recent years, it is seen that some earthen pond enterprises have been transformed into fish adaptation enterprise and fish hatcheries by changing their licenses. Current developments show that the sustainability of the marine fish production sector in earthen ponds is under threat. Therefore, studies to solve the problems faced by these enterprises should be prioritized.

Figure 2. General View of an Earthen Pond Enterprise Producing Marine Fish

PONDS USED IN FISH PRODUCTION AND POND DESIGN

Fish production in earthen ponds is carried out in large volume earthen ponds in terrestrial environment. The size of the ponds used in the enterprises varies excessively. Tezel and Güllü (2017) determined that the average length of the ponds used was 62.75 ± 15.97 m, the average width was 16.66 ± 5.02 m and the average depth was 2.77 ± 0.72 m in earthen pond enterprises. Pond sizes are differs in accordance with the shape and dimensions of the land where the enterprises are established.

The edges of the ponds are sloped to prevent landslides and to prevent the collapse of the pond embankments. Commonly, water is inlet from one of the narrow sides of the pond and water is discharged from the other side through a pipe placed on the bottom of the pond (Figure 3). In some enterprises, the water inlet and outlet are randomly placed at any point in the pond. By creating a slope from the water inlet of the ponds towards the water flow direction, it is aimed to direct the fish feces towards the water discharge point.

Figure 3. Earthen Pond Prepared For Use in Production

The lands where the ponds are established generally have a soil structure with low water permeability. Generally, the bottom of the ponds are tightened with construction machinery to reduce water losses and prevent landslides in the ponds. Gravel is laid on the embankments of the ponds to ensure that the applications made in the ponds are carried out more comfortably.

In earthen pond enterprises, the water drawn from the underground first flows into a water tower and the groundwater that is aerated here is transferred to the ponds through water pipes (Figure 4). After production, the outflow water of ponds is discharged to a channel placed in the enterprise. Then the outflow water is transferred to the water drainage channels established by General Directorate of State Hydraulic Works (Figure 5).

In order to increase the water cycle in the ponds and to increase the dissolved oxygen value of the pond water, a varying number of pedall wheel aerators are positioned at the pond centerline (Figure 6).

Figure 4. Water Tower Where Groundwater Is Aerated

Figure 5. Water Discharge Systems of the Ponds and Water Discharge Channel

Figure 6. Ponds and Pedall Wheel Aerators Used In Production

FISH SPECIES PRODUCED, FISH STOCKING, FISH FEEDING AND OTHER PRODUCTION PRACTICES

Sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are commonly farmed in earthen ponds. In recent years, it is also observed that the production of meagre (*Argyrosomus regius*) has increased. In addition, it is known that other sea fish are also produced in very small quantities in some enterprises.

The earthen pond enterprises produce fish in portion size by procuring fry from fish hatcheries operating in the region. The fry obtained from the hatchery are adapted to the pond water and placed in sufficient number of ponds. The fish placed in the ponds are treated from time to time for vaccination, sizing, drug application and growth monitoring. In these applications, the fish in the pond are directed towards a corner of the pond via using a net (Figure 7). In order to pull the net appropriatly, the aerators in the pond are turned off. If the pedall wheel aerators are not turned off, it will be difficult to pull the net due to the water cycle in the pond and there will be a risk of the net getting entangled to the pedall wheel aerator. Since the aerator is closed during the process, oxygen should be supplemented with oxygen diffusers in the area where the fish are assembled. Fish are usually transferred one or two times during the production process.

In earthen pond enterprises, fish production is carried out at lower stocking densities compared to other aquaculture enterprises. Generally, 6 kg/m^3 final harvest stock densitity are used in enterprises. The fish in the ponds are usually fed twice a day by the personnel (Figure 8). Although the amount of feed given to the fish varies depending on climatic conditions, water quality in the ponds and the physiological condition of the fish, it is usually around 1-2%.

Figure 7. Assembling Fish via Using a Net

Figure 8. Feeding Fish

WATER QUALITY IN PONDS

While the majority of the earth pond enterprises use water drawn from underground by motopumps, a few of enterprises use spring water that comes to the surface spontaneously from undergraund. Water supplied from underground water sources has low dissolved oxygen values and, in addition, underground water may contain undesirable gases such as carbon dioxide and carbon monoxide. For this reason, the water drawn from the underground is first transferred to a water tower and aerated here. The aerated water is then distributed to the ponds.

In production ponds, water is changed at a rate of 1/2 to 1/6 daily. This water change rate varies depending on the fish species produced, stocking density and water quality. With the deterioration of environmental factors in the pond, water exchange is increased to reduce potentially toxic compounds such as ammonia and nitrite (Brambilla et al., 2007; Burford and Lorenzen, 2004; Serpa et al., 2013). Due to the use of large-volume ponds in production, organic matter accumulation occurs at the bottom of the ponds. Organic matter accumulated at the bottom of the ponds causes to changes in the biological and chemical properties of water (Serpa et al., 2007; Tezel and Güllü, 2017). In addition, anaerobic conditions at the bottom of the ponds cause benthic organisms to proliferate and potentially toxic microbial metabolites are passes into the water in consequence of microbial degradation (Boyd, 1995; Xinglong and Boyd, 2006; Tezel and Güllü 2017).

Particles mobilised from the pond bottom and resuspended in water are an important mechanism of nutrient transport in aquaculture ponds. In shallow water bodies such as earthen ponds, sediment may periodically re-suspend due to wind-driven waves. Similarly, sediment in aquaculture ponds can be continuously resuspended due to the movement of fish near the bottom (Avnimelech et al., 1999). Water quality in ponds is directly related to many factors such as vital activities of fish, structure of ponds, accumulation of organic matter in ponds and water movements. In fish production in earthen ponds, fundemental water quality parameters such as water temperature, dissolved oxygen content, salinity, pH, nitrogenous compounds and phosphorus containing compounds are the determining factors of production.

Water temperature is one of the most important parameter influencing production and affecting factors such as food consumption, feeding amount, feed intake, digestion, feed conversion rate, growth, reproduction and respiratory needs of fish (Egemen, 2005). In addition, water temperature affects the natural productivity of the aquatic ecosystem and directly or indirectly regulates water quality variables (Brambilla et al., 2007). Fish are cold-blooded organisms and the body temperature of fish varies depending on daily and seasonal water temperature changes (Boyd and Tucker, 1998). Therefore, water temperature is an important determinant in the selection of the species to be farmed in the available water source.

Due to the use of underground water in earthen ponds, the inflow water temperature of the ponds is generally 18-20 ˚C. However, pond water temperatures vary depending on atmospheric temperature changes. Pond water temperatures, which drop to 10 ˚C in winter season, increase up to 30 ˚C in summer season (Tezel, 2020). The increase in water temperature, especially in summer season, may cause fish to avoid taking feed and slow down growth. When such a situation is experienced in earthen ponds, the water temperature is partially controlled by increasing the water inflow to some extent. During this period, when feeding is usually reduced or stopped and stress increases, the general health of the fish should be carefully monitored and especially the dissolved oxygen content of the water should be kept as high as possible.

Fish need oxygen in order to sustain their lives and to carry out their metabolic activities. Dissolved oxygen content of water is one of the most important water quality parameters in aquaculture (Egemen, 2005). The amount of dissolved oxygen in the pond water is the main limitation of production in the pond. Excessively high or low oxygen levels can affect the fish health, cause the emergence of diseases and even cause mass mortalities, resulting in significant economic losses (Liu et al., 2023). Oxygen consumption of fish varies depending on fish species, fish size, fish activity, time passed after feeding, water temperature and the amount of dissolved oxygen in the ponds. The amount of dissolved oxygen in water varies depending on the transition between water and air, oxygen utilisation in sediment, fish respiration, plankton respiration and photosynthesis (Boyd and Tucker, 1998). In addition, water temperature values directly affect the oxygenation capacity of water and dissolved oxygen values decrease in ponds in summer season due to increasing water temperatures as a result of warming weather. The lowest dissolved oxygen values in ponds are observed at night and before sunrise in summer

(Farrelly et al. 2015; Tezel, 2020). Therefore, dissolved oxygen values should be monitored very carefully during nighttime. Monitoring the dissolved oxygen level in the water and even predicting the changes in advance is very important for the correct management of the amount of dissolved oxygen level in the water (Liu et al., 2023). Although oxygen can pass between water and air by diffusion, pedall wheel aerators are used to accelerate this process and increase the amount of dissolved oxygen in the pond water (Figure 9).

The number and the using duration of pedall wheel aerators in ponds are varies depending on the dissolved oxygen requirement. Especially in summer season, the amount and duration of pedall wheel aerator use increases. One of the most important factors that increase the dissolved oxygen value in the ponds is the diffusion of atmospheric oxygen to the pond water due to the waves caused by the effect of wind. In the windy days, the diffusion of atmospheric oxygen into the water accelerates. The use of pedall wheel aerator can be reduced during these windy days.

Figure 9. Pedall Wheel Aerators Using in Ponds

In the selection of the fish species to be produced, the ability to adapt to the salinity changes that may occur in the water source is also very important as well as the current salinity value. In the region where fish production is carried out in earthen ponds, the salinity of the underground water varies considerably. The salinity of water using in this region, changes between ‰5 to ‰35. However in any earthen pond enterprise, salinity values show very little change during the year. The salinity of the water source is the first factor determines the salinity of the pond water. Due to high precipitation or evaporation processes, the salinity of the pond water varies very little.

The pH value is one of the most important parameters affecting chemical and biological activities in water. In fish farming, the interaction of pH value with other variables is very important. Vital activities of livingthings taking place in the pond causes daily changes in pH of the water (Boyd and Tucker, 1998; Egemen, 2005). High pH values in aquaculture are associated with intensive photosynthesis activity in eutrophic ponds where algae are abundant and algae consume significant amounts of CO_2 during the daylight. As a result of the respiration of algae in the aquatic environment at night and the decomposition of organic material in the ponds due to bacteriological activities, pH levels decrease at night (Svobodova et al., 1993).

Feeds eaten and digested by fish are used to provide energy for metabolic activities, for growth and other physiological processes. Feed that cannot be digested is excreted into the water as faeces (Bregnballe, 2015). The majority of nutrients from feed residues accumulate in pond water. When the nutrients accumulates in pond water exceed the tolerable limit, harmful algal blooms and water quality deterioration may occur in aquaculture ponds (Yang et al., 2017).

Ammonia (NH_3) is an inorganic nitrogen compound that is generally abundant in aquaculture ponds and is highly toxic to fish. Ammonium (NH_4^+) is known as the ionised form of ammonia, which is not toxic to fish. The sum of unionised ammonia and ionised ammonium ions in water is expressed as total ammonia nitrogen (Bregnballe, 2015). The proportion of unionised ammonia in a given amount of total ammonia increases with increasing pH and temperature value and decreasing salinity value. As a result, the amount of ammonia in ponds can change significantly during the daytime (Boyd and Tucker, 1998). Although it does not have a toxic effect on fish, there is a risk about the ammonium that can be turn to toxic ammonia as a result of possible pH, salinity and temperature change. Nonetheless unionised ammonia is more toxic at low dissolved oxygen levels (Pillay, 2004). Ammonia accumulation in aquaculture systems is undesirable due to its toxic effect on fish. In aerobic conditions, ammonia is converted first to nitrite and then to nitrate by two separate bacterial activities (Svobodova et al., 1993; Bregnballe, 2015). Under certain conditions, nitrite accumulation can only occur when the degree of oxidation of ammonia exceeds the degree of oxidation of nitrite (Boyd and

Tucker, 1998). Nitrite rarely accumulates in aquaculture systems and is toxic to aquatic animals. Fish can take up nitrite through the gills and accumulate very high concentrations in blood. In such cases, nitrite reacts with haemoglobin and methaemoglobin form reveals, which inhibits oxygen transport and causes methaemoglobinemia or brown blood disease (Grommen et al., 2002). Nitrate is the last compound of the breakdown of organic nitrogen-containing substances under aerobic conditions. In times when water temperature is high and plants are actively growing, nitrate concentration in aquaculture ponds is usually quite low (Boyd and Tucker, 1998).

Phosphorus is a key metabolic nutrient found in small amounts in waters. Phosphorus in aquaculture systems originates from the water supply and the feeds used. At common pH values of 7-9, most phosphorus is present in the form of orthophosphate ($H_2PO_4^-$ and HPO_4^{-2}), which can be utilised by phytoplankton. Dissolved reactive phosphorus concentrations are often at low levels in waters because the available phosphorus in the water is rapidly absorbed from the water by phytoplankton. In addition, pond sediment adsorbs orthophosphate and removes it from the water (Boyd and Tucker, 1998).

Depending on the amount of nutrients containing nitrogen and phosphorus in the pond waters and water temperature, the amount of phytoplankton in the ponds increases. Phytoplankton increases the amount of dissolved oxygen and contributes to the reduction of ammonia in pond. On the other hand, most water quality problems in ponds are caused by excessive phytoplankton growth (Boyd and Tucker, 1998). Phytoplankton increases the amount of dissolved oxygen in the water by photosynthesis during the daylight and decreases the oxygen value of the environment by respiration during the night. Therefore, an increase in the amount of phytoplankton will cause a negative effect on production.

Tezel (2020) determined that ammonia and nitrite concentrations in the ponds increased especially in summer season but nitrate values in the ponds were generally below 1 mg/L. It was stated that the phosphorus value increased from time to time and this increase was caused by the sediment activity. Occasionally phytoplankton blooms occurs due to the high amount of nutrients and favourable water temperature (Tezel, 2020). During summer season, water quality parameters should be monitored very carefully due to the increase in water temperature and decrease in the amount of dissolved oxygen due to climatic conditions. During these periods when the water quality parameters deteriorate, the pedall wheel aerator use and water change should be increased and the amount of feeding should be reduced. The interactions between pond sediment and the water column have a significant effect on water quality changes in ponds. The removal of organic material accumulated on the pond bottom positively affects the improvement of water quality in ponds (Tezel, 2020).

FISH HARVEST AND MARKETING

The farmed fish are harvested in different sizes depending on the market demand. Generally, fish between 400 g and 1 kg are harvested from the enterprises. In some enterprises, especially sea bass species are farmed up to 2-3 kg according to market demands.

The high visual quality of the fish produced in the earthen pond enterprises has a significant positive effect on consumer preference. Consumer demand for fish produced in earthen ponds is quite high. However, there is no effective marketing network that allows direct sales of fish produced in earthen ponds to the consumer. Produced fish are generally sold by intermediary sailers in wholesale market hall. Since most of the producers have small-scale production and cannot market their products to consumer, many producers sell the fish they produce below their value or do not collect the money for the fish they sell. For this reason, small producers who produce fish by using very limited financial resources stop their production.

MAINTENANCE OF PONDS FOR NEW PRODUCTION PERIOD

In earthen pond enterprises, the water of the production ponds is usually drained at the end of each production period and the sediment accumulated on the bottom of the pond is discharged (Figure 10). In some enterprises, this pond care is done after two production periods. The bottom sediment of the ponds removed via using excavators. Some of removed sediment is used for repair the edges of the ponds and some of it is laid on the embankments between the ponds. The ponds are not used for a certain period of time in order to dry the bottom of the ponds. Due to the high level of groundwater in the region where production is carried out, it is not possible for the pond bottom to dry completely in some enterprises. Macrophytes growing on the edges of the ponds are also uproot. If the ponds remain empty for a long time, the water of other ponds, which are excavated next to them and continue to be used in production, may flow into the empty pond by overcoming the embankments. For this reason, the maintained ponds are filled with water again and kept until the new production period.

Figure 10. Earthen Pond after Production Period

Laying the sediment removed from the bottom of the ponds on the embankments contains significant risks. The organic material in this sediment can be transported back into the pond as a result of a precipitation or wind. Especially in the first rains after the bottom cleaning, the amount of nitrogenous compounds in the ponds may increase significantly. For this reason, it would be more appropriate not to lay this sediment removed from the pond bottom on the embankments of the ponds.

CONCLUSION

The importance of aquaculture is increasing day by day. Many countries in the world are making investments in this field and the aquaculture sector tends to grow continuously. The contribution of small-scale farmers to global production is demonstrates that the targeted production increase should be realised with low-cost technologies (Bosma and Verdegem, 2011). There are two main problems to be solved in earthen pond enterprises. The first one is the problems related to the production processes and the other one is the problems related to the marketing of the fish produced. Increasing the number of studies that will improve the production processes in earthen pond enterprises and adapting cost-effective production techniques will enable a succesfull management of production processes. This will increase the production efficiency of earthen pond enterprises. The high visual quality of fish produced in earthen ponds has a significant positive effect on consumer preference. Establishing an effective marketing network that allows direct sale of fish produced in earthen ponds to the consumer will increase the profitability of the enterprises. New steps to be taken for the improving the production processes and marketing the fish produced will positively impact the sustainability of these enterprises.

REFERENCES

Avnimelech, Y., Kochva, M. & Hargreaves, J.A. (1999). Sedimentation and resuspension in earthen fish ponds. *Journal Of The World Aquaculture Society*, 30(4): 401-409.

Banas, D., Masson, G., Leglize, L., Usseglio-Polatera, P. & Boyd C.E. (2008). Assessment of sediment concentration and nutrient loads in effluents drained from extensively managed fishponds in France. *Environmental Pollution*, 152: 679-685.

Bosma, R.H. & Verdegem M.C.J. (2011). Sustainable aquaculture in ponds: Principles, practices and limits. *Livestock Science*, 139 (1-2): 58-68.

Boyd, C.E. & Tucker, C.S. (1998). Pond aquaculture water quality management. Springer Science+Business Media New York, 700 s. doi/10.1007/978-1-4615-5407-3.

Boyd, C.E. (1995). Bottomsoils, sediment and pond aquaculture. Chapman and Hall, New York City, 348 s.

Brambilla, F., Lalumera, G., Terova, G., Crosa, G. & Saroglia, M. (2007). Inflow and outflow water quality control in aquaculture systems: a case study. *Aquaculture Research*, 38: 1654–1663.

Bregnballe, J. (2015). A guide to recirculation aquaculture. Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation, 95 s.

Burford, M.A. & Lorenzen, K. (2004). Modeling nitrogen dynamics in intensive shrimp ponds: the role of sediment remineralization. *Aquaculture*, 229:129–145.

Ebeling, J.M., Timmons, M.B. & Bisogni, J.J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*, 257: 346-358.

Egemen, Ö. (2005). Water Quality. Ege University Publications, Faculty of Fisheries Publication No: 14, V. Edition, 150 p. (in Turkish)

Farrelly, J.C., Chen, Y. & Shrestha, S. (2015). Occurrences of growth related target dissolved oxygen and ammoniain different catfish pond production systems in southeast Arkansas. *Aquacultural Engineering*, 64: 68-77.

GDFA. (2024). General Directorate Of Fisheries And Aquaculture, Fisheries Statistics Report.

Grommen, R., Van Hauteghem, I, Van Wambeke, M. & Verstraete, W. (2002). An improved nitrifying enrichment to remove ammonium and nitrite from freshwater aquaria systems. *Aquaculture* 211(2002): 115-124.

Guillen, J., Asche, F., Carvalho, N., Polanco, J.M.F., Llorente, I., Nielsen, R., Nielsen, M. & Villasante, S. (2019). Aquaculture subsidies in the European Union: Evolution, impact and future potential for growth, *Marine Policy*, 104: 19-28.

Liu, W., Liu, S., Hassan, S.G., Cao, Y., Xu, L., Feng, D., Cao, L., Chen, W., Chen, Y., Guo, J., Liu, T. & Zhang, H. (2023). A Novel Hybrid Model to Predict Dissolved Oxygen for Efficient Water Quality in Intensive Aquaculture. *IEEE Access* 11: 29162-29174. DOI:10.1109/ACCESS.2023.3260089.

MPDAF. (2022). Official records of Muğla Provincial Directorate of Agriculture and Forestry.

Pillay, T.V.R. (2004). Aquaculture and the environment, Fishing News Books, Blackwell Publishing Ltd, Oxford, Second Ed., UK, 196 s.

SBD. (2014). The tenth development plan declared by The Strategy and Budget Department of The Presidency of the Republic of Türkiye (2014-2018), Ankara, 80 s.

SBD. (2023). The twelfth development plan declared by The Strategy and Budget Department of The Presidency of the Republic of Türkiye (2024-2028). 241 s.

Serpa, D., Falcao, M., Pousao-Ferreira, P., Vicente, M. & Carvalho, S. (2007). Geochemical changes in white seabream (Diplodus sargus) earth ponds during a production cycle. *Aquaculture Research*, 38: 1619-1626.

Serpa, D., Pousão-Ferreira, P., Caetano M., Fonseca, L.C.D., Dinis, M.T. & Duarte, P. (2013). A coupled biogeochemical-dynamic energy budget model as a tool for managing fish production ponds. *Science of the Total Environment*, 463-464: 861-874.

Svobodova, Z., Lloyd, R., Machova, J. & Vykusova, B. (1993). Water quality and fish health. EIFAC Technical Paper No: 54, FAO, Rome, 59 p.

Tezel, R. & Güllü, K. (2017) A Study on Providing Sustainability of Earthen Pond Aquaculture Facilities Producing Marine Fish. *Journal of Aquaculture Engineering and Fisheries Research*, 3(3): 128-140.

Tezel, R. (2020) A Research on Improving the Pond Systems of Earthen Pond Aquaculture Enterprises Produces Marine Fish. Muğla Sıtkı Koçman University, Graduate School of Natural and Applied Sciences, Ph.D. Thesis, 146 p.

TURKSTAT. (2024). Turkish Aquaculture Production Statistics. Turkish Statistical Institute Statistics, Website: https://biruni.tuik.gov.tr/medas/?locale=tr (Erişim Tarihi: 19.09.2024).

Xinglong, J. & Boyd, C.E. (2006). Relationship between organic carbon concentration and potential pond bottom soil respiration. *Aquacultural Engineering*, 35: 147-151.

Yang, P., Lai, D.Y.F., Jin, B., Bastviken, D., Tan, L. & Tong C. (2017). Dynamics of dissolved nutrients in the aquaculture shrimp ponds of the Min River estuary, China: Concentrations, fluxes and environmental loads. *Science of the Total Environment*, 603-604: 256-267.

CHAPTER 8

AQUAPONIC SYSTEMS

Prof. Dr. Gürel TÜRKMEN[1]

[1] Ege University, Faculty of Fisheries, Aquaculture Department, Bornova-İzmir, Türkiye, gturkmen35@gmail.com, Orcid ID 0000-0002-2683-6258

INTRODUCTION

According to United Nations estimates, the world population will reach 9,1 billion in 2050, requiring a quarter more food than today. Globally, although there are still sufficient land resources to feed the world's future population, making more efficient use of limited natural resources and adapting to climate change are expected to be the main challenges facing world agriculture in the coming decades. Today, most of the available land can grow only a few crops, while most of the unused land has chemical, physical, endemic disease and lack of infrastructure problems. Soil, land and water are critical inputs in food production and must be managed sustainably. The United Nations recommends the implementation of sustainable practices for sensible water use through improved irrigation and storage technology in drought mitigation and food production (FAO, 2022a; 2022b; United Nations, 2022). In 2020, agriculture utilized almost 50% of the world's vegetated land. The conversion of forested areas to agricultural land on an ever-increasing scale is thought to further increase atmospheric CO_2 levels, which are driving global warming. Currently, 90% of the water used by human beings in the world is currently used in agricultural production. This growth and resource consumption is not sustainable. Alternative ways to increase food production are needed (Hager et al., 2021). Today, aquaponic systems are one of the most important sustainable practices in food production, requiring less land and water and having a low environmental impact (Gayam, et al., 2022).

Aquaculture is an economically, socially and environmentally important sector, especially in terms of providing products for direct human consumption and raw materials for different industries. According to the Food and Agriculture Organisation, more than 14% of the animal protein consumed in the world comes from aquaculture. Aquaculture has an annual growth rate of over 10% and is the fastest growing industry in the animal protein foods sector. Considering the rapid increase in the world population, it is considered as an important resource provider in terms of meeting the protein deficit in the future. While demand and offer dynamics form the fish products market, variable fishery resources, climate and environmental conditions directly affect the market. Modernised sustainable aquaculture systems such as aquaponics can be a solution to these fluctuations (Oniga et al., 2018).

The term 'aquaponics' is a combination of two words: aquaculture and hydroponics. Aquaculture is the science of fish farming, while hydroponics is the science of growing plants in a soilless environment or nutrient solution. Aquaponics is therefore a combination of these two food production systems (Masabni and Sink, 2020).

Most of the world's aquaculture production takes place in open-cage pens, earthen ponds or canals; these systems are either static or flow-through. Fish in these systems produce nitrogenous and mineral wastes that require extensive filtration. If these wastes are not converted or disposed of, they result in nutrient pollution and subsequent eutrophication. In a hydroponic system, inorganic fertilisers are used as the nutrient source for the plants, requiring regular flushing of the system to replenish the fertiliser solution or remove excess salt build-up. In contrast, aquaponic systems take dissolved nutrients from uneaten fish feed and faeces and, using microbes capable of degrading organic matter, convert nitrogen and phosphorus into bioavailable forms for use by plants in the hydroponic system. Thus, aquaponics is a system that achieves near-zero discharge from both fish and plant production streams, as well as significant reductions in both environmentally harmful discharges from aquaculture sites. The plants act as a water filtration system by absorbing nitrogenous and mineral products, which improves water quality for fish. Nitrifying bacteria convert fish waste products into usable nutrients for plants, and plants in turn filter nutrients from the water to benefit the fish. In other words, aquaponics is the combination of both intensive aquaculture and hydroponic production systems in a recirculating water system (Masabni and Sink, 2020). In this regard, aquaponics is a production technology that can use significantly less land and less water to produce both high-quality fish protein and crops (vegetables and fruit), while minimising the chemical and fertiliser impacts and inputs used in conventional food agriculture (Goddek et al., 2020).

Aquaponic systems, the integration of fish culture and plant production, can provide several opportunities for farmers or producers, such as sustainable agriculture, versatile marketting and the creation of multiple income streams. Environmentally efficient use of water and energy is one of the main advantages of aquaponic systems. Other advantages of aquaponic systems include land use, the ability to intensify crop production throughout the year, and common basic concepts such as use in geographical areas unsuitable for conventional agriculture. Aquaponics does not use fertilizer chemicals, pesticides or weed killers to avoid any residues. Market features such as product forecasting, production for specialized markets, seasonal price advantage, organic products and local fresh foods provide market advantages to producers and farmers in areas close to big cities. In aquaponic production, there are advantages in vertical production and labor force, the ability to make product changes according to market situation and demand, and product transportation. Despite the advantages mentioned above, there are economic challenges such as high initial costs, heating/cooling costs, shortage of qualified personnel, price and market balance, and technical challenges such as management of two different systems, optimizing water quality, daily check and maintenance and mechanization difficulties (Table 1) (Allysha, 2021; Hager et al.,2021).

Table 1: Advantages and Challenges of the Acuponic System (Hager et al., 2021).

Environmental Advantages	Production Advantages	Market Advantages	Economic Challenges	Technical Challenges
85% less water usage	Vertical production and workforce	Year-round production	Initially high cost	Management of two different systems
Zero wastewater discharge	Reduced energy costs	Product estimation	Plant and fish stocking costs	Optimising water quality
Optimum water quality	Modifiability	Production for special markets	Heating / cooling cost	Nutrient balance
75% less energy usage	Advantage in product transport	Price advantage in season	Choosing the right plants and fish	Diseases and medicines
Pesticide-free production	Controlled production	Organic products	Shortage of qualified staff	Electricity outage
Soilles agriculture	Advantage for diseases	Residue-free production	60% comes from plants	Harvesting and quality control
Reduced methane emissions	Better feed conversion rate	Local fresh foods	Price and market balance	Live product transfer
Recycling phosphorus	8 times higher plant production			Daily check and maintenance
	Production on unfavourable soil			Mechanisation difficulties

2. HISTORY OF AQUAPONICS

Aquaponics is a system of agriculture that combines raising aquatic animals (aquaculture) with hydroponics (growing plants in water). The word "aquaponic" is a combination of the Latin words "aqua," which means water, and "ponicos," meaning farming method. Aquaponics is a term that has been 'coined' in the 1970s, but in practice has ancient roots although there are still discussions about its first occurrence. The Aztecs settled on the marshy shores of Lake Texoco, where they built the capital of their empire, Tenochtitlán (now Mexico City). As the swamp and the surrounding rugged soil did not allow the development of sufficient agricultural cultivation to feed the population, the Aztecs invented 'Chinampas', artificial floating gardens (1150-1350 BC at the earliest) made of reeds and covered with mud taken from the bottom of the lake. The plants growing on these islands took up the lake water, rich in nutrients produced by aquatic organisms, through the soil and the roots crossing the reeds, and thrived and produced crops (Figure 1). Since the 1970s, some fish farmers have started to use aquatic and terrestrial plants that utilise nutrients from the water for their growth because they are effective in water purification (Acquacoltura Italia, 2024; Espina and Matulic, 2020).

Figure 1. Primitive Ecological Aquaponic System Used by The Aztecs (Acquacoltura Italia, 2024).

An even older example of aquaponics is believed to have spread in South East Asia, where the Chinese lived. Settlers from Yunnan farmed rice together with fish in paddy fields around 5 AD. The existence and practice of such polyculture cultivation systems dates back to ancient times in many Far Eastern countries such as South China, Thailand and Indonesia. In this system, oriental loach (*Misgurnus anguillicaudatus*), marsh eel (fam. Synbranchidae), common carp (*Cyprinus carpio*) and pond carp (*Carassius carassius*) are reared. The ancient Chinese also used an integrated aquaculture system in which fish, catfish, ducks and plants coexisted in a symbiotic relationship. Although these systems are not really aquaponic systems, they can be recognised as early examples of integrated aquaculture systems.

Aquaponics in the modern context is the result of fish farmers' efforts to reduce their dependence on soil, water and other resources while exploring methods of fish farming. Over the last 50 years, great advances have been made in Recirculating Aquaculture Systems (RAS), making it the most important component of aquaponic aquaculture (Bradley, 2014). In the twentieth century, the first aquaponic attempts to create a practical, efficient and economical system started in the 1970s with the work of Lewis and Naegel (Lewis and Wehr, 1976; Naegel, 1977; Lewis et al., 1978). Other first systems were designed by Waten and Busch in 1984 and Rakocy in 1989 (Espina and Matulic, 2020). Initially, the systems implemented focused on small-scale, fish-centred coupled aquaponics (CAP, aquaculture and hydroponics units are arranged in a single loop and water flows continuously from the fish tanks to the plant unit and back). Rakocy pioneered medium-scale coupled aquaponics systems in the 1980s (Goddek, et al., 2020). Since then, aquaponic system design has been investigated by many institutes in many countries worldwide in terms of plant-fish combination and hydroponic system type. With his research in 2015, Kloas introduced a more representative system for commercial aquaponics using a decoupled aquaponics technique (Kloas, et al., 2015). In this method, the water cycle is independent in each subsystem, resulting in an environmentally friendly cultivation process and mechanisation that allows for optimum fish and plant yields. Aquaponics is currently in the process of developing into an intensive food production system (Rahman, 2010; Aslannidou et al., 2023).

3. SYSTEM DESCRIPTION AND STURUCTURE

3.1. Types of Aquaponic Systems

In general, there are two main types of aquaponic systems, coupled and decoupled. Coupled aquaponic systems share the water resource in an integrated way between the two main components (fish and plants) and the feed given to the fish is expected to provide the full nutritional requirements for the plants being grown. In coupled aquaponic systems, each time water passes through the plant culture unit, nutrients such as nitrate are taken up by the plants and fish feed additions are required to meet new plant needs. Consistency of incoming feed, solid and biological filtration and plant nutrient uptake are critical for continuous and predictable production of market-oriented products. Variability in feeding time, feed quantity, fish and plant size, temperature, pH and water chemistry affect the rate of nutrient uptake (Pattillo, 2017).

Aquaponics was first designed as a “fish-centric” system with vegetable crops as a secondary commodity used for the biofiltration of aquaculture effluents. This option minimizes the phenomenon of eutrophication and the water scarcity status caused by the spread of Recirculating Aquaculture Systems (RAS). Later, the greater profit potential of herbs and vegetables in comparison to fish and the development of decoupled techniques gave a boost to “plant-centric” aquaponic systems. Decoupled aquaponics systems provide separation between the fish and plant portions of the overall aquaponics cycle, providing contingencies for treatment of disease or catastrophic loss in one portion of the system without complete failure of the overall operation. The plant-centric aquaponics reduces the demand for chemical fertilizers and contributes to natural resource protection (Aslannidou et al., 2023). The coupled system recycles water from the hydroponic subsystem immediately back into the aquaculture subsystem, while the segregated system breaks this cycle and allows water to exit the system. Compared to the coupled system, the segregated system requires more water, but provides better control over water quality, resulting in increased vegetable yields (Gibbons, 2020). Moreover, the size of the plants, the intended target and the feasible location also provide reference for classification (Hao et al., 2020).

3.1.1. Coupled Aquaponic Systems

Coupled aquaponics is the archetypal form of aquaponics. Technical complexity increases with the scale of production and the required water treatment. They can be scaled up by gradual fish production, parallel cultivation of different plants and the combination of various hydroponic subsystems. The coupled aquaponics principle combines three classes of organisms: (1) aquatic organisms, (2) bacteria and (3) plants that benefit from each other in a closed recirculated water body. The water serves as a medium of nutrient transport, mainly from dissolved fish waste, which is converted into nutrients for plant growth by bacteria. These bacteria (e.g. *Nitrosomonas* spec., *Nitrobacter* spec.) oxidize ammonium to nitrite and finally to nitrate. Therefore, it is necessary for the bacteria to receive substantial amounts of ammonium and nitrite to stabilize colony growth and the quantity of nitrate production. The main function of coupled aquaponics is the purification of aquaculture process water through the integration of plants with economic benefit (Palm et al., 2020). Coupled aquaponics systems typically have a unidirectional flow of water starting in the fish culture unit, passing through a solids filter, biological filter, hydroponic unit, and sump tank before being pumped back to the fish, completing the cycle (Figure 2) (Pattillo, 2017).

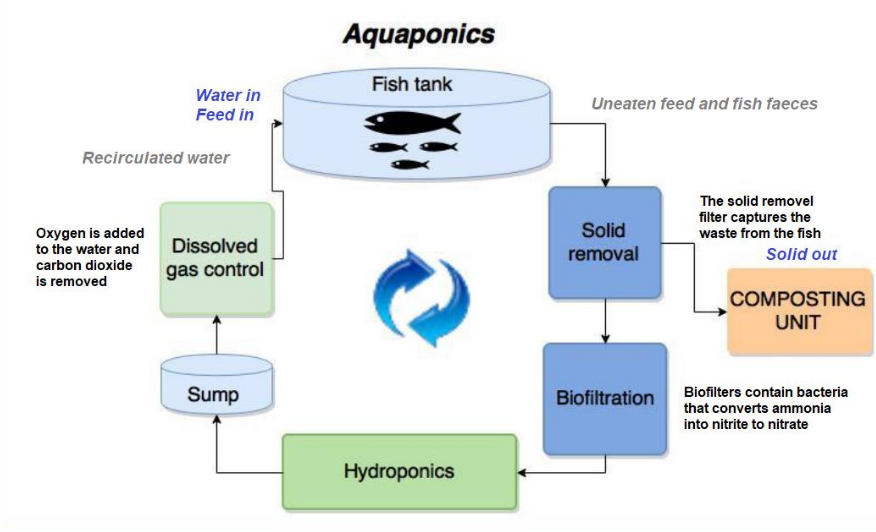

Figure 2. Coupled Aquaponic System (Thorarinsdottir, 2015).

3.1.2. Decoupled Aquaponics Systems

Decoupled aquaponic systems provide separation between the fish and plant parts of the overall aquaponic cycle (Figure 3). Decoupled aquaponic systems use water from fish but do not return the water to the fish after the plants. Through mineralization components and sludge bioreactors, they convert organic matter into bioavailable forms of essential minerals, especially phosphorus, magnesium, iron, manganese and sulfur, which are deficient in typical fish waste, and make them available to plants. In a decoupled system, pH, temperature and other water quality parameters can be adjusted independently. For example, nitrifying bacteria that convert ammonia to nitrate prefer a slightly alkaline pH of 7.0-8.5, while plants are optimized to absorb nutrients best between pH 5.0-6.5 (Pattillo, 2017; Goddek et al., 2020).

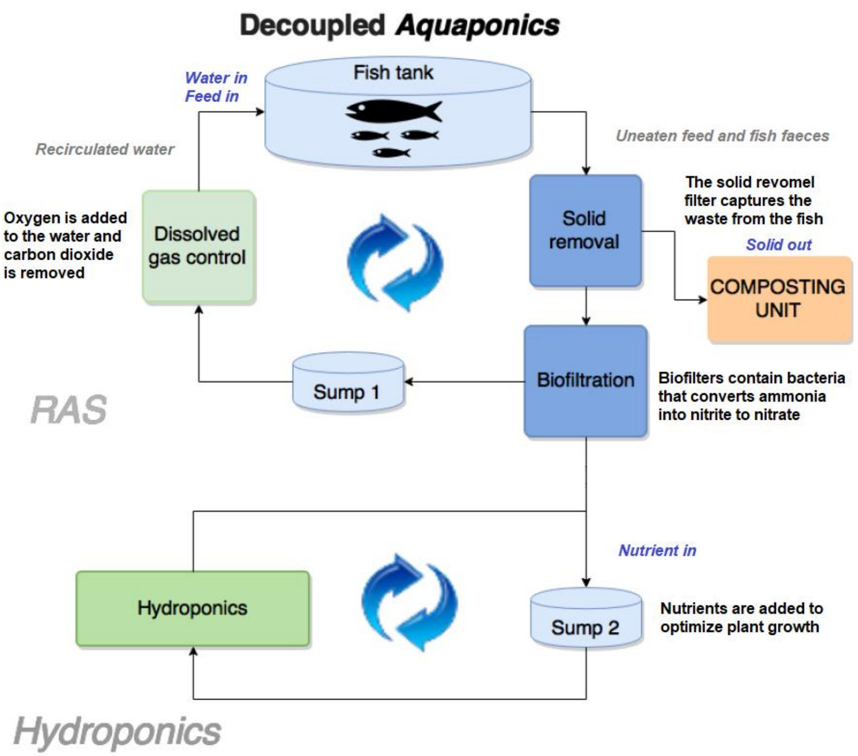

Figure 3. Decoupled Aquaponic System (Thorarinsdottir, 2015).

3.2. Recirculating Aquaculture System (RAS)

Recirculating aquaculture systems (RAS) are intensive fish production systems that facilitate the reuse of fish farming water using a series of water treatment steps. RAS usually include (1) devices to remove solid particles, (2) nitrification biofilters and and (3) a number of gas exchange devices to remove dissolved carbon dioxide and adding oxygen required by the fish and nitrifying bacteria (Figure 4) (Espinal and Matulic, 2020).

To maintain quality in aquaculture, water must be filtered to remove solids, ammonia and CO2. Dissolved oxygen levels, pH and temperature must also be maintained at safe levels at all times. RAS technology has been developed in recent years, especially in relation to sludge treatment and biofiltration. The development of RAS technology, together with stricter environmental requirements and the need to increase profitability, has led to increased interest in integrated multitrophic production methods such as aquaponics, which turns RAS water treatment costs for biofiltration into profit by growing plants that take up nutrients and help control ammonia in fish tanks (Dalsgaard et al., 2012; Thorarinsdottir, 2015).

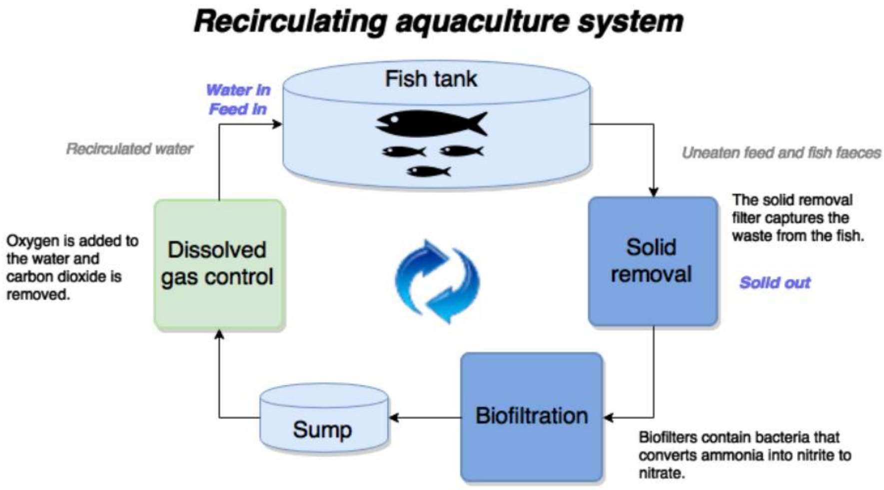

Figure 4. Schematic Overview of A Recirculating Aquaculture System (RAS) (Thorarinsdottir, 2015)

3.2.1. Mechanical Filtration

Mechanical filtration is a fundamental step in removing solids from fish feed and feces in RAS. Effluents not only increase the risk of fish disease and gill damage, but also increase ammonia in the water, reduce oxygen concentration due to higher biochemical oxygen demand (BOD), reduce biofilter efficiency by contaminating the media with heterotrophic bacteria (Thorarinsdottir, 2015). Fish cannot fully utilize the feed; about 25-50 percent of the feed becomes waste. This waste needs to be removed quickly from the system. These waste solids range in size from less than 10 microns (dissolved solids), 30-100 microns (suspended solids) and more than 100 microns (settleable solids). To remove these solids, filters have been developed that both remove the actual solids and increase the operational efficiency of the RAS by performing the services of a biofilter (Table 2) (Pattillo, 2017).

Table 2. Solids Filtration Systems Used in Recirculating Aquaculture Systems.

Type	Quality Grade	Pros	Cons	Cost
Suspended solids filtration				
Bead/sand filter	Moderate/ High	Excellent filtration 2 x with biofiltr.	Frequent back-flushing/maintenance	Moderate/ High
Filter pads	Low/ Moderate	Inexpensive	Frequent maintenan. Moderate effective	Low
Microscreen rotating drum filters	High	Large water volume Very effective Self-cleaning	Expensive	Very High
Radial flow settlers	Moderate/ High	Simple design Very effective	Availability	Moderate
Swirl separators	Moderate	Simple design	Moderate effective	Moderate
Quiescent zone settlers	Low/ Moderate	Simple design	Space requirements Cleaning required	
Dissolved solids filtration				
Foam fractionators/ Protein skimmers	Moderate/ High	Relatively simple design Low maintenance	Expensive Not very effective İn fresh water	Moderate/ High
Cartridge filters	Moderate	Simple	Maintenance	Moderate
Ozonation	High	Extremly. effective Oxygenates water Kills pathogens	Potential bio. hazard Corrosive Expensive	High

3.2.2 Biofiltration

Biofilters are a prominent feature in recirculating aquaculture and in aquaponic systems. Due to the fact that the fish waste is dissolved directly in the water and the size of these particles is too small to be mechanically removed, most fish waste is not filterable using a mechanical filter. The water is treated by converting dissolved ammonia, a toxic metabolite excreted by fish, into harmless nitrate. Because ammonia and nitrite are toxic even at low concentrations but the plants need the nitrates to grow, biofiltration is essential in aquaponics. This conversion, operated by beneficial bacteria, is the main reason for the huge water savings achieved by RAS, since large amounts of water exchange are required to keep ammonia concentrations, which are toxic to fish, below limits. A healthy and matured biofilter is crucial for a stable and well working RAS (Timmons and Ebeling, 2010; Somerville et al., 2014; Thorarinsdottir, 2015; Oniga et al., 2018).

The biofilter consists of a substrate where beneficial bacteria (e.g. *Nitrosomonas* spp., *Nitrobacter* spp. and others) can grow in high densities with high exposure to water and air for the conversion of toxic ammonia and nitrite to nitrate. This biological breakdown process is limited by surface area for bacterial attachment, temperature, dissolved oxygen, alkalinity, pH, availability of ammonia and nitrite, and other factors. In recirculating aquaculture system, there are many choices for biofilter design and substrate material with different advantages and disadvantages, some mechanical filters also functioning as the biofilter (Table 3)(Pattilo, 2017).

Table 3. Types of Biological Filtration Used in Recirculating Aquaculture Systems

Type	Quality Grade	Pros	Cons	Cost
Bead/Sand filters	Moderate/ High	Operational efficiency with solids filtration	Frequent backwashing	Moderate/ High
Fluidized bed biofilter	High	Extremely effective	Can go anoxic if aeration or water flow fails	Moderate/ High
Trickling bed biofilter	Moderate	Effective simple	Can go anoxic if aeration or water flow fails	Moderate
Rotating contact biofilter	Moderate/ Low	Effective	More likely to break down	Moderate

Besides the media filters commonly used in most aquaponic systems, there are also specially designed biofilter media (Table 4) (Estim et al., 2023).

Table 4. Types of Biofilter Media (Estim et al., 2023).

Biofilter media	**Features and performance** (Fickerton, 2018; Wahap et al., 2010)
Fluval BioMax BioRings	Porous rings of silica and aluminum oxide for bacterial activity. Allows easy water flow.
Fluval Bio Foam	Dense, porous foam structure. Effective capturing particulate matter. Requires frequent cleaning. Cleaning is easy but removes bacteria.
Sachem Matrix	10 mm solid pumice bimedia. External/internal macroporous surface area. Does not require replacement. Rinseable and reusable.
Eheim Substrate Pro	Inert product, fine glass particles compacted (sintered) into porous beads. Rinseable and reusable. Comparatively expensive.
CerMedia MarinePure	Porous ceramic material developed at high temperature. Numerous pores and rough surface. Water flows freely. Expensive.
Biofilter Balls	Non-porous plastic 'bio-balls'. No pores to trap particulate matter. Water flows smoothly. Helps nitrification. Inexpensive.
CrystalBio	Lightweight ceramic porous structure. Large surface area for aerobic bacteria. Slightly alkaline. Recyclable and inexpensive.
AquaMat	Ultra-fine yarn structure that can be suspended. Water flows smoothly. Rinseable and reusable. Used for a long time.

When fish are initially introduced into an aquaponic system, between four weeks or 20 to 30 days, nitrogen compounds relatively stabilize in concentration (Figure 5) (Nelson, 2008; Connolly and Trebic, 2010).

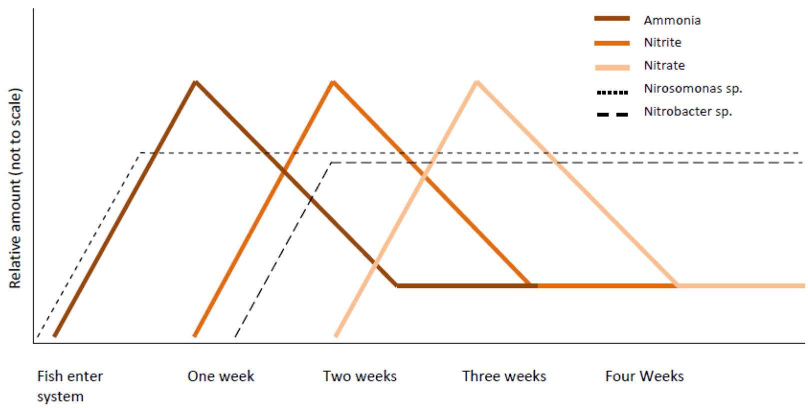

Figure 5. Nitrogen Cycling in Aquaponic Fish Tank (Connolly and Trebic, 2010).

3.3. Hydroponics

Hydroponics is a method to grow crops without soil, and as such, these systems are added to aquaculture components to create aquaponics systems. Thus, together with the recirculating aquaculture system (RAS), hydroponic production forms a key part of the aqua-agricultural system of aquaponics. Many different existing hydroponic technologies can be applied when designing aquaponics systems. This depends on the environmental and financial circumstances, the type of crop that is cultivated and the available space (Maucieri et al., 2020). Various substrates in hydroponics provide plant support and moisture retention. Within these media options, irrigation systems are integrated, providing a nutrient-rich solution to the root zones. All the necessary nutrients for plant growth are supplied by this solution. There are several designs of hydroponic systems, that might serve different purposes, but all have those mentioned basic characteristics. There are three main aquaponics techniques widely in use worldwide; Media-Based Grow Bed (MGB), Deep Water Culture System (DWC) and Nutrient Film Technique (NFT) (Thorarinsdottir, 2015).

3.3.1. Media-Based Grow Bed (MGB)

The most popular designs for small-scale aquaponics are media-based grow bed units (MGB) (Figure 6). It is the simplest aquaponic system that does not require biofilters. The media (Table 5) used to support the plants acts as both a mechanical and biofilter to capture and break down waste. The nitrifying bacteria colonizing the grow media convert fish waste (ammonia) into nitrites and then nitrates that can be used by plants as nutrition (Estim et al., 2023). This technique is best used for backyard gardeners and beginners because it does not require engineering, aquaponics or plant science background to function well. It is inexpensive and efficient on a small scale. Since the medium supports the plants like soil, plants with large root mass such as fruits, flowering plants, vegetables and root vegetables can be produced. A siphon is used to fill and drain the water in order to supply oxygen through direct contact between plant roots and air (Somerville et al., 2014; Oniga et al., 2018; Goering, 2023; Ecolife Conservation, 2024).

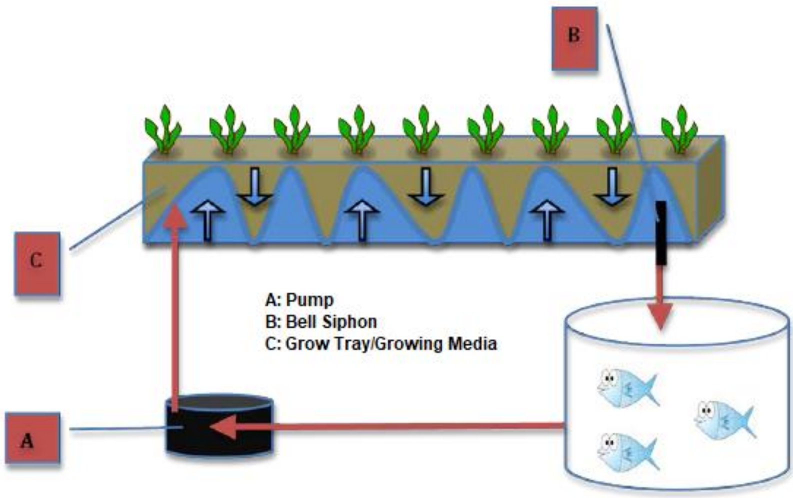

Figure 6. Media-Based Grow Bed Aquaponic System (Ecolife Conservation, 2024).

Table 5. Common Types of Media-Based Grow Bed Media (Estim et al., 2023)

Types of grow-bed media	Advantages	Disadvantages
Gravel	Easily available, long-life, easy to wash for reuse.	Heavy, lower surface area for bacterial activity, gets hot upon long exposure to sun.
Igneous rock (pumice)	Cheap, easily available, light composed of large surface area for bacterial activity. Contains air pockets, does not heat up.	Not easy to clean.
Clay pebbles or Lightweight Expanded Clay Aggregate (LECA), Hydroton	Smooth for sensitive new roots. Oxygenation and easy drainage of water. Less clogging and anaerobic area. Loose form, ease transplant and detaching plant roots.	Smooth surface, biological surface area is less than other substrates such as pumice. Reusable over long periods.
Coral rubble	Easily available and free of cost. It has an enormous structure for bacterial activity. Does not hinder water circulation.	Bioerosion due to organic matrix, $CaCO_3$ dissolution and mechanical abrasion. Requires renewal.

Substrate characteristics, system design, carrying capacity, water flow management and maintenance in Media-Based Grow Bed Systems (MGB) are detailed in Table 6.

Table 6. Rules of Thumb for Media-Based Grow Bed Systems (Hager et al., 2021).

	Rules of Thumb for Media-Based Grow Bed Aquaponic Systems
Substrate Characteristics	• Porous to increase oxygen and water retention • Provide adequate drainage • Easy to handle • Light-weight • Cost effective
System Design	• Plant beds should be at least 12 inches (30 cm) deep • Water should remain 2 inches below the surface of the media • Media displaces 60% of the volume of the plant bed • 1:1 ratio of fish tank volume to plant bed volume for simple design • 2:1 or 3:1 ratio can be achieved by addition of the sump
Carrying Capacity	• Low fish stocking density • Separate solids filtration needed for increase fish density • Feeding rate is 25-40% less than values reported for DWC
Water Flow Management	• Fish tank volume should be circulated through the plant bed/hour • Water flow
Maintenance	• Cleaning required at regular intervals to remove solids • Red worms can be added to move solids trapped in beds

3.3.2. Nutrient Film Technique (NFT)

The Nutrient Film Technique (NFT) is the most popular technique used in hydroponics and can be easily adapted for use in aquaponics (Figure 7 and 8). NFT aquaponics systems are popular in crops applications due to vertical space efficiency, low labor costs, and especially weight limitations. (Somerville et al., 2014) The main advantage of the NFT system is that plant roots are exposed to a continuous supply of water, oxygen, and nutrients. The disadvantage is that it is less buffered against power outages (Ecolife Conservation, 2024). Since plants can also be grown on a vertical plane (or shelf), they are easily accessible and harvestable. This method, the most popular in hydroponic production, is most suitable for leafy greens. This design is not suitable for large fruiting plants as their root mass can clog the channel and their weight may not be supported (Goering, 2023).

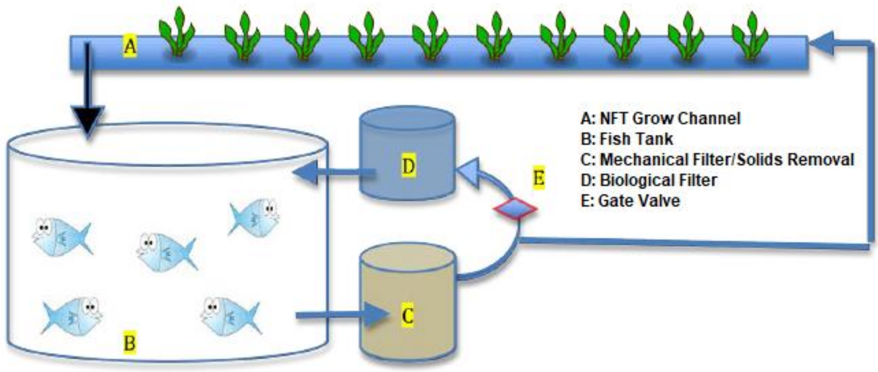

Figure 7. Nutrient Film Technique Aquaponic System (Ecolife Conservation, 2024).

Figure 8. Nutrient Film Technique Aquaponic System
COST-FA1305 Training School 6 (LEGTPA Lozere, La Canourgue, France)

In this method, a thin layer of water containing dissolved nutrients from the fish tank is pumped through a watertight trough or channel towards the bare roots of the plants. Evaporation is very low in this technique because the water is completely protected from the sun. The depth of the recirculating flow is very shallow, ensuring that plenty of oxygen reaches the plant roots. This method facilitates high yields of vegetables. However, the exposure of plant roots to more air and less water can leave them vulnerable to extreme fluctuations in heat and cold (Oniga et al., 2018; Goering, 2023; Ecolife Conservation, 2024).

Substrate characteristics, system design, carrying capacity, water flow management and maintenance in Nutrient Film Technique (NFT) are detailed in Table 7.

Table 7. Rules of Thumb for Nutrient Film Technique (Hager et al., 2021).

	Rules of Thumb for Nutrient Film Technique in Aquaponics
Substrate Characteristics	• Channels can be made from plastic, rain gutter, or PVC pipe • Use white pipes to reflect sunlight and keep cool
System Design	• Square or rounds channels are suitable • Channel diameter should be appropriate for the crop's root size • Leafy greens - 7,5 cm pipe diameter • Fruiting crops-11 cm pipe diameter • Channels should not exceed 12 m to avoid nutrient deficiencies • Slope of channel need to be 1 cm/m to ensure an adequate flow • Efficient solids filtration required as solids can clog tubes • Heavy aeration required
Carrying Capacity	• High fish stocking of 60kg/m^3 with appropriate filtration • Plant need a minimum of 21 cm between
Water Flow Management	• 1-4 hour water retention time in plant troughs • Long, narrow beds help water move through the system
Maintenance	• Channels needs to be cleaned between harvest • Back up pumps and generators are needed

3.3.3. Deep Water Culture (DWC)

Deep Water Culture (DWC) or Raft is the most commonly used technique for large-scale, commercial aquaponics (Figure 8 and 9). In this technique, plants are grown on perforated rafts, usually made of Styrofoam or similarly buoyant material, floating in specialized water tanks about 30 cm deep. The roots of the plants are usually bare and constantly in the water. This is a highly efficient method and requires good aeration and intensive filtration to keep the water clean and free of solid waste. This requires more advanced aquaculture techniques and system requirements and leads to higher upfront costs. This design is common in commercial production because it is the most stable of the three aquaponic system types. Because there is much more water in the system, severe nutrient and temperature fluctuations are much less likely to occur. It is best suited for warmer climates because although it resists daily temperature fluctuations, it is costly to heat water in colder climates (Oniga et al., 2018; Ecolife Conservation, 2024).

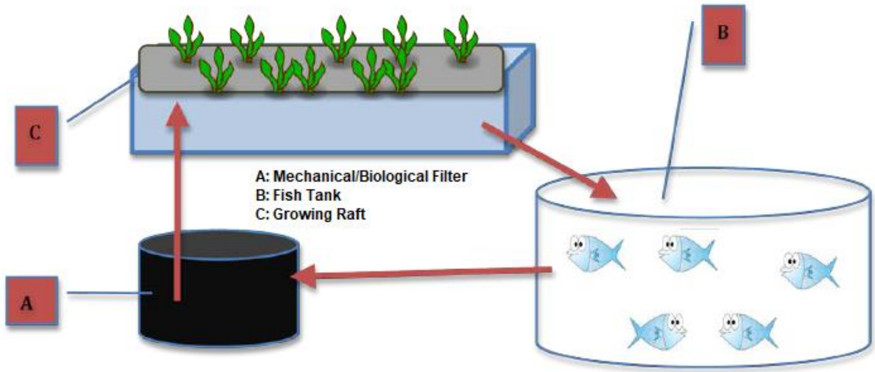

Figure 8. Deep Water Culture Aquaponic System (Ecolife Conservation, 2024).

Figure 9. Deep Water Culture Aquaponic System
COST-FA1305 Training School 6 (LEGTPA Lozere, La Canourgue, France)

Deep Water Culture is more suitable for growing larger-rooted crops in this system because it is much easier to remove plants than in media beds. For large commercial aquaponics growing a specific crop (typically basil, lettuce or salad leaves), this method is most common and the most suitable in terms of mechanization (Somerville et al., 2014; Oniga et al., 2018). Substrate characteristics, system design, carrying capacity, water flow management and maintenance in Deep Water Culture are detailed in Table 8.

Table 8. Rules of Thumb for Deep Water Culture (Hager et al., 2021).

	Rules of Thumb for Deep Water Culture in Aquaponics
Substrate Characteristics	• Rafts are commonly made from HDPE plastic/polystyrene boards • Beds should be insulated to prevent temperature fluctuations
System Design	• Beds should be a 12 inches (30 cm) deep • Width of bed may vary but typically are 120 cm wide • Prevention of solids accumulation in beds by effective filtration • Aeration is needed in fish tanks and plant troughs • Water flow rate of 20-40 L/min.
Carrying Capacity	• High fish stocking density with solids and biological filtration • Fish stocking density not to exceed 60kg/m^3 • Fish consume 1-3% of the body weight in feed per day • Feed (32% protein) input for leafy greens is 40-60g/m^2/day • Feed (32% protein) input for fruiting crops is 60-100g/m^2/day • Leafy greens stocked at 20-25 plants/m^2 • Fruiting crops stocked at 4 plants/m^2
Water Flow Management	• 1-4 hour water retention time in plant troughs • Long, narrow beds help water move through the system
Maintenance	• Removal of fine solids accumulated in the troughs • Clarifier drained daily • Fine solids capture cleaned weekly

3.4. Brackish/Saltwater Aquaponics

There is a growing interest in the use of brackish and saline water resources for agriculture, aquaculture and for aquaponics, as the demand for freshwater is constantly increasing worldwide. The use of brackish water is important as many countries, such as Israel, have underground brackish water resources. The first published research on the use of brackish water in aquaponics was conducted in 2008-2009 in the Negev Desert in Israel (Kotzen and Appelbaum 2010). In the study carried out by Kotzen and Appelbaum (2010) and Appelbaum and Kotzen (2016), they grew Tilapia sp (red x blue). (*Oreochromis niloticus* x *O. aureus*) and various plants and vegetables in brackish water in a deep-water aquaponic system (Palm et al., 2020). Brackish aquaponics using Mediterranean fish and plants provides an alternative opportunity for a combined production of high-quality food products with high commercial and nutritional value. The study by Vlahos et al. (2019) is the first study to investigate the effect of two different salinities (8 and 20 ppt) on growth and survival of *Sparus aurata* and *Crithmum maritimum* in aquaponics. The results suggest that the combined culture of euryhaline fish and halophytes provides good quality products in brackish water aquaponic systems.

Saltwater aquaponics has higher water use efficiency than freshwater aquaponics. Water usege in a marine recirculating aquaculture system (RAS) can be as low as 16 L per kg of seafood produced, while usage in freshwater RAS, is about 50 L per kg of production (Klinger and Naylor, 2012). While marine aquaponics is promising for future food production, it is still at an early stage of development. Finding the optimum salinity and suitable species combination for the development of marine aquaponics is the most important challenge (Chu, 2021). The species combinations in brackish and saltwater that have been evaluated to date are in Table 9 (Brown, 2023).

Table 9: Species Combinations Evaluated for Marine Aquaponics (Brown, 2023).

Animal	Plant	Salinity
Tilapia (*Oreochromis niloticus* x *O. aureus*)	Broccoli (*Brassica oleracea*), kohlrabi (*Brassica oleracea var. gongylodes*), beets (*Beta vulgaris*), Swiss chard (*B. vulgaris*), ruby chard (*B. vulgaris*) basil (*Ocimum basilicum*), celery (*Apium graveolens*), chives (*Apium graveolens*), lettuce (*Lactuca sativa*), and mint (*Mentha spp*.)	2.4
European sea bass (*Dicentrarchus labrax*)	Sea aster (*Tripolium pannonicum*), minutina (*Plantago coronopus*), and samphire (*Salicornia dolichostachya*)	16
Platy (*Xiphophorus sp*.)	Sea purslane (*Sesuvium portulacastrum*) and saltwort or okahijiki (*Salsola komarovii*)	13-17
Pacific white shrimp (*Litopenaeus vannamei*)	Red orache (*Atriplex hortensis*), okahijiki (*S. komarovii*), and minutina (*P.coronopus*)	10,15, 20
Sea bream (*Sparus aurata*)	Rock samphire (*Crithmum maritimum*)	8, 14, 20

4. SYSTEM CONTROL AND OPTIMIZATION

Aquaponic systems need to be balanced. Fish (and therefore fish food) must provide enough nutrients for the plants. The plants need to filter the water for the fish and reduce nitrogenous compounds harmful to the fish to acceptable levels. The biofilter needs to be large enough to process all the fish waste and there needs to be enough water volume to circulate this system. Achieving this balance in a new system takes time and the balance achieved can be maintained with constant control and optimization (FAO, 2015) .

Maintaining good water quality within aquaponic systems is fundamental to the well-being, sustainability and success of the system. Water quality is a broad term encompassing anything that adversely affects the conditions required for maintaining healthy fish and plants. Therefore, requirements for maintaining water quality can vary in different parts of the aquaponic systems. It is necessary to understand the water conditions required in each part of the system and how they affect other parts of the aquaponic system so that these parameters can be monitored and adjusted when necessary to maintain a well-balanced system. (Thorarinsdottir, 2015). In aquaponic systems, there are 5 different parts and functions that are interconnected and will provide the requirement for the next stage. These are (1) Fish Rearing, (2) Solid Removal, (3) Biofilter, (4) Plant Raising System and (5) Sump/Pump (Figure 10). Operational management plans and parameters of these sections vary according to the aquaponic model, fish and plant species. System parameters are checked regularly and necessary optimizations are performed.

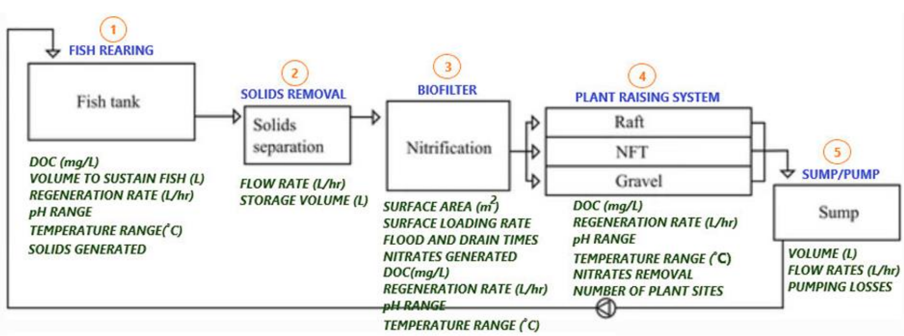

Figure 10. Parameters to Consider in Aquaponics Components (Whittering, 2020).

4.1. Controlling/Monitoring Environmental Parameters

Water is one of the most important media to understand and control in an aquaponic system. Numerous parameters are involved in the aquaponic system, but 5 water quality parameters should be followed closely and even with online monitoring. These parameters are: dissolved oxygen (DO), water acidity (pH), water temperature, nitrogen compounds (NH_3/ammonia, NO_2/nitrite, NO_3/nitrate and electrical conductivity (EC). Each parameter has an impact on the organisms in the unit, such as fish, plants and bacteria. Likewise, each organism has an ideal range of parameters for optimal growth (Thorarinsdottir, 2015).

Environmental monitoring is critical for success in aquaponic systems. The monitoring can be done manually or remotely via monitors. Electrical, temperature, water quality, humidity and safety controls are a necessary practice in risk management, especially for large facilities. In addition, while such systems can be costly for small businesses, they are labour and cost effective for large enterprises. Many automated monitoring systems provide an alarm system to warn of potential hazards during the production phase (Table 10) (Pattillo, 2017).

Table 10. System Monitors and Controls (Pattillo, 2017).

Type	Quality Grade	Pros	Cons	Cost
Hand-Held Meter	Moderate	Convenient Promotes frequent tank inspection	Requires manual use	Moderate
Fixed Position Meter	High	Convenient	May discourage worker attendance	Moderate/ High
Remote Sensing Meter	High	Convenient	May discourage worker attendance	High
Alarm System	Moderate/ High	Risk Management	May discourage worker attendance	Moderate/ High
Data Logging Sensor	Moderate/ High	Long-term data available	May discourage worker attendance	Moderate/ High

Daily, weekly and monthly checks and activities are very important to monitor the health of fish and plants in aquaponic systems (Table 11). In these controls, living organisms such as fish and plants as well as system components and equipment are checked. Water testing provides information about the balance of the system. High ammonia or nitrite indicates insufficient biofiltration. Low nitrate indicates too many plants or not enough fish. Increasing nitrate is desirable, but when nitrate is more than 150 mg per liter, the water needs to be changed (FAO, 2015). As the fish feed, oxygen and pH levels drop and carbon dioxide and ammonia levels rise. Intensive management of water quality is required to identify and mitigate these changes to ensure fish survival. There is a variety of testing equipment available on the market at various price ranges (Table 12) (Pattillo, 2017).

Table 11. Routine Management Practices (FAO, 2015).

Daily activities	Weekly activities	Monthly activities
Check water and air pumps	Check pH, NH_3, NO_2, NO_3	Stock new fish, if required
Check that water is flowing	Adjust the pH, as necessary	Clean out the biofilter
Check for leaks	Check the plants	Clean out all the filters
Check water temperature	Clear fish waste (fish tanks)	Clean of bottom fish tanks
Check water DO	Clear fish waste (biofilter)	Weigh a sample of fish
Feed / remove uneaten feed	Plant vegetables as require	Check fish for any disease
Adjust feeding rates	Plant harvest as require	
Check behaviour of fish	Harvest fish, if required	
Check the plants for pests	Check plant roots	
Remove any dead fish		
Remove sick plants/etc.		
Remove solids in filters		

Table 12. Water Quality Testing Options (Pattillo, 2017).

Type	Quality Grade	Pros	Cons	Cost
Manual Chemical Analysis	Moderate	Fairly accurate	Fairly expensive Time consuming	Moderate/ High
Spectrophotometry Chemical Analysis	High	Accurate Convenience available at a cost	Expensive Can be time consuming	High
Test Strips	Low	Easy, Fast Convenient Inexpensive	Inaccurate	Moderate/ Low

4.2. Temperature

In aquaponics, water temperature is one of the most important parameters for fish, plants and bacteria. Each species has a range of temperatures that it can tolerate and that are optimum for growth. Water may need to be heated and cooled to reach these temperatures. It is important to consider the energy costs associated with raising or lowering water temperatures, especially in relation to the surrounding environment (Table 13). Fish species should be matched to prevailing temperatures. Many plants, especially lettuce, produce leaves best at temperatures (15-18°C). When these temperatures reach 21-27°C in a greenhouse, the plant goes to seed and the crop is not marketable. The ideal temperature range for bacterial growth and reproduction is 17-34°C. Bacterial productivity decreases when the water temperature drops below 17°C, and productivity can decrease by 50 percent or more at temperatures below 10°C. (Somerville et al., 2014; Pattillo, 2017; Oniga et al., 2018).

Table 13. Water Temperature Control Systems Used in Recirculating Aquaculture Systems (Pattillo, 2017).

Type	Quality Grade	Pros	Cons	Cost
Water heating				
Boiler with heat exchanger	High	Selfcontained system Adaptable heat. sources	Requires external heat source	Moderate /High
In-tank heating element	Moderate /High	Effective	Local effectual (needs flow)	Moderate
Ambient air heater	Moderate	Effective Adds efficiency to other heating programs	No direct heat to water Questionable sustainability	Moderate
Solar water heater	Moderate	Renewable energy	Influenced by seasonal changes	Moderate /High
Geothermal	Moderate /Low	Requires little/no energy Unlikely to break down	Limited temp. change Expensive to install	High
Water cooling				
Inline chiller	High	Selfcontained system	Prone to freezing up	Moderate /High
In-tank chiller	Moderate	Less freezing hazard	Local effectual (needs flow) Less efficient	Moderate
Geothermal	Moderate	Requires little/no energy Unlikely to break down	Limited temp. change Expensive to install	High

4.3. Dissolved oxygen

Oxygen is the essential element for the three organisms (fish, plants and nitrifying bacteria) involved in aquaponics. This parameter of water quality has the fastest and strongest impact on aquaponics. In order to maintain high productivity levels of nitrifying bacteria, sufficient dissolved oxygen is needed in the water at all times. The optimal DO level to prevent stress to fish and plants is 5-6 mg/liter. High dissolved oxygen levels promote nitrogen processing and nutrient uptake in plant roots. Air is typically injected in small bubbles through air stones in the fish tank and hydroponic unit. Below DO concentrations of 2.0 mg/L, nitrification is reduced and below 1.7 mg/L, denitrifying bacteria can thrive and convert valuable nitrates back into unusable molecular nitrogen (Somerville et al., 2014; Pattillo, 2017; Oniga et al., 2018).

4.4. Chemical Oxygen Demand/Biochemical Oxygen Demand

The chemical oxygen demand (COD) and the biochemical oxygen demand (BOD) are measures of the amount of organic matter in the system that use up dissolved oxygen, therefore, high CODs and BODs are not desirable, especially for fish health. Both of these parameters are measured using laboratory methods and performed less frequently e.g. once, or a few times a year. COD and BOD can be kept low by maintaining effective filtering of solids in the system and regular cleaning of tanks and pipes (Thorarinsdottir, 2015).

4.5. pH

In any aquaponic system, determining the operating pH of the system is one of the most challenging variables. There are two main beneficial bacteria that determine the pH range of the aquaponic system, these are: Nitrosomanas (pH 7.2-7.8 for maximum nitrification) and Nitrobacteria (pH 7.2-8.2 for maximum nitrification). The pH tolerance range of the most commonly used fish in aquaponics is 6.0-8.5. High pH levels are harmful to fish as ammonia toxicity to fish increases when pH increases. The ideal water for aquaponics has an optimum pH range of 6-7 and is slightly acidic. A pH range of 6-7 allows bacteria to function at a higher capacity and at the same time allows plants to absorb all essential nutrients. A pH higher than 8 or lower than 5 requires urgent attention, without which the entire aquaponic ecosystem can collapse (Oniga et al., 2018; Scattini and Maj 2017; Whittering, 2020). The optimum water temperature and pH ranges for some fish, plant and bacterial species are presented in Table 14.

Table 14. Optimum Temperature and pH for Growth and Yield of Selected Organisms in Aquaponic Culture (Chapman, 2012).

Organisms	Optimum Temperature °C	Optimum pH
Tomatoes, peppers, and cucumbers	27–29 (day) / 17–22 (night)	5.5-6.5
Lettuce	18-24	5.5-6.5
Tilapia	28-32	6.0–8.5
Channel catfish	28-30	6.5–9.0
Common carp	25-30	7.0–9.0
Nitrifying bacteria	25-30	7.0–8.0

4.6. Alkalinity

Alkalinity is generally defined as the pH buffering capacity of water. In RAS, alkalinity control is important for nitrification and the nitrification process suffers as acidity increases. Low alkalinity in RAS causes pH fluctuations and disrupts nitrification biofilter processes. The addition of alkalinity is determined by the nitrification activity in the systems. It is recommended to maintain alkalinity >100 mg/L as $CaCO_3$. In intensively operated RAS and aquaponic systems, the buffering capacity is quite high, since each gram of ammonia nitrogen consumes 7.02 grams of alkalinity (as $CaCO_3$) during nitrification. The daily amount of feed, daily water changes and the presence of denitrifying activity that restores alkalinity are the main factors in this process (Thorarinsdottir, 2015; Espinal and Matulic, 2020).

4.7. Nitrogen Compounds

Nitrogen is one of the critical water parameters. Nitrogen enters the system from fish feces and thus indirectly from feed. Typical protein content in feed for tilapia is 30-32%, whereas carnivorous cold-water species require a higher protein percentage of about 50%. Proteins enable fish growth and have a significant impact on feed conversion ratio (FCR), the rest is released as solid and liquid fish waste. Liquid fish waste is mostly released in the form of ammonium (NH_3 or NH_4+, depending on the pH of the system) through the gills or the fish's urine. Solid waste released into the water in the form of fish feces or uneaten feed is converted to ammonium by microbes. At certain concentrations, nitrogenous wastes are toxic to fish, but ammonia and nitrite are about 100 times more toxic than nitrate. These depend on the species of fish used and the duration of exposure to the relevant concentration. Ammonia and nitrite levels should be close to zero or at most 0.25-1.0 mg/liter in a fully functioning aquaponic unit. It is desirable that almost all ammonia and nitrite are converted to nitrate by bacteria in the biofilter (Somerville et al., 2014; Thorarinsdottir, 2015; Oniga et al., 2018).

4.8. Electrical Conductivity

Electrical conductivity indicates the total concentration of dissolved solids (EC) in the nutrient solution used in hydroponics. EC is easy to measure and is a good guide, although it should be noted that EC may be lower in aquaponic systems compared to the numbers given in hydroponic guidelines. Increases in conductivity indicate that larger scale water changes, more aeration or other treatment measures may be needed (Thorarinsdottir, 2015).

4.9. Macro and Micronutrients

Plants obtain the substances necessary for growth and reproduction from the soil. Since hydroponics is a soilless method of cultivation, these nutrients must be provided in another way. These nutrients are supplied as fish feed and waste. Nutrients are divided into two categories as macro and micro. There are six macronutrients (more essential): Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). The range of micronutrients is much wider. Iron (Fe) is often added to aquaponic systems due to its general deficiency in these systems. Other important micronutrients (trace amounts) include copper (Cu), boron (B), manganese (Mn), molybdenum (Mo) and zinc (Zn) (Thorarinsdottir, 2015).

5. PRODUCTION MANAGEMENT

Aquaponics is a sustainable way of growing vegetables and other plants. The wastewater from aquaculture is used as nutrient solution for hydroponic plant production. Three organisms are involved in the optimal performance of aquaponic systems (Table 15). Fish and plant production must be kept stable and environmental parameters controlled, respectively, to achieve and maintain balance in the system and to secure optimal crops (Thorarinsdottir, 2015).

Table 15. Overview of the roles and factors that affect the growth of the major organisms present in aquaponic systems (Tyson et al.,2008).

Organism	Roles	Sensetive to
Plant	Cash crop, removes water and nutrients from fish tank	Water availability, nutrient concentrations, oxygenation, air/water temperature and pH
Fish	Cash crop, generates bionutrients from feed	Fish density, feed rate, ammonia, oxygenation, water temperature and pH
Nitrifying bacteria	Biofiltration of toxic ammonia to nitrate nitrogen	Water temperature and pH, substrate to grow on, light (keep dark)

Tolerable water quality parameters for fish are in the same range as for plants, except for water temperature and pH in aquaponics (Table 16). Considering the welfare of fish species in aquaponic systems, fish species with high tolerance to pH and water temperature should be preferred. In terms of both fish welfare/health and plant needs, pH and temperature are parameters that have an impact on the optimization of aquaponic production (Yıldız et al., 2017)

Table 16. General Water Quality Tolerances for Fish (warm or coldwater), Hydroponic Plants and Nitrifying Bacteria (Yıldız et al., 2017).

Organism Type	Temperature (°C)	pH	Ammonia (ppm)		Nitrite (ppm)	Nitrate (ppm)	Dissolved Oxygen (ppm)
Warmwater fishes	22-32	6-8.5	< 3		< 1	< 400	4-6
Coldwater fishes	10-18	6-8.5	< 1		< 0.1	< 400	6-8
Plants	16-30	5.5-7.5	< 30		< 1	-	> 3
Bacteria	14-34	6-8.5	< 3		< 1	-	4-8

5.1. Fish Choices/Fish Production

When choosing a specific species one should consider the organism's needs for temperature, salinity, and protein as these affect costs associated with growing, as well as their potential for incorporation into integrated systems such as aquaponics. (Pattillo, 2017). A wide variety of fish and other aquatic animals can be cultured in aquaponic systems. Freshwater, herbivorous or omnivorous fish are ideal choices for their sustainability, ease of feeding and efficient feed conversion. The most recommended fish are presented in Table 17. Other fish options to consider are: Common Carp, Pacu, Trout, Crappie, Barramundi, Silver perch, Golden perch, Yellow perch, Freshwater prawns and Crayfish (Ecolife Conservation, 2024).

Table 17. Fish Species Successfully Grown in Aquaponics (Ecolife Conservation, 2024).

Fish	Aquaponic System	Grow	Temp. (°C)	pH	Disease
Tilapia	Large scale	Very quickly	18-30	Wide pH range	Resistant
Catfish	Large scale	Very quickly	5-33	Wide pH range	Resistant
Koi	Large scale	Quickly	2-30	Wide pH range	Resistant
Goldfish	Home	Quickly	10-24	Wide pH range	Resistant
Tropical Fish	Home/Smal scale	Quickly	20-30	6-8	Noneresistant

One of the most common species in aquaponics is tilapia due to its omnivorous nature, fast reproduction and growth. This species of fish is very resistant and tolerant to a wide range of water parameters, such as wide temperature range (15-30 °C) and free ammonia (NH_3) concentration (0.2-3.0 mg/L) (Rakocy et al., 2004). The daily amount of feed for fish in aquaponics depends on the type of plant. Fruiting vegetables require about one-third more nutrients than leafy greens to support flower and fruit development (Table 18) (FAO, 2015).

Table 18. Feeding, Planting and Fish Stocking Depending on Plant Types (FAO, 2015).

Plant types	Daily fish feed	Planting density	Fish feding rate/day	Fish stocking density
Leafy Green Plants	40-50 g/m^2	20-25 plants/m^2	1-2 % body weight	10-20 kg/m^3
Fruiting Vegetables	50-80 g/m^2	4 plants/m^2	1-2 % body weight	10-20 kg/m^3

5.2. Plant Choices/Plant Production

A wide variety of plants can be grown using aquaponics. Some fruiting plants require higher nutrient requirements and therefore need to be grown in densely stocked, well established systems. It is recommended to add potassium sulphate to the water to trigger flowering. Here are a few crops and some fruit crops that have been successfully grown using aquaponics (Table 19) (Ecolife Conservation, 2024).

Table 19. A Few Plants and Some Fruitings Plants That Have grown Successfully Using Aquaponics (Ecolife Conservation, 2024).

Plants	Plants	Fruitings Plants
Leafy Lettuces	Watercress	Tomatoes
Bok Choi	Chives	Pappers
Swiss Chard	Microgreens	Cucumbers
Arugula	Tropical Plants	Beans
Basil	House Plants	Peas
Mint		Squash

Lettuce is the most widely grown crop in aquaponics, both because of its short harvest time (3-4 weeks) and the high demand in western diets. It is also a very lucrative crop as a large part of its final mass is harvestable and edible. Another reason for the success of these crops is the lack of a fruiting phase, resulting in consistent nutrient requirements and a more reliable harvest (Rocky et al., 2006). Although all types of fruiting plants have been successfully grown in aquaponic systems, they are mostly grown by hobbyists or researchers who grow them for consumption. As these plants have longer harvest times, they are more suitable for cultivation in regions with a longer growing season, such as the tropics, where they can be grown all year round. Tomatoes, peppers and cucumbers are popular fruit crops grown in aquaponic systems (Nelson, 2008).

However, aquaponic commercial plant production combined under intensive fish production is struggling to compete with normal plant production and commercial hydroponics on a large scale. Solutions need to be developed that allow optimal plant growth while at the same time providing the required water quality for fish (Palm et al., 2020).

5.3. Risk Analysis

The risk analysis showing the main risks in an integrated aquaponic production system and how they can be minimized is presented in Table 20. The results show that monitoring and control of key environmental parameters is essential to maintain a healthy and stable system. The risk contingency plan includes preventing pollution leading to fish and plant diseases, maintaining a safe system control for a balanced environment, assuring good quality products and understanding market needs (Thorarinsdottir, 2015). However, potential hazards to aquatic animal health in aquaponics must be considered. These factors include abiotic, biotic, feeding, management, welfare and diseases (Yildiz et al., 2020).

Table 20. Risk Analysis for Aquaponics (Thorarinsdottir, 2015)

Risk	Probability	Severeness	Contingency plan
Fish disease	Low	Medium	Strict management procedures (SMP), dividing production systems into units, controlling and keeping a healthy environment and cleaning tanks between stocking
Plant pest	Low	Medium	SMP, organic defences, integrated pest management
Failure of temperature control	Low	High	Online monitoring and automatic control with alarm system
Oxygen level too low	Low	High	Monitoring and control, improvement of aeration, reduction of stock
Failure of pH control	Low	High	Monitoring and control
Sodium levels too high	Low	Medium	Monitoring and control, shift to different feed sources
Failure of EC control	Low	Medium	Monitoring and control
Contamination of water	Low	Medium	Monitoring and control, safety procedures
Marketing failure	Low	High	Keeping good quality, fulfil official requirements, and inform consumers about the production processes
Extreme weather conditions, etc.	Very low	High	Initiate emergency plan minimizing losses

6. CONCLUSION AND FUTURE PERSPECTIVES

Technological advances in agriculture have enabled agricultural productivity to increase exponentially over the last century, thereby supporting significant population growth and the associated need for food. However, these advances raise serious concerns about the ability of ecosystems to sustain food production, conserve freshwater and forest resources, and help regulate climate and air quality (Foley et al. 2005).

Aquaponic systems offer a unique opportunity for year-round plant and fish production. Off-season production of leafy greens, herbs and vegetables can offer producers an important source of income, where they can benefit from much higher seasonal prices. The high quality and freshness of aquaponic produce is highly desired by chefs in metropolitan areas. As aquaponic producers are able to fill seasonal gaps with fresh produce, their ability to gain market traction and market share will increase. Furthermore, the local food movement and consumers' willingness to pay more for a superior product seems to be a major advantage for aquaponic producers (Pattillo, 2017).

In recent years, national and international aquaponics projects and training programs have been organized in many countries. These activities help the promotion and dissemination of aquaponics. An example of one of them, the Cost-FA1305 program, is presented in Figure 11. Currently, aquaponics research mainly focuses on system components, wastewater treatment, nutrient management and system production. Aquaponics research has spread from the US to Europe, research topics have expanded from internal components to external characteristics, and the identity of aquaponics has shifted from production to multiple roles. Aquaponics development has great potential, but faces management and market challenges. For management, more research is needed on the influencing factor, nutrients and microbial community in aquaponics. The identity of aquaponics in urban production and living needs to be clarified in the new conceptual framework for market recognition. The inclusion of aquaponics in the green infrastructure category can provide it with research directions, development environment and policy support, and more promotion chances and opportunities (Hao et al., 2020).

Figure 11. Group Picture of The COST-FA1305 in Lozera, France, 2017

The demand for food between now and 2050 has been estimated at 70-100% over the current food supply. Conventional food production uses 70% of global freshwater resources. Current open field crop production results in biodiversity loss. In addition, salinization of soils and freshwater is a growing problem. New food production systems that use less freshwater and produce high quality, fresh food are sorely needed. Marine aquaponics systems appear to be one of the options for our future food supply, but current research is extremely limited (Brown, 2023).

Aquaponics is a production model in which completely natural processes are applied on a commercial scale and two different healthy products are obtained with an environmentally friendly approach in an integrated system. It is not difficult to foresee that aquaponic production will become widespread in the future in today's world where the importance of food without the use of chemicals and drugs is increasing day by day.

REFERENCES

Acquacoltura Italia. 2024. Aquaponics History And Benefits. https://acquacolturaitalia.com/wpcontent/uploads/2016/10/aquaponics_history_and_benefits.pdf (18 June 2024).

Allysha, N.S. 2021. Aquaponıc Theory and Overvıew. https://www.rasaquaculture.com/post/aquaponic-theory-and-overview (03 June 2024).

Appelbaum, S., Kotzen, B. 2016. Further investigations of aquaponics using brackish water resources of the Negev desert. Ecocycles Sci J Eur Ecocycles Soc, 2(2): 26–35. https://doi.org/10.19040/ecocycles.v2i2s.53

Aslanidou, M., Elvanidi, A., Mourantian, A., Levizou, E., Mente, E., Katsoulas, N. 2023. Nutrients Use Efficiency in Coupled and Decoupled Aquaponic Systems. Horticulture, 9: 1-22. https://doi.org/10.3390/horticulturae9101077.

Bradley, K. 2014. Aquaponics: A Brief History of This Integrated Fish and Veggies System. https://www.milkwood.net/2014/01/20/aquaponics-a-brief-history/ (15 October 2024).

Brown, P.B. 2023. Marine Aquaponics: An Ocean Full of Potential for Sustainable Food Production. Examines Mar Biol Oceanogr. 6(1). EIMBO. 000627. 2023. DOI: 10.31031/EIMBO.2023.06.000627

Connolly, K., Trebic, T. 2010. Optimization of A Backyard Aquaponic Food Production System. Faculty of Agricultural and Environmental Sciences Macdonald Campus, McGill University. (14 September 2024). https://www.mcgill.ca/bioeng/files/bioeng/KeithTatjana2010.pdf

Chu, Y.T. 2021. The Development of Marine Aquaponics. Doctor of Philosophy. Department of Forestry and Natural Resources, West Lafayette, Indiana, 152p.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K. and Pedersen, P.B. 2012. Farming different species in RAS in Nordic countries: Current status and future perspectives, Aquacultural Engineering. doi:10.1016/j.aquaeng.2012.11.008

Ecolife Conservation, 2024. Introduction to Aquaponics. https://www.ecolifeconservation.org/resource/introduction-to-aquaponics/ (13 September 204).

Espinal, C.A., Matulic, D. 2020. Recirculating Aquaculture Technologies. In: Aquaponics Food Production Systems. Combined Aquaculture and Hydroponic Production Technologies for the Future (eds: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M.) https://doi.org/10.1007/978-3-030-15943-6

Estim, A., Rossita, S., Muhamad Shaleh, S.R., Saufie, S., Mustafa, S. 2023. Decarbonizing Aquatic Food Production Through Circular Bioeconomy of Aquaponic Systems. Aquaculture Studies, 23(4), AQUAST963. http://doi.org/10.4194/AQUAST963

FAO, 2015. Management of the aquaponic systems. https://openknowledge.fao.org/server/api/core/bitstreams/fa1b9972-1963-488e-bc19-0f1f134fea8f/content (23 July 2024).

FAO, 2022a. News article: 2050: a third more mouths to feedMay https://www.fao.org/news/story/en/item/35571/icode/.

FAO, 2022b. Food and agriculture projections to 2050 | Global Perspectives Studies | Food and Agriculture Organization of the United Nations, https://www.fao.org/global-perspectivesstudies/food-agriculture-projections-to-2050/en/.

Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K. 2005. Global consequences of land use. Science, 309: 570–574. https://doi.org/10.1126/science.1111772

Gayam, K.K., Jain, A., Gehlot, A., Singh, R., Akram, S.V., Anand, D., Noya, I.D. 2022. Imperative Role of Automation and Wireless Technologies in Aquaponics Farming. Hindawi Wireless Communications and Mobile Computing, https://doi.org/10.1155/2022/8290255.

Gibbons, G.M. 2020. An Economic Comparison of Two Leading Aquaponic Technologies Using Cost Benefit Analysis: The Coupled and Decoupled Systems. All Graduate Theses and Dissertations. 7823. https://digitalcommons.usu.edu/etd/7823.

Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M. 2020. Aquaponics Food Production Systems. Combined Aquaculture and Hydroponic Production Technologies for the Future. https://doi.org/10.1007/978-3-030-15943-6

Goering, C. 2023. Designs for Aquaponic Systems: Pros and Cons. Ecolife Conservation. https://www.ecolifeconservation.org/updates/types-aquaponics-systems-design/ (04 October 2024).

Hager, J., Bright, L.A., Dusci, J., Tidwell, James. 2021. Kentucky State University Aquaponics Production Manual A Practical Handbook for Growers. https://www.ksuaquaculture.org/PDFs/Aquaponics%20Handbook%202021%20Updated%20.pdf.

Hao, Y., Ding, K., Xu, Y., Tang, Y., Liu, D., Li, G. 2020. States, Trends, and Future of Aquaponics Research. Sustainability 2020, 12, 7783; doi:10.3390/su12187783.

Klinger, D., Naylor, R., 2012. Searching for solutions in aquaculture: charting a sustainable course. Annu. Rev. Environ. Resour. 37: 247–276. https://doi.org/10.1146/annurev-environ-021111-161531.

Kloas, W., Groß, R., Baganz, D., Graupner, J., Monsees, H., Schmidt, U., Rennert, B. 2015. A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. Aquac. Environ. Interact, 7: 179–192.

Lewis, W.M., Wehr, L.W. 1976. A fish-rearing system incorporating cages, water circulation, and sewage removal. Prog Fish Cult 38(2):78–81. https://doi.org/10.1577/1548-8659(1976)38[78:AFSICW]2.0.CO;2

Lewis, W.M., Yopp, J.H., Schramm, H.L., Jr, Brandenburg, A.M. 1978. Use of hydroponics to maintain quality of recirculated water in a fish culture system. Trans Am Fish Soc 107(1):92–99. https://doi.org/10.1577/1548-8659(1978)107<92:UOHTMQ>2.0.CO;2

Kotzen, B., Appelbaum, S. 2010. An investigation of aquaponics using brackish water resources in the Negev Desert. J Appl Aquac 22(4):297–320. https://doi.org/10.1080/10454438.2010.527571

Masabni, J., Sink, T. 2020. What is Aquaponics? Texas A&M AgriLife Extension.https://agrilifelearn.tamu.edu/s/product/what-is aquaponics/01t4x000004OfvjAAC. (17 July 2024)

Maucieri, C., Nicoletto, C., Os, E., Anseeuw, D., Havermaet, R.V., Junge, R. 2020. Hydroponic Technologies. In: Aquaponics Food Production Systems. Combined Aquaculture and Hydroponic Production

Technologies for the Future (eds: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M.) https://doi.org/10.1007/978-3-030-15943-6

Naegel, L.C.A. 1977. Combined production of fish and plants in recirculating water. Aquaculture 10(1):17–24. https://doi.org/10.1016/0044-8486(77)90029-1.

Nelson, R.L. 2008. Aquaponic Food Production - Raising fish and plants for food and profit. Montello, WI: Nelson and Pade, Inc.

Oniga, C.C., Jurcoane, Ş., Mocuța, D., Rahoveanu, A.T. 2018. Studies About The Fish Farming Development In Aquaponic Systems: A Review. Scientific Bulletin. Series F. Biotechnologies, 22: 237-246.

Palm, H.W., Knaus, U., Appelbaum, S., Strauch, S.M., Kotzen, B. 2020. Coupled Aquaponics Systems. In: Aquaponics Food Production Systems. Combined Aquaculture and Hydroponic Production Technologies for the Future (eds: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M.) https://doi.org/10.1007/978-3-030-15943-6

Pattillo, D.A. 2017. An Overview of Aquaponic Systems: Aquaculture Components. Technical Bulletin Series, 124. North Central Regional Aquaculture Center.

Rahman, S. 2010. Effluent Water Characterization of Intensive Tilapia Culture Units and Its Application in an Integrated Lettuce Aquaponic Production Facility. Ph.D. Thesis, Auburn University, Auburn, AL, USA.

Rakocy, J.E., Charlie Shultz, R., Bailey, D.S., Thoman, E.S. 2004. Aquaponic Production of Tilapia and Basil: Comparing a Batch and Staggered Cropping System. Acta Horticulturae 648(648):63-69.

Scattini, N., Maj, S.P. 2017. Aquaponics–A Process Control Approach. Modern Applied Science, vol. 11, no. 11. https://doi.org/10.5539/mas.v11n11p43

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., Lovatelli A. 2014. Small-scale aquaponic food production. Integrated fish and plant farming. FAO Fisheries and Aquaculture Technical Paper No. 589. Rome, FAO. 262 pp.

Tyson, R.V., Simonne, E.H., Treadwell, D.D., White, J.M., Simonne, A. 2008. Reconciling pH for ammonia biofiltration and cucumber yield in a recirculating aquaponic system with perlite biofilters. HortScience, 43: 719-724.

Thorarinsdottir, R.I. 2015. Aquaponics Guidelines. Co-founded by the Eco-innovation Initiative of the European Union. 63p.

Timmons, M.B. and Ebeling, J.M. (2010). Recirculating Aquaculture. NRAC.

United Nations, 2022. Food security and nutrition and sustainable agriculture. Sustainable development knowledge platform. https://sustainabledevelopment.un.org/opics/foodagriculture.

Vlahos, N., Levizou, E., Stathopoulou, P., Berillis, P., Antonopoulou, E., Bekiari, V., Krigas, N., Kormas, K., Mente, E. 2019. An Experimental Brackish Aquaponic System Using Juvenile Gilthead Sea Bream (*Sparus aurata*) and Rock Samphire (*Crithmum maritimum*). Sustainability, 11, 4820; doi:10.3390/su11184820.

Whittering, M. 2020. Design and Evaluation of a Recirculating Aquaponic System. ENG4111 and 4112 Research Project. University of Southern Queensland, Faculty of Health, Engineering and Sciences.

Yıldız, H.Y., Robina, L., Pirhonen, J., Mente, E., Dominguez, D., Parisi, G. 2017. Fish Welfare in Aquaponic Systems: Its Relation to Water Quality with an Emphasis on Feed and Faeces—A Review. Water, 9, 13. doi:10.3390/w9010013.

Yildiz, H.Y., Radosavljevic, V., Parisi, G., Cvetkovikj, A. 2020. Insight into Risks in Aquatic Animal Health in Aquaponics. In: Aquaponics Food Production Systems. Combined Aquaculture and Hydroponic Production Technologies for the Future (eds: Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M.) https://doi.org/10.1007/978-3-030-15943-6

CHAPTER 9

UNDERSTANDING HEAVY METAL ACCUMULATION IN FISH: IMPLICATIONS FOR HUMAN HEALTH AND AQUACULTURE SUSTAINABILITY

Assoc. Prof. Dr. Osman Sabri KESBİÇ [1]
Assoc. Prof. Dr. Ümit ACAR [2]
Assoc. Prof. Dr. Burak Evren İNANAN [3]
Prof. Francesco FAZIO [4]

[1] Department of Animal Nutrition and Nutritional Diseases in Veterinary Faculty of Kastamonu University, 37150, Kastamonu, Turkey, okesbic@kastamonu.edu.tr, Orcid ID: 0000-0002-1576-1836
[2] Department of Forestry in Bayramiç Vocational School of Çanakkale Onsekiz Mart University, 17100, Çanakkale, Turkey, umitacar@comu.edu.tr, Orcid ID: 0000-0003-2515-5490
[3] Department of Fisheries and Diseases in Faculty of Veterinary Medicine of Aksaray University, 68100 Aksaray, Turkey, burakinanan@aksaray.edu.tr, Orcid ID: 0000-0002-2888-8457
[4] Department of Veterinary Sciences, University of Messina. Viale Giovanni Palatucci, 13 -98168 Messina, Italy, ffazio@unime.it, Orcid ID: 0000-0003-3198-2580

INTRODUCTION

Fish is widely recognized as a valuable dietary source of essential nutrients, including high-quality protein, essential amino acids, micronutrients, and omega-3 fatty acids that are vital for human health. Given its nutritional benefits, fish holds a prominent position in global food security and nutrition, making it a highly sought-after and widely traded food commodity.[1] The demand for fish has been increasing and traditional capture fisheries have faced limitations in meeting this demand (Beveridge et al., 2013). Right here, aquaculture has emerged as a significant contributor to fish production to compensate for the limitations of capture fisheries (Anderson et al., 2017). By cultivating fish in controlled environments, aquaculture has the potential to reduce pressure on wild fish populations and ecosystems. It can also provide a more reliable and consistent supply of fish to meet the increasing demand. However, it is essential to ensure that aquaculture practices are carried out sustainably and responsibly to minimize environmental impacts and maintain the health and welfare of farmed fish (Belton et al., 2014; Thomas et al., 2021). Unfortunately, fish is significantly affected by heavy metal pollution resulting from anthropogenic environmental effects that threaten human health (Djedjibegovic et al., 2020).

Although some heavy metals such as copper (Cu) and Zinc (Zn) are essential trace elements for the vital activities of living organisms, high concentrations could cause toxic effects. Other heavy metals with high toxic effects are lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni) and chromium (Cr). In addition, heavy metals accumulate in the organism. This accumulation could occur in two patterns, biomagnification and bioaccumulation. Biomagnification refers to the process by which the concentration of certain substances, including heavy metals, increases progressively along the food chain. In aquatic ecosystems, for example, small organisms such as plankton may contain low levels of heavy metals. As these organisms are consumed by larger fish, the heavy metals accumulate in their tissues. When even larger predators consume these larger fish, the heavy metal concentrations further increase. This can result in the highest heavy metal concentrations in top predatory species (Rand, 2020; Des W. Connell, 2018). On the other hand, bioaccumulation is the process by which an organism accumulates and retains a substance, such as a heavy metal, at a higher concentration than that found in its surrounding environment. It occurs when the rate of intake or absorption of a substance is greater than its rate of elimination or excretion. This can lead to an increase in heavy metal concentrations in an organism's tissues over time.

1.1. Toxicological Aspects of Heavy Metals in Fish

Contamination of fish with heavy metals is indeed a significant concern for human health, and fish consumption is a common route of heavy metal exposure for humans (Zohra & Habib, 2016). Heavy metals are classed as essential or non-essential in biological systems based on their functions. Essential heavy metals are necessary for live organisms and may be required in very low amounts in the body. There is no known biological purpose for non-essential heavy metals in living organisms. Mn, Fe, Cu, and Zn are examples of necessary heavy metals (Lelièvre et al., 2020) although Cd, Pb, and Hg are hazardous and considered physiologically nonessential (Sabir et al., 2019; Fu and Xi, 2020). Micronutrients or trace elements for plants include the heavy metals Mn, Fe, Co, Ni, Cu, Zn, and Mo. They are required for growth, stress tolerance, and the manufacture and function of many biomolecules like carbohydrates, chlorophyll, nucleic acids, growth chemicals, and secondary metabolites (Sytar et al., 2021). Diseases or abnormal circumstances result from either a lack or an excess of an important heavy metal. However, the lists of required heavy metals for various categories of creatures, such as plants, animals, and microbes, may differ. It indicates that heavy metal may be vital for one group of organisms but not for another. Heavy metal interactions with many organism groups are quite complicated (Olawale, 2019). Different heavy metals have varying toxicological profiles and can affect various organs and systems in the human body. For example, mercury primarily targets the nervous system. At the same time, lead affects multiple systems, including the central nervous, kidneys, and cardiovascular system (Akiyama et al., 2022). Understanding the specific health effects of each heavy metal is crucial for accurate risk assessment. On the other hand, people at the top of the food pyramid must be extremely cautious about what they eat. Metals that have not accumulated to hazardous levels in a species that has become a source of vegetables, fruit, and/or animal protein can cause significant harm to people.

Authorities have published many reports on the highest amounts of heavy metals that fish and aquatic organisms can contain due to the toxic effects of heavy metals and their negative effects on human health (Alwes et al., 2012).

1.2. Arsenic (As) and Toxicity

Arsenic is a metalloid element found in the Earth's crust and is present in the environment due to natural and human activities. It can be released into the environment through natural processes such as volcanic activity, rock erosion, and weathering minerals containing arsenic (Rossman, 2003). Exposure to arsenic can occur through various routes, including ingestion of contaminated water and food, inhalation of airborne particles, and dermal contact. The levels of arsenic (As) in the muscles of both Japanese seabass (*Lateolabrax japonicus*) and red seabream (*Pagrus major*) from all eight fish cage sites along the coast of Fujian province in China, as investigated by Onsanit, S., Ke, C., Wang, X., Wang, K. J., & Wang (2010) generally exceeded the safety limit set by China (1.0 μg/g) among all the trace elements analyzed. The arsenic levels ranged from 1.5 to 4.5 μg/g for the seabass and 1.1 to 2.9 μg/g for the red seabream. Another study in India measured for total arsenic content in the muscle of *Labeo catla*, *Labeo rohita*, and *Cirrhinus mrigal*a (i.e., edible part) and the mean value of total arsenic concentration in the fishes ranged from 0.05 to 2.18 mg kg^{-1} w.wt (Kumar et al., 2021). Existing documentation clearly indicates that zooplankton possesses the capacity to accumulate arsenic concentrations that are 10 to 20 times higher compared to fish. Surprisingly, when fishes consume zooplankton as their food source, they exhibit higher levels of arsenic concentration without any instances of biomagnification occurring (Chen et al., 2000).

1.3. Cadmium (Cd) and Toxicity

Cadmium (Cd) is a naturally occurring element in the environment in small concentrations (ATSDR, 1999). However, it can become polluted by both natural sources and human activities, including industrial processes, urban development, and agricultural practices. Additionally, soil erosion caused by rainfall can contribute to the contamination of cadmium (Drąg-Kozak et al., 2019). Given its high toxicity potential, long-lasting presence in the environment, and tendency to accumulate in aquatic organisms, even at low levels, cadmium poses significant harm. In aquatic ecosystems, cadmium is not easily metabolized by organisms, leading to its accumulation in soft tissues and subsequent toxicity. This accumulation directly affects the contamination of the food chain, thereby impacting the overall ecological functioning (Helmy NA, 2019; Mahjoub et al., 2021). In a previous study conducted by (Saeed et al., 2019), it was reported that the Cd level in the muscle of *Oreochormis niloticus* from Manzala Lake, Egypt, was recorded at 10.36 μg/g. Furthermore, found that in the Danube River, Grocka, the average Cd content in carp muscle was 0.082 μg/g. Studies have reported elevated levels of cadmium (Cd) in two fish species, *Mycteroperca fusca* and *Mycteroperca olfax*. (Franco-Fuentes et al., 2021) found Cd levels to be 2.54 ± 10.3 μg/g in *M. fusca*, while (Lozano et al., 2019) recorded levels of 10.70 ± 7.93 μg/g in *M. olfax*. The authors attributed these variations to factors such as age, size, metabolic activity, and dietary preferences of the organisms. Notably, other studies have also suggested that organisms with a higher proportion of fish in their diet tend to exhibit higher levels of heavy metals compared to those primarily feeding on invertebrates (Escobar-Sánchez et al., 2016).

1.4. Copper (Cu) and Toxicity

Copper (Cu) is released into aquatic environments through various human activities such as mining, industrial processes, and the use of copper-based pesticides and fungicides and is found in freshwater bodies at concentrations up to 100 μg/L (Irwin RJ et al., 1997). In marine environments, copper concentrations are typically much lower than in freshwater bodies. This is due to several factors, including the larger volume and greater dilution capacity of the oceans compared to freshwater systems. Additionally, the chemistry and characteristics of seawater can influence the behaviour and fate of copper, resulting in lower (Cao et al., 2019). While copper is naturally present in the environment, human activities have significantly increased its concentration in water bodies, leading to potential ecological and health risks (Gupta & Siddique, 2020). Copper is often used in industrial applications, including plumbing, electrical wiring, and manufacturing processes. When these materials corrode or are improperly disposed of, copper can leach into water sources (Pourbeyram & Mehdizadeh, 2016). Besides the harmful effects, Cu is an essential redox-active transition metal and has some important functions in enzymatic reactions, photosynthetic electron transport, connective tissue formation, iron metabolism, pigmentation and immune function of organisms (Yruela, 2015; Adams et al., 2016).

1.5. Chromium (Cr) and Toxicity

Chromium (Cr) presents a significant risk to both the environment and public health within water ecosystems. The pollution caused by Cr can lead to various health issues, including vomiting, diarrhea, liver damage, and lung infections.[38] Even at low concentrations, Cr exhibits high levels of bioaccumulation and is resistant to biological degradation, thereby adversely affecting aquatic organisms, including fish (Velma et al., 2009). It is well-known that Cr is toxic, neurotoxic, genotoxic, carcinogenic, and mutagenic, and it has been classified as one of the 25 hazardous substances (Farombi et al., 2007; Kawade & Khillare, 2012). The primary sources of environmental Cr releases are industrial activities, particularly in the processing and manufacturing of chemicals, minerals, steel, metal plating, leather tanning, textile dyeing, electroplating, cement production, and metallurgical works (Nakkeeran et al., 2018; Lian et al., 2019). Fish organs can accumulate Cr up to 4,000 times higher than the concentration in their surrounding environment (Duffus, 1980).

1.6. Iron (Fe) and Toxicity

Iron (Fe) is a widely abundant metal, comprising approximately 5% of the Earth's crust. It plays a crucial role in supporting life in oxygen-rich environments (Bury & Grosell, 2003). Iron is essential for cellular respiration in animals, as its flexible redox activity enables it to be a vital component. Additionally, its presence in hemoglobin enhances the oxygen-carrying capacity of blood while also supporting photosynthesis in plants (Bury et al., 2001). However, excessive amounts of iron can be toxic and lead to various detrimental effects. One such effect is oxidative stress and tissue damage caused by iron's involvement in the Fenton reaction, as documented by Yang, Z., Shan, C., Pan, B., & Pignatello, J. J (2021). Iron overload can occur in iron-storing organs like the liver, heart, and pancreas, resulting in their malfunctioning. This condition is associated with disorders such as hemochromatosis, as noted by Kew (2014) and Siri-Angkul (2018). Concerns regarding iron toxicity in freshwater environments typically arise downstream from activities such as iron ore mining and acid mine drainage. These activities can lead to increased iron concentrations in water bodies, exceeding the recommended freshwater quality guidelines. The existing guidelines, as established by the US Environmental Protection Agency and Canadian Council of Resources and Environment Ministers [51-52], generally range between 300 and 1000 μg L^{-1}.

1.7. Mercury (Hg) and Toxicity

Mercury (Hg) is a toxic metal considered a global pollutant that can be found in both freshwater and marine ecosystems, as highlighted by Visha, A., Gandhi, N., Bhavsar, S. P., & Arhonditsis, G. B. (2018). It poses harm to all organisms, even at very low concentrations, with 0.05 μg/g being noteworthy, according to (Zahir et al., 2005). Hg is released into the environment through both natural and human activities. Natural sources include volcanic emissions and the erosion of soil and rock, as well as fluxes from marine systems, as discussed by Mason and Pirrone (2009). Human-based sources of mercury include the extraction of natural resources, such as using mercury to obtain gold and other precious metals, as well as industrial and agricultural uses. Additionally, the combustion of fossil fuels contributes to human-based mercury emissions, as outlined by Outridge, P. M., Mason, R. P., Wang, F., Guerrero & Heimburger-Boavida (2018) and Streets, Horowitz, H. M., Levin, L., Thackray & Sunderland (2019). Hg is a versatile element that readily forms inorganic and organic compounds by combining with other elements. Exposure to elevated levels of metallic, inorganic, and organic mercury can have detrimental effects on various aspects of health. Specifically, it can lead to damage to vital organs such as the kidneys and the brain. Furthermore, exposure to mercury during pregnancy can pose risks to the developing fetus. It is important to note that methyl mercury, a form of organic mercury, is particularly concerning as it has been identified as highly carcinogenic, as indicated by Boffetta, Merler & Vainio (1993).

1.8. Nickel (Ni) and Toxicity

Nickel (Ni) is a transition metal that plays a crucial role in plants and microorganisms as it is involved in the activity of various enzymes responsible for important processes like carbon, nitrogen, and oxygen cycling (Ragsdale, 1998). However, it should be noted that elevated concentrations of nickel can have detrimental effects (Pane et al., 2003). Human activities such as mining, smelting, refining, alloy processing, sewage sludge disposal, fossil fuel combustion, waste incineration, stainless steel production, and coin minting have contributed to the contamination of aquatic environments with nickel (Nriagu, 1980). Also, Ni is an essential trace element for human health in small amounts too. It can be adverse effects on humans in excessive exposure such as allergic reactions (Saito et al., 2016), respiratory issues (Buxton et al., 2019), carcinogenic potential (Kasprzak et al., 2003), gastrointestinal effects (Mlinaric et al., 2019) and reproductive and developmental effects (Forgacs et al., 2019).

1.9. Lead (Pb) and Toxicity

Lead is a versatile metal used in various industries, including cloth tinge, varnish, pesticide, explosives, batteries, and paint manufacturing (Johnson, 1998). Its widespread use creates numerous pathways for it to enter environmental systems, including Occupational Exposure, Soil Contamination, Water Contamination, Food Chain Contamination, and Atmospheric Deposition (Giri et al., 2021). Lead (Pb) is a toxic substance with significant implications for human health, particularly concerning the immune system. Throughout history, lead and its compounds have been utilized due to their advantageous physical and chemical properties such as softness, malleability, ductility, poor conductivity, and resistance to corrosion. However, the utilization of lead in various applications carries significant risks. In industrial settings, the inhalation of lead dust and fumes poses a major concern as it can result in lead poisoning. Furthermore, organic compounds containing lead can be absorbed through the skin, adding to the overall lead exposure. The toxic effects of lead encompass a broad spectrum of physiological, biochemical, and neurological dysfunctions in humans (Burbure et al., 2006). These effects can manifest in diverse ways and have serious consequences for overall health and well-being.

1.10. Zinc (Zn) and Toxicity

Human activities have contributed to increased levels of trace elements, including zinc (Zn), in natural water bodies. Anthropogenic factors such as the use of zinc-containing fertilizers and pesticides in agriculture, burning fossil fuels and industrial emissions, sewage and wastewater discharged from residential, industrial, and commercial sources and mining moreover, Zn is naturally present in the Earth's crust, and natural weathering and erosion processes can release it into the environment. Erosion of zinc-rich rocks have led to the enrichment of zinc in the aquatic environment (O'Sullivan et al., 2012). Zinc (Zn) is an essential element that is vital for many biochemical processes in the body such as immune function, growth and development, cognitive function, or reproductive health. Despite being present in relatively low levels, around 10% of the proteome consists of Zn-dependent proteins. These proteins rely on zinc for their proper structure and function, playing important roles in various physiological functions. The significance of zinc as a micronutrient underscores its critical contribution to cellular processes and emphasizes the importance of maintaining adequate zinc levels for overall health and well-being (Hogstrand et al., 2009). Despite all this, high levels of zinc have adverse effects on human inhalation, dermal, gastrointestinal, ocular, ingestion and cardiovascular system (Nriagu J., 2007).

1.11. Cobalt (Co) and Toxicity

Cobalt is an essential element required for various biological processes, including the formation of vitamin B12 and its role as a cofactor for enzymes such as dehydrogenases, hydratases, and transferases (Banerjee and Ragsdale, 2003). However, high concentrations of cobalt can be found in industrial wastewater, near cobalt-mining facilities, and in the runoff of fertilizers used in agriculture (Comhaire et al., 1998; Majmudar and Burleson, 2006). These elevated levels of cobalt can pose a threat to human health, as well as terrestrial and aquatic organisms. In higher concentrations, cobalt exhibits toxicity to cells, animals, and plants (Gál J et al., 2008). It can inhibit cellular respiration and enzymes involved in the citric acid cycle, leading to detrimental effects on cellular function. Studies have shown that cobalt toxicity can disrupt various physiological processes and have adverse effects on organisms. It is important to monitor and regulate cobalt concentrations in industrial processes and environmental settings to prevent excessive exposure and minimize the potential toxic effects on both human health and the ecosystem (Tripathi and Srivastava, 2007).

1.12. Manganese (Mn) and Toxicity

Manganese (Mn) is an essential trace metal that is widely distributed in bacteria, plants, humans, and fish. It plays a crucial role in various metabolic processes, including amino acid, lipid, protein, and carbohydrate metabolism

(Erikson et al., 2007). This essentiality is reflected in its presence in all tissues. Manganese is naturally occurring and can be found in the earth's mantle, as well as in most rocks and soil types (Homoncik et al., 2010). It is one of the most abundant elements and finds extensive use in the production of steel, batteries, and various products such as ceramics, varnishes, fertilizers, and pesticides (Bader et al., 1999; Gerber et al., 2002). Upon entering the body through oral or inhalation routes, manganese is rapidly absorbed. While it has a relatively short half-life in the bloodstream, it exhibits longer half-lives in tissues. Recent research indicates a significant accumulation of manganese in bone, with an estimated half-life of approximately 8-9 years in human bones. This accumulation highlights the potential long-term effects of manganese exposure on bone health. Manganese toxicity has been associated with dopaminergic dysfunction, as evidenced by neurochemical analyses and synchrotron X-ray fluorescent imaging studies. These studies have shed light on the potential impact of manganese on the function of dopamine-related pathways in the brain (O'Neal and Zheng, 2015).

2. MAIN FACTORS AFFECTING HEAVY METAL BIOACCUMULATION IN WILD AND FARMED FISH

When considering the health risk assessment of heavy metals for both fish and humans, it is crucial to understand factors affecting heavy metal bioaccumulation in fish. Basically, fish are exposed to heavy metals through water, sediments, and their diet (Olsson et al., 1998; Zeitoun and Mehana, 2014). Once absorbed, these metals tend to accumulate and biomagnify in the food chain, leading to higher concentrations in fish at the top of the aquatic food chain. As a result, predatory fish species often exhibit higher levels of heavy metal contamination compared to lower trophic-level species (Eisler R., 1994). Moreover, different patterns of effecting factors took place in wild and farmed fish.

2.1. Farmed Fish

Aquaculture, or fish farming, plays a significant role in meeting the increasing global demand for seafood. In aquaculture systems, fish are reared in semi-controlled environments, such as ponds, tanks, or cages, where their diets and living conditions can be regulated. However, heavy metal bioaccumulation remains a concern in aquaculture settings due to various factors:

2.1.1. Feed Contamination

Aquaculture fish are commonly fed formulated feeds that often contain fishmeal and fish oil derived from wild-caught fish. If these wild-caught fish contain high levels of heavy metals, they can transfer those metals to the farmed fish through feed (Janbakhsh et al., 2017; Kundu et al., 2017). Therefore, careful monitoring of feed sources and quality control measures are essential to minimize heavy metal contamination in aquaculture fish.

2.1.2. Water Quality

The water sources used in aquaculture systems can contain heavy metals due to agricultural runoff, industrial discharges, or natural sources. Fish raised in contaminated water may accumulate heavy metals through direct uptake from the water or via their diet (Datta S., 2012; Nyamete et al., 2022). Therefore, regular water quality monitoring and implementing appropriate water treatment strategies are crucial to mitigate heavy metal contamination in aquaculture fish.

2.1.3. Stocking Density and Fish Health

High stocking densities in aquaculture systems can lead to increased stress levels among fish, making them more susceptible to heavy metal accumulation. Stress weakens their immune systems and alters their physiological functions, potentially affecting their ability to metabolize and eliminate heavy metals. Maintaining optimal stocking densities and implementing proper fish health management practices can help reduce the risk of heavy metal bioaccumulation in aquaculture fish (Cretì et al., 2010).

2.2. Wild Fish

In contrast to aquaculture fish, capture fish refers to fish caught from natural water bodies, such as oceans, rivers, and lakes. These fish are exposed to heavy metals present in their natural habitats, and several factors contribute to the bioaccumulation of heavy metals in captured fish:

2.2.1. Habitat Contamination

Natural water bodies can become contaminated with heavy metals through various sources, including industrial discharges, agricultural runoff, and atmospheric deposition (Zhang et al., 2022). Fish living in contaminated habitats are more likely to accumulate heavy metals in their tissues over time (Sonone et al., 2020). Therefore, monitoring and controlling pollution sources are crucial for maintaining the quality of aquatic environments and minimizing heavy metal bioaccumulation in captured fish.

2.2.2. Trophic Position

Fish occupying higher trophic levels, such as predatory species, are more prone to heavy metal accumulation. As these fish consume smaller fish or organisms lower in the food chain, they inherit the accumulated heavy metals from their prey (Zhang et al., 2016). Consequently, predatory fish species often exhibit higher levels of heavy metal contamination compared to lower trophic-level species (Kumar et al., 2020). Understanding the trophic dynamics and the bioaccumulation potential of different fish species is essential for assessing the health risks associated with consuming captured fish (Tao et al., 2012).

2.2.3. Migration Patterns

Some fish species undertake long-distance migrations, moving between different habitats during their life cycles. These migrations can expose them to various environmental conditions, including different levels of heavy metal contamination. As a result, their heavy metal accumulation patterns can vary depending on their location and the specific habitats they inhabit during different stages of their lives. Considering the migratory patterns of fish species is crucial for comprehensive risk assessment and management strategies (Visnjic-Jeftic et al., 2010; Copaja et al., 2017).

In both aquaculture and capture fish scenarios, implementing good agricultural and aquaculture practices, monitoring water quality, and enforcing strict regulatory standards are vital for minimizing heavy metal bioaccumulation in fish and ensuring the safety of fish consumption for human health. However, many studies have been and continue to be carried out on the accumulation of heavy metals in fish farmed and/or wild-caught in different locations and their effects on human health.

3. STUDIES ON HEAVY METAL ACCUMULATION IN FISH ALWAYS BEEN SET UP IN DIFFERENT LOCATIONS

Spatial variation in heavy metal contamination in aquatic ecosystems, including fish, can vary significantly on a regional scale. Different regions may have distinct sources of pollution, varying industrial activities, agricultural practices, and geographical characteristics, which can affect the levels of heavy metal accumulation in fish (Liang et al., 2016; Amundsen et al., 1997). Conducting studies on a regional scale allows for a comprehensive understanding of the specific contamination patterns and associated health risks within a particular geographic area. In addition to this, regional studies take into account the local environmental conditions that influence heavy metal accumulation in fish. Factors such as proximity to industrial activities, mining sites, urban areas, or agricultural practices can significantly contribute to heavy metal contamination in aquatic ecosystems (Al Naggar et al., 2018). By focusing on specific regions, researchers can better assess the environmental factors that contribute to heavy metal accumulation and the subsequent health risks for the local population. Dietary habits and cultural practices related to fish consumption can vary among regions and populations. Different fish species, caught or cultured in specific regions, may exhibit varying levels of heavy metal contamination. Moreover, local cultural practices such as traditional diets, food preparation methods, and preferences for specific fish species may influence the extent of exposure to heavy metals. Conducting regional studies enables researchers to consider these dietary and cultural factors and provide context-specific health risk assessments. Also regional studies also take into account the characteristics of the local population, including demographics, socio-economic status, and health profiles. Certain populations may have specific vulnerabilities or susceptibilities to heavy metal exposure due to genetic factors, nutritional status, or co-exposure to other contaminants (Tamale et al., 2017; Naz et al., 2022). By focusing on a regional perspective, researchers can tailor the health risk assessment to the specific population and better understand the potential health impacts of heavy metal accumulation in fish. Regional studies provide valuable information for policymakers, regulatory agencies, and local authorities responsible for managing and mitigating the health risks associated with heavy metal contamination in fish. By conducting assessments on a regional scale, policymakers can make more informed decisions regarding regulatory standards, monitoring programs, and risk management strategies tailored to the specific regional context. This regional approach allows for targeted interventions and better protection of public health. Conducting regional studies facilitates collaboration among researchers, institutions, and stakeholders within a specific geographic area. It encourages the sharing of data, methodologies, and expertise, which can lead to more robust and comprehensive assessments. Regional studies also foster opportunities for cross-disciplinary collaborations, involving experts in environmental science, toxicology, public health, and fisheries management, among others, to collectively address the complex issue of heavy metal accumulation and health risk assessment in fish.

In conclusion, regional studies on heavy metal accumulation and health risk assessment in fish are conducted to account for spatial variations in contamination levels, consider local environmental conditions and cultural practices, tailor assessments to specific populations, inform regulatory and management decisions, and promote collaboration and data sharing. This regional perspective enhances the accuracy and relevance of the findings and supports effective measures to protect public health.

4. HUMAN HEALTH RISK ASSESSMENT INDICES OF HEAVY METALS IN FISH

The availability of heavy metals in the aquatic environment could be enriched by anthropogenic activities and natural processes. This enriched availability of heavy metals is threatening both fish and eventually human health; therefore, determination and assessment of heavy metals in fish become necessary. In this point, different statistical indices for assessing bioaccumulation and health risk levels of heavy metals based on measurement of the concentration of heavy metals have been designed.

The indices are composite indicators commonly used in pollution assessment including heavy metals in organisms and abiotic environments such as water resources and sediment. These basic statistics summarize and classify relevant measurements in a standardized way. They are representative of a pollution status, e.g. heavy metal pollution status. Moreover, the indices have two practical advantages: *i*) the non-specialist people can efficiently comprehend the pollution level, and *ii*) the pollution levels of different organisms (including vertebrate and invertebrate animals, edible plants) or environments can be contrasted (Tomlinson et al., 1980; Lipton and Gillett, 1991; Han et al., 1996).

Using the indices becomes necessary when evaluating heavy metal pollution in aquatic environments and assessment of heavy metals for human health. The indices are prominent among different risk assessment methods dealing with the effects of heavy metals on humans (Hoang et al., 2021). These indices could be classified into two main groups: ecological risk indices and health risk indices. The first group assesses heavy metal concentrations measured in water and sediment; the second group evaluates heavy metal concentrations obtained from fish and other water products that can be consumed by humans. Moreover, some indices are specific either for carcinogenic or non-carcinogenic risk assessments. This chapter addresses that the indices used for the estimation of human health risk are applied to heavy metals measured in fish tissues. In terms of human health risk assessment and fish consumption limits, it should be noted that the name fish in the document series by the United States Environmental Protection Agency (USEPA) appertains to sport and subsistence-caught fish and invertebrate such as oysters and shrimp in different aquatic environments, and even fish products like caviar (MacDonald DD et al., 2000; Cui et al., 2004; Sobhanardakani et al., 2018).

4.1. Metal Pollution Index (MPI)

Metal pollution index (MPI) is designed for the calculation of the total heavy metal concentration detected in a fish species. MPI was calculated by taking the geometrical mean of each metal concentration measured in the target tissue (Usero et al., 1997). The following equation is used for the calculation of MPI.

$$MPI = (Cf_1 \times Cf_2 \dots Cf_n)^{1/n} \qquad \text{(X 1.)}$$

where

MPI: the metal pollution index, and

Cf_n: the concentration of metal in tissue (µg/g).

Any value of the index greater than 0 expresses increasing bioaccumulation of heavy metals in samples. Moreover, MPI is deployed to compare samples' overall heavy metal contents from different sampling locations (Islam et al., 2017).

4.2. Daily Intake Rate (EDI)

Estimated daily intake (EDI) is used for the estimation of the average amount of daily intake of a heavy metal (USEPA, 2000). Also, its formula could be adapted to the estimation of daily intake of multi-heavy metals, presenting as estimated daily intakes (EDIs). EDI is calculated by the following equation:

$$EDI(\mu g \ / \ kg - bw \ / \ \ day) = \frac{FIR \times C}{WAB} \quad \text{(X 2.)}$$

where

EDI: the estimated daily intakes,

FIR: the fish ingestion rate (g/person/day),

C: the measured heavy metal concentration in fish (µg/g wet weight),

WAB: the average body weight.

This index could be adapted for weekly intake termed as the estimated weekly intake (EWI) (Copat et al., 2012).

4.3. Health Risk Index and Hazard Index

The health risk index abbreviated as HRI is suggested by Tchounwou PB, Abdelghani AA, Pramar YV, Heyer LR & Steward (1996) and indicates possible health risks for the consumer in terms of heavy metal accumulation in fish. HRI describes the relationship between the reference dose and the estimated daily intake level. Some studies have used the term hazard quotients (HQ) instead of HRI. HRI is determined using the following equation:

$$HRI = \frac{EDI}{RfDo} \quad \text{(X 3.)}$$

where

EDI: the estimated daily intakes,

RfDo: a reference dose (oral) of the metal.

Values of HRI less than 1 are assumed as safe, while greater values than 1 for a heavy metal in fish imply its risk to consumer human health (Cui et al., 2004). The sum of HRI or HQ calculated for each metal named Hazard index (HI) could be used for the estimation of their cumulative risk to human health (Ali and Khan, 2018).

4.4. Target Hazard Quotient (THQ)

The target hazard quotient has been established by the United States Environmental Protection Agency (USEPA) and could be applied to heavy metals measurements in fish and other water products like oysters and shrimp.

THQ as an estimate of non-carcinogenic risk level b was originated EPA Region EQ Risk-Based Concentration Table in 1995, and its methodology was provided in USEPA (2000). It is mainly derived from the ratio of exposure and reference values, and useful for non-carcinogenic risk assessment. used following formula for THQ:

$$THQ = \frac{EFr \times EDtot \times FIR \times C}{RfDo \times BWa \times ATn} \times 10^{-3} \quad \text{(X 4.)}$$

where

THQ: the target hazard quotient,

EFr: the exposure frequency (365 days/year),

EDtot: the exposure duration (average lifetime),

FIR: the food ingestion rate (g/day),

C: the heavy metal concentration in fish (mg/g),

RfDo: is the oral reference dose (mg/kg/day),

Bwa: the average adult body weight,

ATn: the averaging exposure time for non-carcinogens (365 days/years × average lifetime).

Accordingly, THQ values range from 0 to 1 meaning that a daily exposure level causes any adverse effects during the lifetime in humans (**Figure 1**). On the contrary, THQ values calculated equal to or greater than 1, in other words, greater than reference doses, refer to deleterious effects on human health. The USEPA Integrated Risk Information System (IRIS) program database provides RfDo values for each heavy metal. For instance, RfDo value for As is 3 x 10^{-4} mg/kg-day for of non-carcinogenic risk assessment.

On the other hand, the integrated and interactive effects of multiple pollutant might be critical when considering chemically reactive substances like heavy metals (Cunningham, 1986). Therefore, an approach to measuring cumulative THQ is needed. the total THQ (TTHQ) is the arithmetic sum of the individual heavy metal THQ values. TTHQ of heavy metals is calculated using the following equation (Yang and Zhang, 2011).

$$TTHQ = THQ\ (\,heavy\ metal\ 1) + THQ\ (\,heavy\ metal\ 2) + \cdots + THQ\ (\,heavy\ metal\ n) \quad \text{(X 4.)}$$

where

TTHQ: the total target hazard quotient,

THQ (heavy metal n): calculated THQ for a heavy metal.

Additionally, THQ allow comparisons among heavy metal concentrations in different foodstuff types such as fish, cereals, vegetables, fruit, meat (Liang G et al., 2019).

4.5. Target Cancer Risk (TR)

The target cancer risk index (TR) is calculated to reveal carcinogenic health hazards due to fish consumption over a human lifetime (USEPA, 1989). TR is also termed as the risk of cancer or lifetime cancer risk index (Raknuzzaman et al., 2016). With this index, carcinogenic health risks generated through carcinogenic heavy metals are determined by the additional probability of a person developing cancer over the lifelong exposure. The equation is used for estimating TR:

$$TR = \frac{EF \times ED \times FIR \times C \times CSFo}{WAB \times AT} \times 10^{-3} \quad \text{(X 5.)}$$

where

TR: the target cancer risk,

EF: the exposure frequency (350 days/year),

ED: the exposure duration (average lifetime),

FIR: the fish ingestion rate (kg/person/ day),

C: the metal concentration in fish (mg/kg),

CSFo: the oral carcinogenic slope factor (mg/kg/day);

WAB: the average body weight (kg),

ATc: the average time for carcinogens (365 days/years × average lifetime).

Essentially, TR values between 10^{-6} and 10^{-4} are generally considered as an acceptable range regarding to lifetime carcinogenic risk (**Figure 1**). On the other hand, the cancer risk values above 10−4 are considered as carcinogenic risk for a measured heavy metal (USEPA, 2000; USEPA, 2011).

Moreover, in TR formula, CSFo values for heavy metals could be found in the USEPA IRIS program database. The IRIS database is a useful tool for assessments of human health affected by exposure of chemicals in the environment and contains more than 500 assessments including heavy metals (Gehlhaus et al., 2011). For instance, CSFo value for as is 1.5 mg/kg/day in calculation of carcinogenic risk from oral exposure, according to IRIS database.

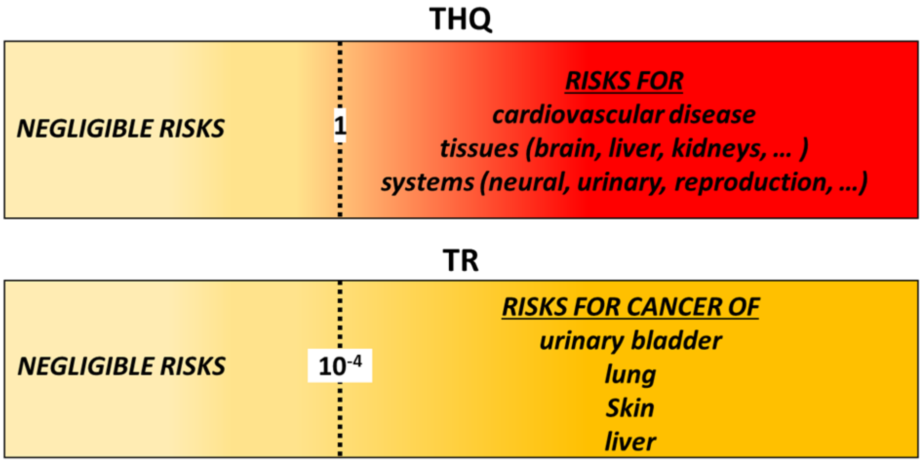

Figure 1. The benchmark levels for target hazard quotient (THQ) and target cancer risk (TR) are represented by an arbitrary color scale, remarking non-carcinogenic and carcinogenic possible disease emerged in fish consumers.

REFERENCES

Adams, M. S., Dillon, C. T., Vogt, S., Lai, B., Stauber, J., & Jolley, D. F. (2016). Copper uptake, intracellular localization, and speciation in marine microalgae measured by synchrotron radiation X-ray fluorescence and absorption microspectroscopy. Environmental Science & Technology, 50(16), 8827-8839.

Al Naggar, Y., Khalil, M. S., & Ghorab, M. A. (2018). Environmental pollution by heavy metals in the aquatic ecosystems of Egypt. Open Acc. J. Toxicol, 3, 555603.

Ali, H., & Khan, E. (2018). Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. Environmental chemistry letters, 16(3), 903-917.

Amundsen, P. A., Staldvik, F. J., Lukin, A. A., Kashulin, N. A., Popova, O. A., & Reshetnikov, Y. S. (1997). Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Science of the total environment, 201(3), 211-224.

ATSDR (1999) Toxicol. Profile of cadmium. Agency for toxic substances and drug registry, Atlanta, GA. US Deptt of Health and Human Services

B. Belton and S. H. Thilsted, Fisheries in transition: Food and nutrition security implications for the global South. Glob. Food Sec, 2014, **3(1),** 59-66. https://doi.org/10.1016/j.gfs.2013.10.001

B.S. Zohra and A. Habib, "Assessment of heavy metal contamination levels and toxicity in sediments and fishes from the Mediterranean Sea (southern coast of Sfax, Tunisia)," Environ. Sci. Pollut. Res., 2016, **23**, 13954-13963. https://doi.org/10.1007/s11356-016-6534-3

Bader, M., Dietz, M. C., Ihrig, A., & Triebig, G. (1999). Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. International archives of occupational and environmental health, 72, 521-527.

Banerjee, R., & Ragsdale, S. W. (2003). The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. Annual review of biochemistry, 72(1), 209-247.

Boffetta, P., Merler, E., & Vainio, H. (1993). Carcinogenicity of mercury and mercury compounds. Scandinavian journal of work, environment & health, 1-7.

Bury, N. R., Grosell, M., Wood, C. M., Hogstrand, C., Wilson, R. W., Rankin, J. C., ... & Jensen, F. B. (2001). Intestinal iron uptake in the European flounder (Platichthys flesus). Journal of Experimental Biology, 204(21), 3779-3787.

Bury, N., & Grosell, M. (2003). Iron acquisition by teleost fish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 135(2), 97-105.

Buxton, S., Garman, E., Heim, K. E., Lyons-Darden, T., Schlekat, C. E., Taylor, M. D., & Oller, A. R. (2019). Concise review of nickel human health toxicology and ecotoxicology. Inorganics, 7(7), 89.

Canadian Council of Resources and Environment Ministers. (1987). Canadian water quality guidelines.

Cao, R., Zhang, T., Li, X., Zhao, Y., Wang, Q., Yang, D., ... & Zhao, J. (2019). Seawater acidification increases copper toxicity: A multi-biomarker approach with a key marine invertebrate, the Pacific Oyster Crassostrea gigas. Aquatic Toxicology, 210, 167-178.

Chen, C. Y., & Folt, C. L. (2000). Bioaccumulation and diminution of arsenic and lead in a freshwater food web. Environmental science & technology, **34(18)**, 3878-3884. https://doi.org/10.1021/es991070c

Chien, L. C., Hung, T. C., Choang, K. Y., Yeh, C. Y., Meng, P. J., Shieh, M. J., & Han, B. C. (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Science of the total environment, 285(1-3), 177-185.

Comhaire, S., Blust, R., Van Ginneken, L., Verbost, P. M., & Vanderborght, O. L. J. (1998). Branchial cobalt uptake in the carp, Cyprinus carpio: Effect of calcium channelblockers and calcium injection. Fish Physiology and Biochemistry, 18, 1-13.

Connell, D. W. (2018). Environmental routes leading to the bioaccumulation of lipophilic chemicals. In Bioaccumulation of Xenobiotic Compounds (pp. 59-74). CRC Press. https://doi.org/10.1201/9781351070126

Copaja, S. V., Pérez, C. A., Vega-Retter, C., & Véliz, D. (2017). Heavy metal content in Chilean fish related to habitat use, tissue type and river of origin. Bulletin of environmental contamination and toxicology, 99, 695-700.

Copat C, Bella F, Castaing M, Fallico R, Sciacca S, Ferrante M (2012) Heavy metals concentrations in fish from Sicily Mediterranean Sea) and evaluation of possible health risks to consumers. Bull Environ Contam Toxicol 88:78–83.

Cretì, P., Trinchella, F., & Scudiero, R. (2010). Heavy metal bioaccumulation and metallothionein content in tissues of the sea bream Sparus aurata from three different fish farming systems. Environmental monitoring and assessment, 165, 321-329.

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qui, Y., Liang, J.Z., 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China.

Cunningham KM 1986. Exposure Characterization Hallenbeck WH. Cunningham Quantitaive risk assessment for environmental and occupational health. Chelsea, MI: Lewis,. P 9-17

Datta, S. (2012). Management of water quality in intensive aquaculture. Respiration, 6, 602.

de Burbure, C., Buchet, J. P., Leroyer, A., Nisse, C., Haguenoer, J. M., Mutti, A., ... & Bernard, A. (2006). Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environmental health perspectives, 114(4), 584-590.

Drąg-Kozak E, Pawlica-Gosiewska D, Gawlik K, Socha M, Gosiewski G, Łuszczek-Trojnar E, Solnica E, Popek W (2019) Cadmium-induced oxidative stress in Prussian carp (Carassius gibelio Bloch) hepatopancreas: ameliorating effect of melatonin. Environ Sci Pollut Res 26:12264–12279. https://doi.org/10.1007/s11356-019-04595-3

Duffus, J. H. (1980). Environmental Toxicology. Edward Arnold (Publishers) Ltd..

Eisler, R. (1994). A review of arsenic hazards to plants and animals with emphasis on fishery and wildlife resources. Advances in Environmental Science and Technology-New York-, 27, 185-185.

Erikson, K. M., Thompson, K., Aschner, J., & Aschner, M. (2007). Manganese neurotoxicity: a focus on the neonate. Pharmacology & therapeutics, 113(2), 369-377.

Escobar-Sánchez, O., Ruelas-Inzunza, J., Moreno-Sánchez, X. G., Romo-Piñera, A. K., & Frías-Espericueta, M. G. (2016). Mercury concentrations in pacific angel sharks (Squatina californica) and prey fishes from Southern Gulf of California, Mexico. Bulletin of environmental contamination and toxicology, 96, 15-19.

Farombi, E. O., Adelowo, O. A., & Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. International journal of environmental research and public health, 4(2), 158-165.

Festa, R. A., & Thiele, D. J. (2011). Copper: an essential metal in biology. Current Biology, 21(21), R877-R883.

Food and Agriculture Organization (FAO), The State of the World Fisheries and Aquaculture, 2023

Forgacs, Z., Massányi, P., Lukac, N., & Somosy, Z. (2012). Reproductive toxicology of nickel–review. Journal of Environmental Science and Health, Part A, 47(9), 1249-1260.

Franco-Fuentes, E., Moity, N., Ramírez-González, J., Andrade-Vera, S., Hardisson, A., González-Weller, D., ... & Gutiérrez, Á. J. (2021). Metals in commercial fish in the Galapagos Marine Reserve: Contribution to food security and toxic risk assessment. Journal of Environmental management, 286, 112188. https://doi.org/10.1016/j.jenvman.2021.112188

Gál, J., Hursthouse, A., Tatner, P., Stewart, F., & Welton, R. (2008). Cobalt and secondary poisoning in the terrestrial food chain: data review and research gaps to support risk assessment. Environment international, 34(6), 821-838.

Gehlhaus MW 3rd, Gift JS, Hogan KA, Kopylev L, Schlosser PM, Kadry AR. Approaches to cancer assessment in EPA's Integrated Risk Information System. Toxicol Appl Pharmacol. 2011 Jul 15;254(2):170-80.

Gerber, G. B., Leonard, A., & Hantson, P. H. (2002). Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. Critical reviews in oncology/hematology, 42(1), 25-34.

Giri, S. S., Kim, M. J., Kim, S. G., Kim, S. W., Kang, J. W., Kwon, J., ... & Park, S. C. (2021). Role of dietary curcumin against waterborne lead toxicity in common carp Cyprinus carpio. Ecotoxicology and Environmental Safety, 219, 112318.

Gupta, N., & Siddique, R. (2020). Utilization of Copper Slag in Self-compacting Concrete-Strength and Permeation Properties. In Rheology and Processing of Construction Materials: RheoCon2 & SCC9 2 (pp. 544-551). Springer International Publishing.

Han BC, Jeng WL, Hung TC, Wen MY (1996) Relationship between copper speciation in sediments and bioaccumulation by marine bivalves of Taiwan. Environ Pollut 91:35–39

Helmy NA, Hassan MA, Hassanien FS, Maarouf AA (2018) Detection of heavy metals residues in fish and shellfish. Benha Veterinary Medical Journal 34(2):255–264

Hoang, H. G., Chiang, C. F., Lin, C., Wu, C. Y., Lee, C. W., Cheruiyot, N. K., ... & Bui, X. T. (2021). Human health risk simulation and assessment of heavy metal contamination in a river affected by industrial activities. Environmental Pollution, 285, 117414.

Hogstrand, C., Kille, P., Nicholson, R. I., & Taylor, K. M. (2009). Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. Trends in molecular medicine, 15(3), 101-111.

Homoncik, S. C., MacDonald, A. M., Heal, K. V., Dochartaigh, B. É. Ó., & Ngwenya, B. T. (2010). Manganese concentrations in Scottish groundwater. Science of the total environment, 408(12), 2467-2473.

Irwin RJ, Van Mouwerik M, Stevens L, Seese MD, Basham W (1997) Environmental Contaminants Encyclopedia. National Park Service, Water Resources Division, Fort Collins, Colorado. Distributed within the Federal Government as an Electronic Document.

Islam, G. R., Habib, M. R., Waid, J. L., Rahman, M. S., Kabir, J., Akter, S., & Jolly, Y. N. (2017). Heavy metal contamination of freshwater prawn (Macrobrachium rosenbergii) and prawn feed in Bangladesh: a market-based study to highlight probable health risks. Chemosphere, 170, 282-289.

J. Djedjibegovic, A. Marjanovic, D. Tahirović, K. Čaklovica, A. Turalic, A. Lugusic, E. Omeragic, M. Sober and F. Čaklovica, Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. Sci Rep.2020, **10(1),** 13238. https://doi.org/10.1038/s41598-020-70205-9

J. L. Anderson, F. Asche, T. Garlock and J. Chu, Aquaculture: Its role in the future of food, Emerald Publishing Limited, 2017, vol. 17, Pp. 159-173. https://doi.org/10.1108/S1574-871520170000017011

Janbakhsh, S., Hosseini Shekarabi, S. P., & Shamsaie Mergan, M. (2018). Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea. Caspian Journal of Environmental Sciences, 16(4), 307-317.

Johnson, F. M. (1998). The genetic effects of environmental lead. Mutation Research/Reviews in Mutation Research, 410(2), 123-140.

Jovanović, D. A., Marković, R. V., Teodorović, V. B., Šefer, D. S., Krstić, M. P., Radulović, S. B., ... & Baltić, M. Ž. (2017). Determination of heavy metals in muscle tissue of six fish species with different feeding habits from the Danube River, Belgrade—public health and environmental risk assessment. Environmental Science and Pollution Research, 24, 11383-11391. https://doi.org/10.1007/s11356-017-8783-1

Kasprzak, K. S., Sunderman Jr, F. W., & Salnikow, K. (2003). Nickel carcinogenesis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 533(1-2), 67-97.

Kawade, S. J., & Khillare, Y. K. (2012). Toxicty of chromium on glycogen content of certain tissues of freshwater fish, Channa gachua (Ham). J. Recent Trends Biosci, 2(1), 29-37.

Kew, M. C. (2014). Hepatic iron overload and hepatocellular carcinoma. Liver cancer, 3(1), 31-40.

Kumar, M., Gupta, N., Ratn, A., Awasthi, Y., Prasad, R., Trivedi, A., & Trivedi, S. P. (2020). Biomonitoring of heavy metals in river ganga water, sediments, plant, and fishes of different trophic levels. Biological trace element research, 193, 536-547.

Kundu, G. K., Alauddin, M., Akter, M. S., Khan, M. S., Islam, M. M., Mondal, G., ... & Huque, A. (2017). Metal contamination of commercial fish feed and quality aspects of farmed tilapia (Oreochromis niloticus) in Bangladesh. Bioresearch Communications-(BRC), 3(1), 345-353.

Kwan, T., Leber, B., Ahuja, S., Carter, R., & Gerstein, H. C. (1998). Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene. Clinical and investigative medicine, 21(6), 251.

Lelièvre, P., Sancey, L., Coll, J. L., Deniaud, A., & Busser, B. (2020). The multifaceted roles of copper in cancer: A trace metal element with dysregulated metabolism, but also a target or a bullet for therapy. Cancers, **12(12)**, 3594. https://doi.org/10.3390/cancers12123594

Lian, G., Wang, B., Lee, X., Li, L., Liu, T., & Lyu, W. (2019). Enhanced removal of hexavalent chromium by engineered biochar composite fabricated from phosphogypsum and distillers grains. Science of the Total Environment, 697, 134119.

Liang G, Gong W, Li B, Zuo J, Pan L, Liu X. Analysis of Heavy Metals in Foodstuffs and an Assessment of the Health Risks to the General Public via Consumption in Beijing, China. Int J Environ Res Public Health. 2019

Liang, P., Wu, S. C., Zhang, J., Cao, Y., Yu, S., & Wong, M. H. (2016). The effects of mariculture on heavy metal distribution in sediments and cultured fish around the Pearl River Delta region, south China. Chemosphere, 148, 171-177.

Lipton J, Gillett JW (1991) Uncertainty in ocean-dumping health risks: influence of bioconcentration, commercial fish landings and seafood consumption. Environ Toxicol Chem 10:967–976

Lozano, G., Brito, A., Hardisson, A., Gutiérrez, Á., González-Weller, D., & Lozano, I. J. (2009). Content of lead and cadmium in barred hogfish, Bodianus scrofa, island grouper, Mycteroperca fusca, and Portuguese dogfish, Centroscymnus coelolepis, from Canary Islands, Spain. Bulletin of environmental contamination and toxicology, 83, 591-594.

M. Akiyama, Y. Shinkai, H. Yamakawa, Y.G. Kim, and Y. Kumagai, "Potentiation of methylmercury toxicity by combined metal exposure: In vitro and in vivo models of a restricted metal exposome," Chemosphere, 2022, **299,** 134374. https://doi.org/10.1016/j.chemosphere.2022.134374

M. C. Beveridge, S. H. Thilsted, M. J. Phillips, M. Metian, M. Troell and S. J. Hall, Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculturea. J. Fish Biol., 2013, **83(4),** 1067-1084. https://doi.org/10.1111/jfb.12187

M. Thomas, A. Pasquet, J. Aubin, S. Nahon and T. Lecocq, When more is more: taking advantage of species diversity to move towards sustainable aquaculture. Biol. Rev, 2021, **96(2),** 767-784. https://doi.org/10.1111/brv.12677

MacDonald DD, Ingersoll CG, Berger TA (2000) Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch Environ Contam Toxicol 39:20–31

Mahjoub M, Fadlaoui S, El Maadoudi M, Smiri Y (2021) “Mercury, lead, and cadmium in the muscles of five fish species from the mechraâ-hammadi dam in morocco and health risks for their consumers”, Journal of Toxicology 8865869. https://doi.org/10.1155/2021/8865869

Majmudar, K., & Burleson, M. L. (2006). An evaluation of cobalt chloride as an O2-sensitive chemoreceptor stimulant in channel catfish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 142(1-2), 136-141.

Mason, R., & Pirrone, N. (Eds.). (2009). Mercury fate and transport in the global atmosphere: Emissions, measurements and models. Springer-Verlag New York.

Mlinaric, M. R., Durgo, K., Katic, V., & Spalj, S. (2019). Cytotoxicity and oxidative stress induced by nickel and titanium ions from dental alloys on cells of gastrointestinal tract. Toxicology and Applied Pharmacology, 383, 114784.

Nakkeeran, E., Patra, C., Shahnaz, T., Rangabhashiyam, S., & Selvaraju, N. J. B. T. R. (2018). Continuous biosorption assessment for the removal of hexavalent chromium from aqueous solutions using Strychnos nux vomica fruit shell. Bioresource Technology Reports, 3, 256-260.

Naz, S., Fazio, F., Habib, S. S., Nawaz, G., Attaullah, S., Ullah, M., ... & Ahmed, I. (2022). Incidence of Heavy Metals in the Application of Fertilizers to Crops (Wheat and Rice), a Fish (Common carp) Pond and a Human Health Risk Assessment. Sustainability, 14(20), 13441.

Nriagu, J. (2007). Zinc toxicity in humans. School of Public Health, University of Michigan, 1-7.

Nriagu, J. O. (1980). Nickel in the Environment.

Nyamete, F., Chacha, M., Msagati, T., & Raymond, J. (2022). Bioaccumulation and distribution pattern of heavy metals in aquaculture systems found in Arusha and Morogoro regions of Tanzania. International Journal of Environmental Analytical Chemistry, 102(17), 5961-5978.

O. Sytar, S. Ghosh, H. Malinska, M. Zivcak, and M. Brestic, "Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants," Physiologia Plantarum, 2021, **173(1),** 148-166. https://doi.org/10.1111/ppl.13285

O'Neal, S. L., & Zheng, W. (2015). Manganese toxicity upon overexposure: a decade in review. Current environmental health reports, 2, 315-328.

O'Sullivan, A., Wicke, D., & Cochrane, T. (2012). Heavy metal contamination in an urban stream fed by contaminated air-conditioning and stormwater discharges. Environmental Science and Pollution Research, 19, 903-911.

Obasi, P. N., & Akudinobi, B. B. (2020). Potential health risk and levels of heavy metals in water resources of lead–zinc mining communities of Abakaliki, southeast Nigeria. Applied Water Science, 10(7), 1-23.

Olsson, P. E., Kling, P., & Hogstrand, C. (1998). Mechanisms of heavy metal accumulation and toxicity in fish. Metal metabolism in aquatic environments, 321-350.

Onsanit, S., Ke, C., Wang, X., Wang, K. J., & Wang, W. X. (2010). Trace elements in two marine fish cultured in fish cages in Fujian province, China. Environmental Pollution, **158(5)**, 1334-1342. https://doi.org/10.1016/j.envpol.2010.01.012

Outridge, P. M., Mason, R. P., Wang, F., Guerrero, S., & Heimburger-Boavida, L. E. (2018). Updated global and oceanic mercury budgets for the United Nations Global Mercury Assessment 2018. Environmental science & technology, 52(20), 11466-11477.

Pane, E. F., Richards, J. G., & Wood, C. M. (2003). Acute waterborne nickel toxicity in the rainbow trout (Oncorhynchus mykiss) occurs by a respiratory rather than ionoregulatory mechanism. Aquatic toxicology, 63(1), 65-82.

Panigrahi A K, Bakshi A, Pattanayak S (2021) A comprehensive review on the uptake by and accumulation of some heavy metals in fresh water fishes. Biosc.Biotech.Res.Comm. 14(1). http://dx.doi.org/10.21786/bbrc/14.1/55

Pourbeyram, S., & Mehdizadeh, K. (2016). Nonenzymatic glucose sensor based on disposable pencil graphite electrode modified by copper nanoparticles. journal of food and drug analysis, 24(4), 894-902.

Ragsdale, S. W. (1998). Nickel biochemistry. Current opinion in chemical biology, 2(2), 208-215.

Ragsdale, S. W. (1998). Nickel biochemistry. Current opinion in chemical biology, 2(2), 208-215.

Raknuzzaman, M., Ahmed, M. K., Islam, M. S., Habibullah-Al-Mamun, M., Tokumura, M., Sekine, M., & Masunaga, S. (2016). Trace metal contamination in commercial fish and crustaceans collected from coastal area of Bangladesh and health risk assessment. Environmental Science and Pollution Research, 23, 17298-17310.

Raman, R. K., Talukder, A., Mahanty, A., Sarkar, D. J., Das, B. K., Bhowmick, S., ... & Mohanty, B. P. (2022). Arsenic bioaccumulation and identification of low-arsenic-accumulating food fishes for aquaculture in arsenic-contaminated ponds and associated aquatic ecosystems. Biological trace element research, **200(6)**, 2923-2936. https://doi.org/10.1007/s12011-021-02858-0

Rand, G. M., Wells, P. G., & McCarty, L. S. (2020). Introduction to aquatic toxicology. In Fundamentals of aquatic toxicology (pp. 3-67). CRC Press. https://doi.org/10.1201/9781003075363

Rossman, T. G. (2003). Mechanism of arsenic carcinogenesis: an integrated approach. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, **533(1-2)**, 37-65. https://doi.org/10.1016/j.mrfmmm.2003.07.009

S. Sabir, M.S.H. Akash, F. Fiayyaz, U. Saleem, M.H. Mehmood, and K. Rehman, "Role of cadmium and arsenic as endocrine disruptors in the metabolism of carbohydrates: inserting the association into perspectives," Biomedicine & Pharmacotherapy, 2019, **114,** 108802. https://doi.org/10.1016/j.biopha.2019.108802

S.A. Olawale, "Biosorption of heavy metals: a mini review," Acta Sci Agric, 2019, **3(2),** 22-25

Saeed, A. N., Shaker, I. M., 2008. Assessment of heavy metals pollution in water and sediments and their effect on Oreochromis niloticus in the Northern Delta lakes, Egypt. In: Proceedings of the 8thInternational Symposium on Tilapia in Aquaculture 8:475–490. https://doi.org/ 10.5555/20133318746

Saito, M., Arakaki, R., Yamada, A., Tsunematsu, T., Kudo, Y., & Ishimaru, N. (2016). Molecular mechanisms of nickel allergy. International journal of molecular sciences, 17(2), 202.

Sobhanardakani S, Tayebi L, Hosseini SV. Health risk assessment of arsenic and heavy metals (Cd, Cu, Co, Pb, and Sn) through consumption of caviar of Acipenser persicus from Southern Caspian Sea. Environ Sci Pollut Res Int. 2018 Jan;25(3):2664-2671.

Sonone, S. S., Jadhav, S., Sankhla, M. S., & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. Lett. Appl. NanoBioScience, 10(2), 2148-2166.

Streets, D. G., Horowitz, H. M., Lu, Z., Levin, L., Thackray, C. P., & Sunderland, E. M. (2019). Five hundred years of anthropogenic mercury: Spatial and temporal release profiles. Environmental Research Letters, 14(8), 084004.

Tamale, A., Ejobi, F., Muyanja, C., Naigaga, I., Nakavuma, J., Drago, C. K., & Amulen, D. R. (2017). Sociocultural factors associated with fish consumption in Lake Albert fishing community: Guidelines for lead and mercury. Cogent Environmental Science, 3(1), 1304604.

Tao, Y., Yuan, Z., Xiaona, H., & Wei, M. (2012). Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihu lake, China. Ecotoxicology and environmental safety, 81, 55-64.

Tchounwou PB, Abdelghani AA, Pramar YV, Heyer LR, Steward CM. Assessment of potential health risks associated with ingesting heavy metals in fish collected from a hazardous-waste contaminated wetland in Louisiana, USA. Rev Environ Health. 1996 Oct-Dec;11(4):191-203

Tomlinson, D.L., Wilson, J.G., Harris, C.R. et al. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Helgolander Meeresunters 33, 566–575 (1980).

Tripathi P, Srivastava S. Mechanism to combat cobalt toxicity in cobalt resistant mutants of Aspergillus nidulans. Indian J Microbiol, 2007; 47, 336-44

U.S. Environmental Protection Agency. (1976). Quality criteria for water (p. 534).

USEPA (1989) Risk assessment guidance for superfund, vol. I: Human Health Evaluation Manual. EPA/540/1-89/002. Office of Emergency and Remedial Response, Washington, DC

USEPA (2000) Guidance for assessing chemical contamination data for use in fish advisories volume II Risk assessment and fish consumption limits. EPA/823–B94-004. United States Environmental Protection Agency, Washington

USEPA (US Environmental Protection Agency). (2014). Framework for human health risk assessment to inform decision making. Washington, DC, USA: Office of the Science Advisor, Risk Assessment Forum.

USEPA Exposure Factors Handbook 2011 Edition (Final Report). U.S. nvironmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011.

Usero, J., Gonzalez-Regalado, E., & Gracia, I. (1997). Trace metals in the bivalve molluscs Ruditapes decussatus and Ruditapes philippinarum from the Atlantic Coast of Southern Spain. Environment International, 23(3), 291-298.

Velma, V., Vutukuru, S. S., & Tchounwou, P. B. (2009). Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. Reviews on environmental health, 24(2), 129-146.

Visha, A., Gandhi, N., Bhavsar, S. P., & Arhonditsis, G. B. (2018). Assessing mercury contamination patterns of fish communities in the Laurentian Great Lakes: A Bayesian perspective. Environmental Pollution, 243, 777-789.

Visnjic-Jeftic, Z., Jaric, I., Jovanovic, L., Skoric, S., Smederevac-Lalic, M., Nikcevic, M., & Lenhardt, M. (2010). Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (Alosa immaculata Bennet 1835) from the Danube River (Serbia). Microchemical journal, 95(2), 341-344.

Yang, Z., Shan, C., Pan, B., & Pignatello, J. J. (2021). The Fenton reaction in water assisted by picolinic acid: accelerated iron cycling and co-generation of a selective Fe-based oxidant. Environmental Science & Technology, 55(12), 8299-8308.

Yi, Y., Yang, Z., & Zhang, S. (2011). Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environmental pollution, 159(10), 2575-2585.

Yruela, I. (2005). Copper in plants. Brazilian Journal of Plant Physiology, 17, 145-156.

Z. Fu and S. Xi, "The effects of heavy metals on human metabolism," Toxicology Mechanisms and Methods, 2020**, 30(3),** 167-176. https://doi.org/10.1080/15376516.2019.1701594

Zahir, F., Rizwi, S. J., Haq, S. K., & Khan, R. H. (2005). Low dose mercury toxicity and human health. Environmental toxicology and pharmacology, 20(2), 351-360.

Zeitoun, M. M., & Mehana, E. E. (2014). Impact of water pollution with heavy metals on fish health: overview and updates. Global Veterinaria, 12(2), 219-231.

Zhang, L., Tan, X., Chen, H., Liu, Y., & Cui, Z. (2022). Effects of Agriculture and Animal Husbandry on Heavy Metal Contamination in the Aquatic Environment and Human Health in Huangshui River Basin. Water, 14(4), 549.

Zhang, Y., Lu, X., Wang, N., Xin, M., Geng, S., Jia, J., & Meng, Q. (2016). Heavy metals in aquatic organisms of different trophic levels and their potential human health risk in Bohai Bay, China. Environmental Science and Pollution Research, 23, 17801-17810.

CHAPTER 10

POTENTIAL USE OF SATELLITE TECHNOLOGIES IN AQUACULTURE

Assoc. Prof. Dr. Levent DOĞANKAYA[1]
PhD Student Bilal ATEŞ[2]

[1] Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture, Ankara, Türkiye. dogankaya@ankara.edu.tr, Orcid ID: 0000-0001-8517-7654
[2] Ankara University Graduate School of Natural and Applied Sciences, Ankara, Türkiye. Bilal.Ates@ankara.edu.tr, Orcid ID: 0000-0003-1111-5914

INTRODUCTION

Human position occupying the top of the food pyramid is a significant factor for the pressure they exert on the environment and their susceptibility to environmental problems. The rapid depletion in wild stocks as food reveals the importance of aquaculture. The observation, examination, and analysis are essential to effectively address any issue and ensuring sustainable life requires the maximum utilization of current technological capabilities. Radar and satellite technology, which were called as the most crucial technical solutions of the Second World War and the Cold War, are applied in many fields such as urban planning, geology, meteorology, space research and military operations. It is likely that the use of remote sensing methods for monitoring aquaculture and farming systems and the affecting factors may provide unique perspectives.

Satellite technologies enable the acquisition of new data from physical, biological and chemical observation on global, regional or local scale (Thies and Bendix, 2011; Wu et al., 2019; Giménez et al., 2023). While some data from satellites can be evaluated visually through software, some data can be obtained using calculation algorithms.

Considering the aquaculture production volume and aquaculture in Türkiye, it is expected that the use of technological possibilities and capabilities will be important.

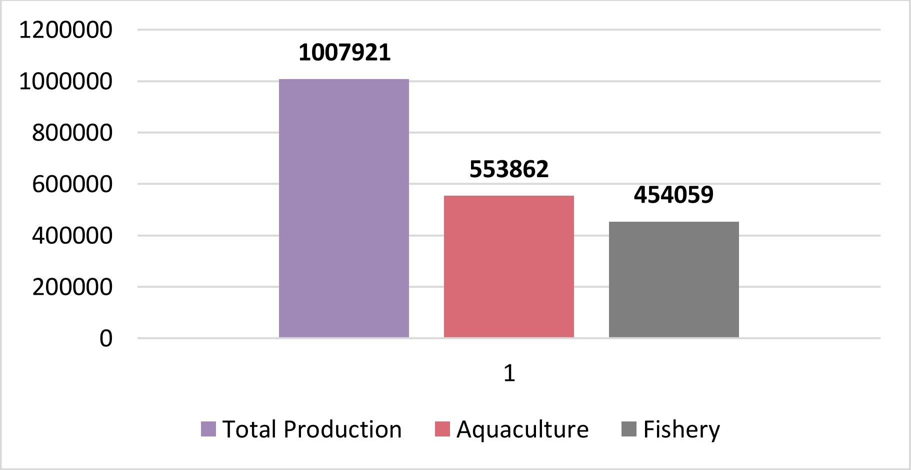

Figure 1: Turkish Aquaculture Production (tons/year) (TUIK, 2023)

1. BRIEF ASSESSMENT OF THE REGIONAL PERSPECTIVE IN MARINE AQUACULTURE PRODUCTION

1.1 Economic Importance of Bivalves and Marine Fish

Although fishing activity started with catch, current aquaculture production has reached over half of the total production. The most important feature of aquaculture is the adaptation to the environment. The cultured species must both adapt to its environment and its negative impact on the environment must be minimized for sustainability.

In 2020, fisheries and aquaculture produced an economic value of 151 billion USD; while 122.6 billion dollars of income was provided by farming alone. Global fish consumption per capita was 20.2 kg. Global ship fleets, especially in China and European countries, have shrunk by 10% due to the impact of the sustainability approach. While China is the country that exports the most aquatic products in the world, the USA is the country that imports the most. (FAO, 2022).

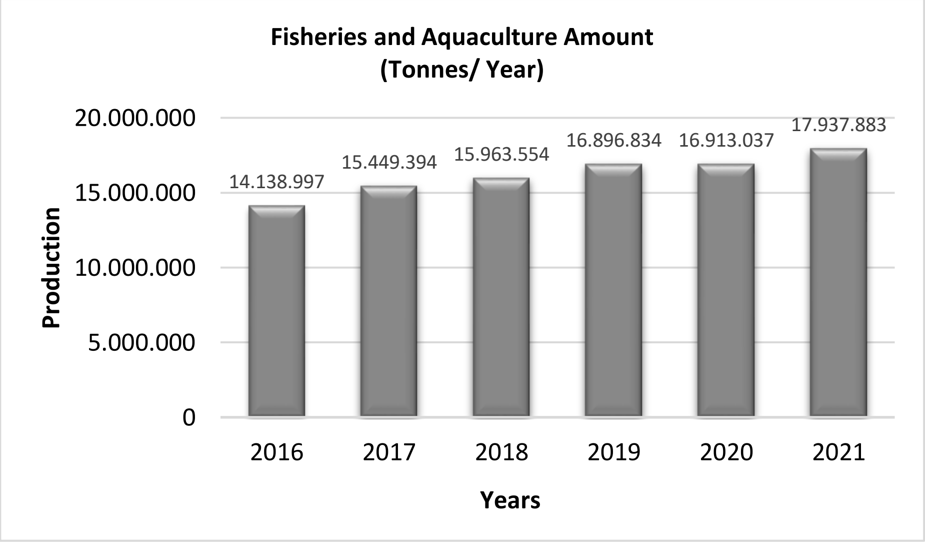

Figure 2: Global Shellfish Production (FAO, 2024)

When the changes in Mediterranean mussel farming over the years are examined, while the global production amount decreases, significant increases in Turkey's production are noticeable:

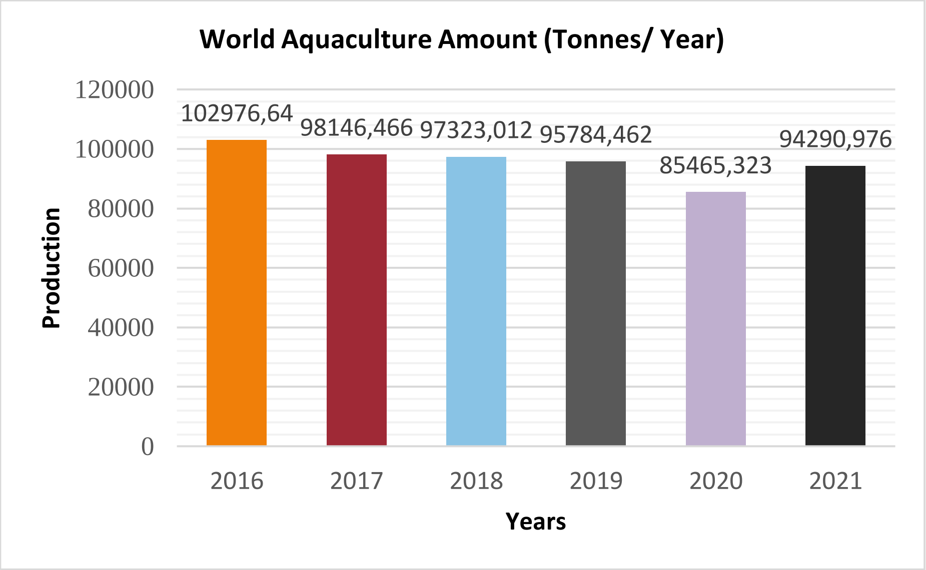

Figure 3: Mediterranean Mussel Global Production (FAO, 2024)

1.2 The Status of the Mediterranean and the Europe in Aquaculture

The European Union has been working on the continuity of aquaculture activities since 2002. In 2009, the European Commission published two communiqués on the development and sustainability of aquaculture products. In 2013, it announced its participation in the Integrated European Principles and in 2019 the Green Deal, which will also cover climate and environmental issues (Puszkarski and Śniadach, 2022).

In 2021, Turkey was the leading country in the world in terms of sea bream (*Sparus aurata*) and sea bass (*Dicentrarus labrax*) production, with a total production of 534.753 tons (FAO, 2024).

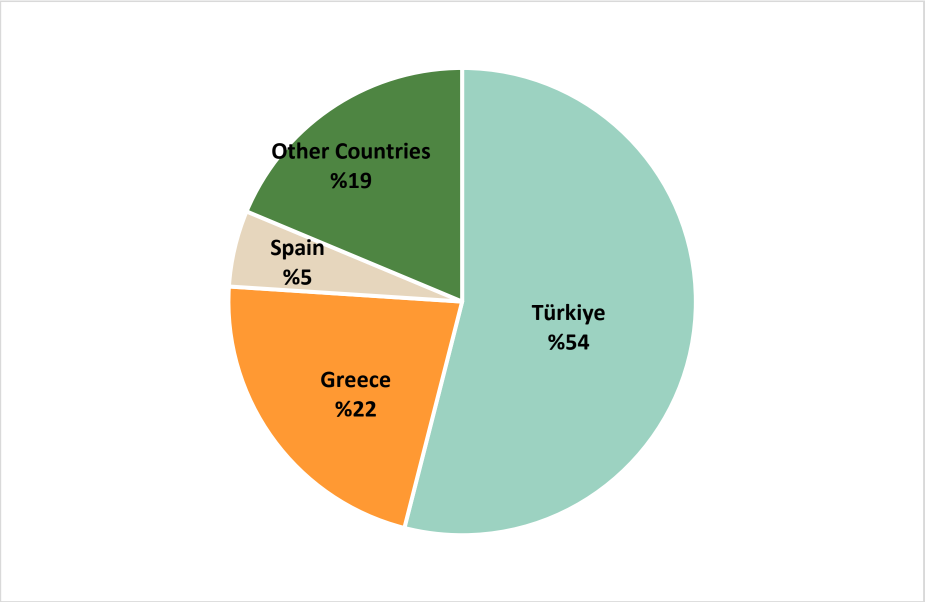

Figure 4: Country Shares in Global Sea Bream (*Sparus aurata*) and Sea Bass (*Dicentrarchus labrax*) Production (%) (FAO, 2024)

In 2021, about 10 million tons of aquaculture products were consumed in European countries. Two out of every three products consumed were obtained from fisheries, and the rest from aquaculture. 1 million tons of products consumed were cultured in Europe; 3 million tons were imported. European aquaculture production reached a value of 4.17 billion euros. The main producers are Spain (24%), France (17%), Italy (13%) and Greece (13%). The distribution of the highest aquaculture amounts by marine species is as follows: Greece, sea bream and sea bass; France, Pacific oyster (*Crassostrea gigas*); Italy, Manila oyster (*Ruditapes philippinarum*); Malta, Atlantic bluefin tuna (*Thunnus thynnus*); Spain, Mediterranean mussel (*Mytilus galloprovincialis*); Ireland, salmon. Freshwater aquaculture accounts for 20% of aquaculture in Europe. The top freshwater species are carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) (EPRS, 2024).

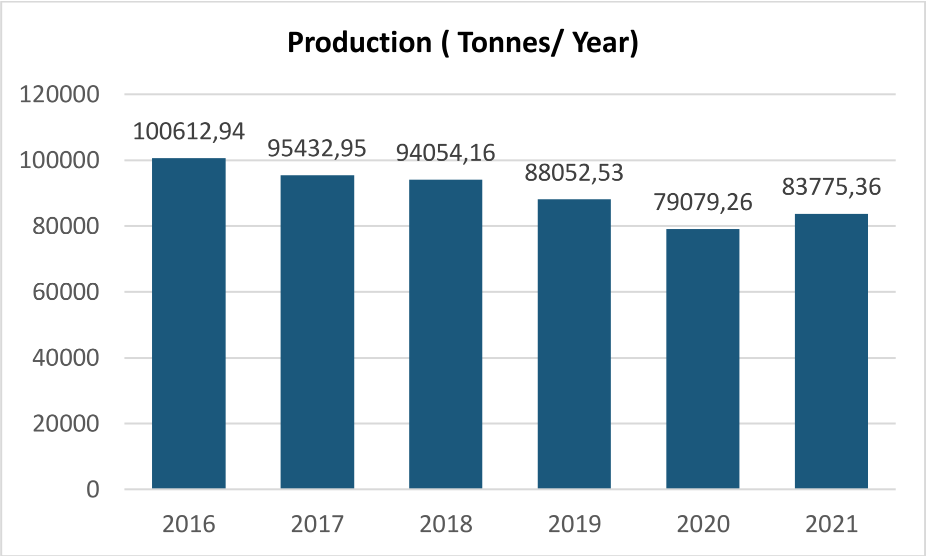

Figure 5: European Mediterranean Mussel Production (FAO, 2024)

2. POTENTIAL OF OCEANOGRAPHIC SATELLITE DATA FOR AQUACULTURE

The limited, regional and uncertain nature of in situ measurements performed in oceans and seas complicate the assessment of standardized data. Satellites are considered to be one of the most important technological tools with their capacity to produce operational data with larger scales, long term series and standards.

Ocean color, salinity, surface wind, ocean topography, wave height and frequency, and surface temperature (SST) measurements can be made with satellites as ocean observations. In addition, special and multi-purpose sensors can be integrated to satellites (Anonymous 2024a, Bayler et al., 2024, Rajeesh and Dwarakish, 2015).

It is possible to capture data with different remote sensing methods of active and passive sensors on satellites (Bayler et al., 2024, Rajeesh and Dwarakish, 2015). It is also possible to obtain diverse data sets by using different methods and algorithms such as machine learning, deep learning, artificial neural networks applied on satellite observation data.

2.1 Use of Oceanographic Satellite Data in Bivalve and Marine Fish Culture

2.1.2 Monitoring of Environmental Conditions

The set of terms used to express the physical, biological and chemical attributes of the aquatic ecosystem can be defined as water quality characteristics (Pulatsü et al., 2014).

The temperature value measured at different depths up to 20 m from the water surface is accepted as the sea surface temperature (Sea Surface Temperature – SST) (Kent et al., 2010, Rajeesh and Dwarakish, 2015), though modern satellite technologies and different measurement techniques now focus on the upper 10 meters. thermal convection between the sea surface and the atmosphere, the mixing ratio of water and wind are accepted as key factors affecting water temperature (Anonymous, 2024c).

Water temperature is the most significant physicochemical factor affecting life, as the oceans cover a large part of the planet surface (approximately 3/4). Depending on annual, seasonal and daily transformations and changes, it has an important role in the formation of air mass formation, the continuity of ocean currents, the migration or adaptation of marine organisms, and is considered a good indicator for determining the status of the global climate trends over extended periods.

Aside from aquatic mammals, all aquatic species are poikilothermic, therefore, changes in normal water temperature can cause physiological and behavioral disorders in fish (Tanyolaç, 2011). Embryonic formation and hatching periods of fish develop according to water temperature (Timur, 2011). Increased water temperature can also increase the toxicity of heavy metals and ammonia (Pulatsü and Topçu, 2012).

Spectroradiometer, infrared radiometer and microwave radiometer sensors used in satellites can perform SST measurements (Anonymous, 2024d). In modern satellites such as Sentinel-3 and Landsat 9, SST measurements are based on infrared radiometer sensor technology. The opportunity to evaluate the data dating back to 1981 contributes to the formation of robust predictions (Anonymous, 2024e).

Anthropogenic energy imbalance is the main factor in the warming of the oceans (Hansen et al., 2011, Forester et al., 2021). The vast majority of the heat capacity (~90%) created as a result of the energy imbalance is retained by the oceans. The remaining heat capacity melts the ice, warms the continents and the atmosphere (von Schuckman et al., 2020). Therefore, monitoring the oceans stands out as a crucial tool in future planning (von Schuckmann et al., 2023, Cheng et al., 2024).

The development of tools for the observation and estimation of the chlorophyll-a density, dissolved organic matter, suspended matter amount and water classification in the oceans, which are important parameters for monitoring algal blooms, climate change, changes in coastal waters, and biological productivity are based on sensor measurements called OLCI (Ocean and Land Color Instrument) (Anonymous 2024b, Bayler et al., 2024)

Algae constitute the primary production in the food chain. There are few harmful species of these microscopic organisms. When species that negatively affect the ecosystem increase their biomass in water, it is called harmful algal bloom (HAB). HAB in freshwater is generally caused by cyanobacteria. HAB in the sea is caused by some dinophlogellates and diatom species. HAB can only change the color tones of red, green and brown on the water surface, or no color change can be seen (Gilbert and Li, 2023)

Harmful algal blooms (HABs) are events that threaten human health as well as aquatic organisms. A cross-section from 1997 to 1998, when algal blooms affected the coasts of Hong Kong and damaged aquaculture in the Pearl River estuary in the South China Sea, was taken and the observations and measurements for November 1998 were examined. In order to determine the chemical and physical properties of the water in the northern South China Sea between the 16th and 18th of November, water temperature, salinity, oxygen, turbidity and chlorophyll-a values were measured with a multiparameter device at 23 stations and water samples were taken. Algal blooms were detected at 9 stations in the Pearl River mouth on 17 November 1998. In addition, water surface temperature (SST) and SeaWiFS data from the NOAA satellite between 17 and 20 November were obtained and analyzed. While previous studies focused on *Phaeocystis globosa* causing algal blooms, this study determined that *Gymnodinium catenatum* was the responsible. Considering that chlorophyll-a increases before algal blooms, it has been suggested that it may be possible to minimize damages by using satellite data (Tang et al., 2003).

Ramesh et al. (2023) tried to prove that harmful algal blooms in the Indian Ocean occur during the time periods when extreme water temperature values occur, using SST and ocean color parameters of the MODIS satellite with 4 km resolution.

The convolutional neural network (CNN) model and the ocean and land color detector (OLCI) of the Sentinel satellites (3A AND 3B) with six spectral bands, 300 m spatial resolution, and high signal-to-noise ratio were used to distinguish harmful algal blooms in coastal areas of South Korea. Depending on the given inputs in the model learning, the consistency in predictions for mixed HAB events decreases, while the consistency increased for HAB caused by a single species (Shin et al., 2022).

Determination and estimation of water quality parameters (Chlorophyll-a, dissolved oxygen, total suspended solids, Secchi disk depth, total dissolved solids and pH) of Gala Lake in Meriç Delta were carried out using linear regression analysis (MLR), artificial neural network (ANN) and support vector machine (SWM) models with principal component analysis (PCA) algorithm with validated Göktürk2, Sentinel 2A and LANDSAT 8 satellite data (Batur and Maktav, 2019).

Dissolved oxygen is an important factor for marine life. The amount of dissolved oxygen on the surface of the sea shores is high (Mavropoulou et al., 2020). Dissolved oxygen in water is formed as a result of atmospheric convection and photosynthesis (Tanyolaç, 2011). There is an inversely proportional interaction between water temperature and dissolved oxygen level (Matear and Hirst, 2003; Mavropoulou et al., 2020). Thus, naturally the dissolved oxygen level decreases when descending to equatorial regions (Kocataş, 2012). Eutrophication, triggered by excessive nutrient enrichment from both human and natural sources, can trigger harmful algal blooms, creating oxygen-deficient zones in bottom waters and leading to ecosystem degradation and biodiversity loss (ESA, 2024).

Li et al (2023) studied dissolved oxygen deficiency in the northern Gulf of Mexico by training MODIS functional data analysis (FDA), linear regression (LLR) and random forest regression (RFR) models using water quality parameters (dissolved oxygen, temperature, and depth) from the Louisiana Universities Marine Consortium (LUMCON) and the Southeast Regional Monitoring and Assessment Program (SEAMAP). RFR has provided the best oxygen estimation.

2.1.3. Assessment of Habitat Suitability

Marine mammals are considered to be one of the indicator organisms that can provide insights about the general health status of the oceans due to the ecosystem they live in. In order to monitor marine mammals to obtain information about the health status of the aquatic habitat for providing early response and mitigation, research studies can be carried out with 27 high-resolution satellites (Very High-Resolution Satellites - VHR) excluding three military satellites. modern VHR satellites can provide a resolution of up to 0.29 m and data analysis through machine learning allows comprehensive information on marine mammals and their habitat (Clarke et al., 2021).

Acidic waters are expected to have adverse effects on the marine food web. For instance, calcium carbonate is essential for the skeletons of many organisms in the oceans, including corals. Increased CO_2 absorption hinders the formation and maintenance of calcium carbonate and accelerates coral reef erosion (Tribollet, 2009). Increases in water temperatures and acidification, as well as decreases in oxygen levels, are also expected to move aquaculture areas to higher latitudes (De Silva, 2009).

2.1.4. Analysis of Biological Cycles

Global warming is accepted as a significant risk factor for biological productivity by reducing vertical water exchanges that enable nutrient transportation from deeper layers in the oceans (Keeling et al., 2010). Warming and changing ocean circulation are also expected to reduce oxygen supply to deep waters, leading to the expansion of subsurface low-oxygen zones in the future. Both the decrease in nutrients at the surface and reduced dissolved oxygen at depth have the potential to change ocean productivity. Satellite observations indicate that the annual warming of the ocean surface leads to a decrease in biological productivity in tropical and subtropical ocean areas and an expansion of the area of surface waters with low phytoplankton biomass (Doney, 2014).

Phytoplankton have been the subject of many studies due to their photosynthetic pigment chlorophyll-a (Cetinić et al., 2024). The presence of mesozooplankton can also be evaluated through the chlorophyll-a value (Druon et al., 2019).

Phytoplankton have a critical role in ocean carbon cycle by absorbing atmospheric carbon dioxide thereby mitigate climate change. Although fluctuations in chlorophyll-a levels measured by satellites in different areas and seasons are considered a natural process, high and persistent concentrations can signal algal blooms (ESA, 2024).

Today, ocean color sensors on satellites enable chlorophyll-a monitoring through various models and algorithms (Harvey et al., 2015, He et al. 2020). While phytoplankton can be detected by remote sensing methods because they have photosynthetic pigments, zooplankton are usually colorless to avoid predation. However, they can be detected by remote sensing because their food contains colored carotenoids (Basedow, 2019).

In the Mar Menor Lagoon in Spain —the largest in Europe— chlorophyll-a was calculated and compared between 2016 and 2022 using field samples from 12 stations and data from Sentinel 3 A and B satellites using machine learning and deep learning techniques and convolutional neural network algorithm. Accordingly, a good correlation was obtained between Sentinel 3 satellite data and in situ measurements (Giménez et al., 2023).

In the Taiwan Strait, the Convolutional Neural Network with Dual Attention Mechanism Optimization (CBAM) model was applied to the data set obtained from MODIS satellites with a resolution of 500 m for chlorophyll-a values between May to September 2022 and compared with the data obtained from 17 marine observation stations of the Fujian Marine Forecast Institution. The results showed that the CNN-CBAM model had more accurate diagnoses than machine learning methods (Yu et al., 2024).

As a result of the synthesis of chlorophyll-a data from SeaWiFS and MODIS satellites in the Bohai Sea of China and the application of Experimental Orthogonal Function (EOF) analysis negative values were presented in coastal areas. These negative values in the marine coastal areas can be interpreted as exposure to anthropogenic effect and the high organic and organic matter transportation by streams (Du et al., 2024).

For the efficient and accurate calculation of chlorophyll-a value in the North Arabian Sea, South Pacific, Red Sea and Florida coasts, the MLPNN (Multilayer Neural Networks) model was trained on NASA Bio-Optical Marine Algorithm Dataset (NOMAD) and validated using satellite matching and simulated datasets with in situ Chl-a concentration. The trained MLPNN model was also successfully applied to global satellite data from SeaWiFS, MODIS, VIIRS and Hawkeye sensors to detect spatial characteristics of phytoplankton increase and physical processes in various aquatic environments. (Kolluru and Tiwari, 2022).

2.1.5. Disease Monitoring

The basic approach in disease research is based on evaluating the environment, host and pathogen (Eisenlord et al., 2016). Warmer winters than average can allow pathogens to persist longer (Harvel et al., 2009). Population declines in some species such as corals, snails and snake grasses are due to the excess of warm days during the year (Doney et al., 2014). Bacterial diseases that increased with extreme summer temperatures between 2005 and 2010 caused major losses in the snail population (*Haliotis cracherodii*) of Chesapeake Bay.

Warming of the oceans causes an increase in the average sea level and changes in ocean currents (Gulev et al., 2021). Warming can lead to deterioration in ecosystems and biodiversity, destruction of coral reefs, increase in disease risk and behavioral changes in aquatic organisms (von Schuckmann et al., 2024).

A statistical model was performed using satellite and in situ observation data to predict the spread of epizootic shell disease correlated on water quality parameters, especially water temperature. An economically important marine species for the USA and Canada, the American Lobster (*Homarus americanus*), was evaluated as an example for modeling. The study showed increased disease incidence during warmer seasons (Maynard ,2016).

Boosted Regression Trees (BRT), a machine learning algorithm which can use SST data, biotic parameters and disease spread metrics for estimating *Montipora* bleaching syndrome, *Porites* growth anomalies and *Porites* tissue loss disease in Hawaii have been applied using SST values from NOAA/NESDIS satellites and disease information from the Hawaiian Coral Disease Database (HICORDIS). This temperature-based prediction model aimed to provide help to scientists and managers with a prediction for mass, acute and fatal diseases (Caldwell et al., 2016).

3. DATA ANALYSIS AND MODELING

The diversity in satellite data measured from a specific sensor necessitates various detection (band and spectrum), algorithm and control processes along with the use of artificial intelligence methods.

Total phosphorus is considered as one of the key factors of eutrophication. In order to estimate the total phosphorus amount in the Yangtze River of China, dependency analyses were carried out using all bands from Senitnel-2 satellite and water quality parameters (total phosphorus, total nitrogen, water temperature, pH, dissolved oxygen, electrical conductivity, turbidity, chemical oxygen demand, ammonium nitrate). As a result of this analysis, a positive correlation was found between total phosphorus and turbidity. The total phosphorus values were measured daily between January 2020 and May 2023 and evaluated using the Center-expansion Radial Based Functional Neural Network (RBFNN) machine learning method, and it was revealed that a prediction could be made from the total phosphorus and turbidity data set obtained from the satellite (Yang et al., 2024).

Chatziantoniou et al. (2022) suggested that dissolved oxygen could be estimated with the Support Vector Regression (SVR) machine learning model using satellite and field data obtained from the Copernicus Marine Area Monitoring Service (CMEMS) of a cage aquaculture farm located in the south-east of the island of Lesvos in the Aegean Sea (Figure 6).

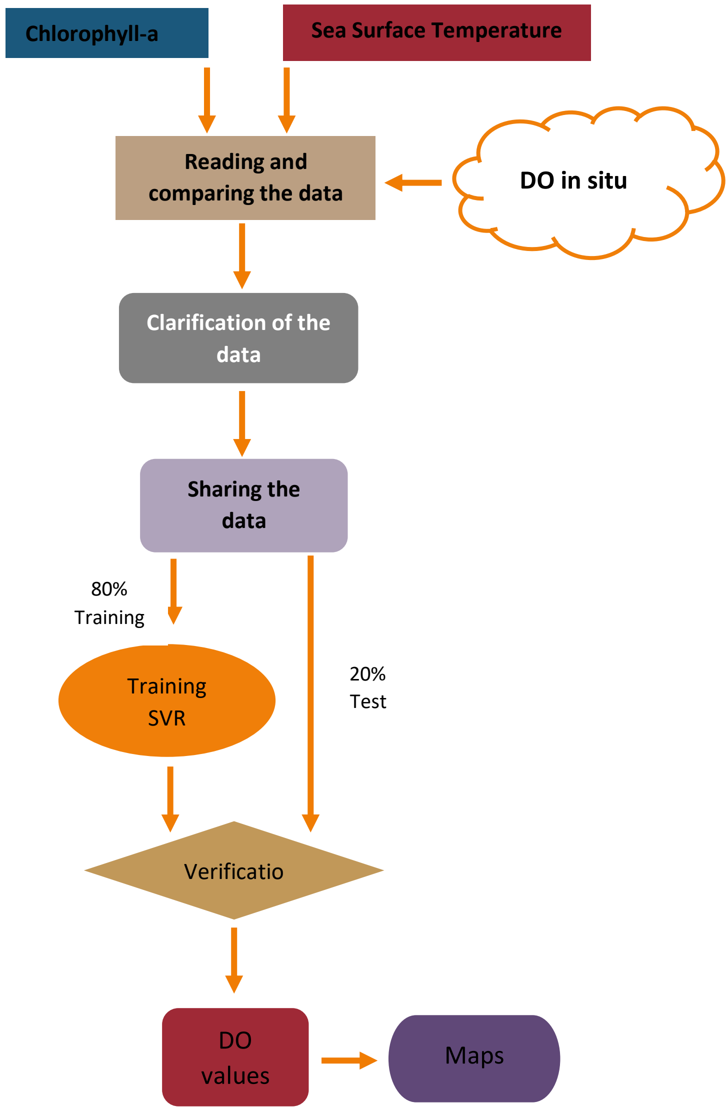

Figure 6: Flowchart for Dissolved Oxygen (DO) Prediction with SVR Machine Learning (Chatziantoniou et al., 2022)

4. CASE STUDIES

Sentinel satellites have been launched in the Copernicus project since 2014 to monitor activities such as the atmosphere, sea, land, climate change, security and emergencies in Europe. With this project, a total of 20 satellites are planned to be in Earth orbit by 2030 (Copernicus, 2024).

In a study for identifying suitable aquaculture areas for sea bream and seabass in the Mediterranean in accordance with the procedures of Maritime Spatial Planning (MSP) and Spatial Multi-Criteria Assessment (SMCE), Porporato et al. (2020), used water temperature (SST) data from CMEMS (marine.copernicus.eu) and wave height data from the Hellenic Marine Research Center (HCMR). An indicator was created based on macro criteria (fish market weight, distance to the port, wave height, etc.) according to the economic, environmental and blue growth scenarios of farming and the site selection. A suitability scale between 0-1 was created for the numerical equivalents of the indicator evaluations. A scale value above 0.6 was accepted for the suitability of the area. In addition, attention was paid to the fact that the selected area was from the place declared as the Special Economic Zone of Italy. According to all the results, the Tyrrhenian and Ionian seas were validated as proper sites for aquaculture.

Satellite data and various water quality parameters were used to evaluate the proper sites for Pacific Oyster (*Crassostrea gigas*) in the Gulf of Bourgneuf, France. While SST, Chlorophyll-a and Total Suspended Solids were evaluated as satellite data, water temperature, chlorophyll-a radiation, turbidity and salinity parameters were measured in situ from 3 different stations on the field. Regular field measurements were used to calculate the growth potential of the organism with Dynamic Energy Budget (DEB), and data sets (2003-2011) obtained from NOAA's MODIS and AVHRR satellites were used to create a prediction model on the productivity and health of the culture stocks according to the quality characteristics of the water (Palmer et al., 2020).

The evaluation of cultivation areas is generally carried out through annual or shorter-term field measurements and observations in many countries. More frequent and long-term data integrity of on-site measurements will enable us to make clear and accurate assessments. With the development of satellite technology, it is possible to obtain continuous and large-scale information with higher resolution. The operational work of satellite data and the regular recording of sensor measurement values to databases are the key factors facilitating access.

5.CONCLUSION AND RECOMMENDATIONS

As rising sea levels have escalated risks for coastal flooding, storms and tropical cyclones, the resulting damage has also increased (IPCC, 2022). Combined with overfishing, coastal ecosystem degradation, and pollution - especially in low-lying areas and on islands- the climate change causes infrastructure fragility and endangers food security (Doney et al., 2014).

It is possible to access many physical, chemical and biological information via satellites such as water temperature, chlorophyll-a, suspended matter, water levels, waves, oxygen. The fact that all living creatures are components of a large ecosystem necessitates interaction with each other. Negative trends in a single species can ripple through ecosystems, sometimes imperceptibly, but often with cumulative effects, studies conducted for longer time periods can lead to different results.

While satellites provide large-scale valuable data, field observations and measurements enable detailed and precise information. The combination of these two methods offers researchers to validate and calibrate satellite-derived data and capture fine-scale variability, providing a more comprehensive understanding of marine biodiversity and ecosystems. This integrated approach supports to develop precise predictive models, monitoring temporal variations, and fostering the management and conservation of aquatic environments (Kavanaugh, 2021).

The Intergovernmental Science-Policy Solidarity for Biodiversity and Ecosystem (IPBES), held in Paris in 2019, highlighted that living species accelerating at an unpredictable rate. It was concluded that the main problems of our ecosystem are the inefficient and healthy use of land and sea areas, over-exploitation, invasive species, climate change and environmental pollution (UN, 2019).

It is an undeniable fact that oceans and seas are the main elements of climate and economy. Since the vast majority of world trade is carried out on seas by commercial ships, coastal population densities are increasing day by day. With a global population of 8 billion and 2.9 billion residing in coastal areas (UNFPA, 2023) pressure on both marine and freshwater bodies is set to intensify (Reimann et al., 2023).

Although the water temperature parameter seems to be a fundamental metric that directly affects ecosystems, biotic factors such as age and size of populations and abiotic factors such as pH, chlorophyll-a, salinity should also be integrated in future studies carried out to determine and predict environmental problems.

Satellite data and its integration with on-site observations is a promising tool for evaluation, observation and validation of marine ecosystems and aquaculture lands for eco-friendly and sustainable production. Supporting current technologies with machine learning and artificial intelligence will provide valuable outputs that will both enrich the scientific database and strengthen field applications.

REFERENCES

Altstatt, J. M., Ambrose, R. F., Engle, J. M., Haaker, P. L., Lafferty, K. D., & Raimondi, P. T. (1996). Recent declines of black abalone Haliotis cracherodii on the mainland coast of central California. *Marine Ecology Progress Series*, 142, 185-192.

Anonymous. (2024a). Retrieved from: https://earth.esa.int/eogateway/news/satellite-data-central-to-ocean-monitoring.

Anonymous. (2024b). Access Date: https://sentiwiki.copernicus.eu/web/s3-olci-instrument.

Anonymous. (2024c). Access Date: https://www.ghrsst.org/ghrsst-data-services/for-sst-data-users/products/.

Anonymous. (2024d). Access Date: https://marine.copernicus.eu/explainers/operational-oceanography/monitoring-forecasting/satellites.

Anonymous. (2024e). Access Date: https://cds.climate.copernicus.eu/datasets/satellite-sea-surface-temperature?tab=overview.

Basedow, S. L., McKee, D., Lefering, I., Gislason, A., Daase, M., Trudnowska, E., ... & Falk-Petersen, S. (2019). Remote sensing of zooplankton swarms. *Scientific Reports*, 9(1), 686.

Batur, E., & Maktav, D. (2018). Assessment of surface water quality by using satellite images fusion based on PCA method in the Lake Gala, Turkey. *IEEE Transactions on Geoscience and Remote Sensing*, 57(5), 2983-2989.

Bayler, E., Chang, P. S., De La Cour, J. L., Helfrich, S. R., Ignatov, A., Key, J., ... & DiGiacomo, P. M. (2024). Satellite Oceanography in NOAA: Research, Development, Applications, and Services Enabling Societal Benefits from Operational and Experimental Missions. *Remote Sensing*, 16(14).

Caldwell, J. M., Heron, S. F., Eakin, C. M., & Donahue, M. J. (2016). Satellite SST-based coral disease outbreak predictions for the Hawaiian Archipelago. *Remote Sensing*, 8(2), 93.

Cheng, L., von Schuckmann, K., Minière, A., Hakuba, M. Z., Purkey, S., Schmidt, G. A., & Pan, Y. (2024). Ocean heat content in 2023. *Nature Reviews Earth & Environment*, 5(4), 232-234.

Clarke, P. J., Cubaynes, H. C., Stockin, K. A., Olavarría, C., de Vos, A., Fretwell, P. T., & Jackson, J. A. (2021). Cetacean strandings from space: challenges and opportunities of very high resolution satellites for the remote monitoring of cetacean mass strandings. *Frontiers in Marine Science*, 8, 650735.

Copernicus. (2024). Copernicus in detail. https://www.copernicus.eu/en/about-copernicus/copernicus-detail. (Access date: 07.06.2024).

De Silva, S. S., & Soto, D. (2009). Climate change and aquaculture: Potential impacts, adaptation and mitigation. *Climate Change Implications for Fisheries and Aquaculture: Overview of Current Scientific Knowledge*. FAO Fisheries and Aquaculture Technical Paper No. 530, 151-212.

Doney, S., Rosenberg, A. A., Alexander, M., Chavez, F., Harvell, C. D., Hofmann, G., ... & Ruckelshaus, M. (2014). Oceans and marine resources. *Climate Change Impacts in the United States: The Third National Climate Assessment*. U.S. Global Change Research Program.

Druon, J. N., Hélaouët, P., Beaugrand, G., Fromentin, J. M., Palialexis, A., & Hoepffner, N. (2019). Satellite-based indicator of zooplankton distribution for global monitoring. *Scientific Reports*, 9(1), 4732.

Eisenlord, M. E., Groner, M. L., Yoshioka, R. M., Elliott, J., Maynard, J., Fradkin, S., ... & Harvell, C. D. (2016). Ochre star mortality during the 2014 wasting disease epizootic: Role of population size structure and temperature. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1689), 20150212.

EPRS. (2024). Briefing Report of "EU aquaculture: State of play".

ESA. (2024). Chlorophyll in Europe's transitional, coastal and marine waters. Available: https://www.eea.europa.eu/en/analysis/indicators/chlorophyll-in-transitional-coastal-and?activeAccordion= .

FAO. (2022). The State of World Fisheries and Aquaculture Report: Towards Blue Transformation. Rome.

FAO. (2024). FishStat. Available at: https://www.fao.org/fishery/statistics-query/en/home.

Forster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J.-L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M., & Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks and Climate Sensitivity. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis*. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 923–1054). Cambridge University Press. https://doi.org/10.1017/9781009157896.009

Giménez, J. G., Martínez-España, R., Cano, J. C., & Cecilia, J. M. (2023, June). Estimation of Chl-a in highly anthropoized environments using machine learning and remote sensing. In *2023 19th International Conference on Intelligent Environments (IE)* (pp. 1-8). IEEE.

Glibert, P. M., & Li, M. (2023). Warming, Wheezing, Blooming Waters: Hypoxia and Harmful Algal Blooms.

Gulev, S. K., Thorne, P. W., Ahn, J., Dentener, F. J., Domingues, C. M., Gerland, S., Gong, D., Kaufman, D. S., Nnamchi, H. C., Quaas, J., Rivera, J. A., Sathyendranath, S., Smith, S. L., Trewin, B., von Schuckmann, K., & Vose, R. S. (2021). Changing State of the Climate System. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis*. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 287–422). Cambridge University Press. https://doi.org/10.1017/9781009157896.004

Hansen, J., Sato, M., Kharecha, P., & von Schuckmann, K. (2011). Earth's energy imbalance and implications. *Atmospheric Chemistry and Physics*, 11, 13421–13449. https://doi.org/10.5194/acp-11-13421-2011

Harvell, D., Altizer, S., Cattadori, I. M., Harrington, L., & Weil, E. (2009). Climate change and wildlife diseases: When does the host matter the most? *Ecology*, 90, 912-920. https://doi.org/10.1890/08-0616.1

IPCC. (2022). Summary for Policymakers. In H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegria, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, & B. Rama (Eds.), *Climate Change 2022: Impacts, Adaptation, and Vulnerability*. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 3–33). Cambridge University Press. https://doi.org/10.1017/9781009325844.001

Kavanaugh, M. T., Bell, T., Catlett, D., Cimino, M. A., Doney, S. C., Klajbor, W., Messié, M., Montes, E., Muller-Karger, F. E., Otis, D., Santora, J. A., Schroeder, I. D., Triñanes, J., & Siegel, D. A. (2021). Satellite remote sensing and the Marine Biodiversity Observation Network: Current science and future steps. *Oceanography*, 34(2), 62–79. https://doi.org/10.5670/oceanog.2021.215

Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. *Annual Review of Marine Science*, 2, 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

Kent, E.C., Kennedy, J.J., Berry, D.I., & Smith, R.O. (2010). Effects of instrumentation changes on sea surface temperature measured in situ. *WIREs Clim Change, 1*, 718-728. https://doi.org/10.1002/wcc.55

Kocataş, A. (2012). *General Oceanology, Introduction to Marine Sciences* (10th ed.). Bursa.

Kolluru, S., & Tiwari, S.P. (2022). Modeling ocean surface chlorophyll-a concentration from ocean color remote sensing reflectance in global waters using machine learning. *Science of The Total Environment, 844*, 157191. https://doi.org/10.1016/j.scitotenv.2022.157191

Li, Y., Li, S., Song, K., Liu, G., Wen, Z., Fang, C., Shang, Y., Lyu, L., & Zhang, L. (2023). Sentinel-3 OLCI observations of Chinese lake turbidity using machine learning algorithms. *Journal of Hydrology, 622*, 129668.

Matear, R.J., & Hirst, A.C. (2003). Long-term changes in dissolved oxygen concentrations in the ocean caused by protracted global warming. *Global Biogeochemical Cycles, 17*(4).

Mavropoulou, A.M., Vervatis, V., & Sofianos, S. (2020). Dissolved oxygen variability in the Mediterranean Sea. *Journal of Marine Systems, 208*, 103348.

Maynard, J., van Hooidonk, R., Harvell, C.D., Eakin, C.M., Liu, G., Willis, B.L., Williams, G.J., Groner, M.L., Dobson, A., Heron, S.F., Glenn, R., Reardon, K., & Shields, J.D. (2016). Improving marine disease surveillance through sea temperature monitoring, outlooks and projections. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 371*(1689), 20150208. https://doi.org/10.1098/rstb.2015.0208

Melillo, J. M., Richmond, T. C., & Yohe, G. W. (Eds.). (2021). *Climate Change Impacts in the United States: The Third National Climate Assessment* (pp. 557-578). U.S. Global Change Research Program. https://doi.org/10.7930/J0RF5RZW

Palmer, S.C., Gernez, P.M., Thomas, Y., Simis, S., Miller, P.I., Glize, P., & Barillé, L. (2020). Remote sensing-driven Pacific oyster (Crassostrea gigas) growth modeling to inform offshore aquaculture site selection. *Frontiers in Marine Science, 6*, 802.

Pastor, F. (2021). Sea surface temperature: From observation to applications. *Journal of Marine Science and Engineering, 9*(11), 1284. https://doi.org/10.3390/jmse9111284

Porporato, E.M., Pastres, R., & Brigolin, D. (2020). Site suitability for finfish marine aquaculture in the Central Mediterranean Sea. *Frontiers in Marine Science, 6*, 772.

Pulatsü, S., Topçu, A., & Atay, D. (2014). *Water Pollution and Control*. Ankara University Press.

Puszkarski, J., & Śniadach, O. (2022). Instruments to implement sustainable aquaculture in the European Union. *Marine Policy, 144*, 105215. https://doi.org/10.1016/j.marpol.2022.105215

Rajeesh, R., & Dwarakish, G.S. (2015). Satellite Oceanography– A review. *Aquatic Procedia, 4*, 10.1016/j.aqpro.2015.02.023

Ramesh, N.V.K., Spandana, A.P., Sri, N.N., Kumar, D.R., Ratnam, D.V., & Vani, B.V. (2023). Monitoring harmful algal blooms in the Indian Ocean region using satellite remote sensing data. In *2023 IEEE Renewable Energy and Sustainable E-Mobility Conference (RESEM)* (pp. 1-5). IEEE.

Reimann, L., Vafeidis, A.T., & Honsel, L.E. (2023). Population development as a driver of coastal risk: Current trends and future pathways. *Cambridge Prisms: Coastal Futures, 1*, e14.

Shin, J., Khim, B.K., Jang, L.H., Lim, J., & Jo, Y.H. (2022). Convolutional neural network model for discrimination of harmful algal bloom (HAB) from non-HABs using Sentinel-3 OLCI imagery. *ISPRS Journal of Photogrammetry and Remote Sensing, 191*, 250-262.

Tang, D., Kester, D.R., Ni, I.H., Qi, Y., & Kawamura, H. (2003). In situ and satellite observations of a harmful algal bloom and water condition at the Pearl River estuary in late autumn 1998. *Harmful Algae, 2*(2), 89-99.

Tanyolaç, L. (2011). *Limnology, Freshwater Science* (6th ed.). Hatipoğlu Publishing House.

Thies, B., & Bendix, J. (2011). Satellite-based remote sensing of weather and climate: Recent achievements and future perspectives. *Meteorological Applications, 18*(3), 262-295.

Tribollet, A., Godinot, C., Atkinson, M., & Langdon, C. (2009). Effects of elevated pCO2 on dissolution of coral carbonates by microbial euendoliths. *Global Biogeochemical Cycles, 23*, GB3008. https://doi.org/10.1029/2008GB003286

UN. (2009). *United Nations. Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) Report*. 29 April – 4 May 2019. Paris.

UNFPA. (2023). *World Population Status Report*.

von Schuckmann, K., Cheng, L., Palmer, M.D., Hansen, J., Tassone, C., Aich, V., Adusumilli, S., Beltrami, H., Boyer, T., Cuesta-Valero, F.J., Desbruyères, D., Domingues, C., García-García, A., Gentine, P., Gilson, J., Gorfer, M., Haimberger, L., Ishii, M., Johnson, G.C., Killick, R., King, B.A., Kirchengast, G., Kolodziejczyk, N., Lyman, J., Marzeion, B., Mayer, M., Monier, M., Monselesan, D.P., Purkey, S., Roemmich, D., Schweiger, A., Seneviratne, S.I., Shepherd, A., Slater, D.A., Steiner, A.K., Straneo, F., Timmermans, M.-L., & Wijffels, S.E. (2020). Heat stored in the Earth system: Where does the energy go? *Earth System Science Data, 12*, 2013–2041. https://doi.org/10.5194/essd-12-2013-2020

von Schuckmann, K., Moreira, L., Cancet, M., Gues, F., Autret, E., Baker, J., Bricaud, C., Bourdalle-Badie, R., Castrillo, L., Cheng, L., Chevallier, F., Ciani, D., de Pascual-Collar, A., De Toma, V., Marie, D., Claudia, F., Garric, G., Gehlen, M., Giesen, R., Hodges, K., Iovino, D., Jandt-Scheelke, S., Jansen, E., Juza, M., Karagali, I., Lavergne, T., Masina, S., McAdam, R., Minière, A., Morrison, H., Panteleit, T.R., Pisano, A., Pujol, M., Stoffelen, A., Thual, S., Gennip, S.V., Veillard, P., Yang, C., & Zuo, H. (2024). The state of the global ocean. *State of the Planet, 4*, 1-30.

Wu, X., Xiao, Q., Wen, J., You, D., & Hueni, A. (2019). Advances in quantitative remote sensing product validation: Overview and current status. *Earth-Science Reviews, 196*, 102875.

Yang, F., Feng, Q., Zhou, Y., Li, W., Zhang, X., & He, B. (2024). Monitoring total phosphorus concentration in the middle reaches of the Yangtze River using Sentinel-2 satellites. *Remote Sensing, 16*(9), 1491.

CHAPTER 11

CRISPR TECHNOLOGY AND ITS AREAS OF USE IN AQUACULTURE

Assoc. Prof. Dr. Şükrü ÖNALAN [1]
Zilan YILMAZ[2]
Aybike ÇELİK[3]

[1] Van Yuzuncu Yil University, Fisheries Faculty, Department of Diseases, Van, Türkiye. sukruonalan@yyu.edu.tr, Orcid ID: 0000-0003-0058-5232
[2] Zonguldak Bülent Ecevit University, Faculty of Science, Molecular Biology and Genetics, Zonguldak/Van, Türkiye. zilan.yilmaz@fef.karaelmas.edu.tr, Orcid ID: 0009-0006-6364-6951
[3] Middle East Technical University, Faculty of Arts and Science, Department of Biological Sciences, Department of Molecular Biology and Genetics, Ankara, Türkiye. aybike.celik@metu.edu.tr, Orcid ID: 0009-0002-5974-5050

INTRODUCTION

CRISPR stands for “clustered regularly interspaced short palindromic repeats,” which identifies unique DNA sequences within other constantly repeating DNA sequences in bacterial genomes (Leung & Jia, 2018). Bacteria then transcribe these DNA elements into RNA upon viral infection. The RNA directs a nuclease (a protein that cuts DNA) to the viral DNA, cutting it and providing defense against the virus. The nucleases were named “Cas”, meaning “CRISPR-associated” (Westra et al., 2014). It makes it possible to correct existing errors in the genome and turn genes on and off in cells and organisms quickly, cheaply and easily. It has a number of laboratory applications to enable rapid generation of cellular and animal models, functional genomic screens, and live imaging of the cellular genome (Redman et al., 2016). Researchers have placed emphasis on developing new tools that increase the scope and efficiency of genome editing, especially in eukaryotic cells and animal models of human diseases (Komor et al., 2017).

CRISPR/Cas9 is a gene editing technology consisting of two main components: A guide RNA that matches the targeted gene and Cas9 (CRISPR-associated protein 9), an endonuclease that breaks double-stranded DNA allowing changes to the genome (Redman et al., 2016).

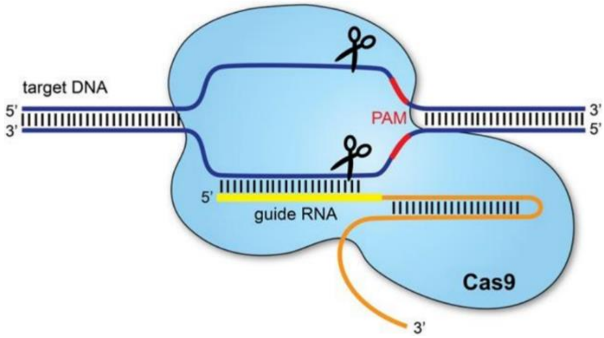

Figure 1. Schematic Illustration of Guide RNA Directing Cas9 to Target DNA for Cutting (Redman et al., 2016).

In nature, the CRISPR-Cas system is a prokaryotic adaptive immune mechanism used to degrade invading nucleic acids. A variety of CRISPR-Cas systems exist among various species of bacteria and archaea, differing in their components and mechanisms of action. For example, class 1 CRISPR-Cas systems consist of multiprotein effector complexes, whereas class 2 systems have a single effector protein; overall, there are six CRISPR-Cas types and at least 29 subtypes (Makarova et al., 2015).

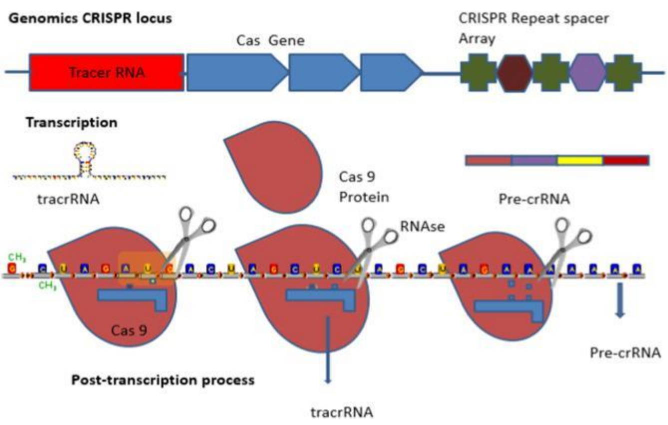

Figure 2. General Biology of CRISPR Cas Genome Editing Technology (Hussain et al., 2019).

HISTORY OF CRISPR

CRISPR began to become widespread as archaeal and bacterial genomes emerged from the first wave of genome sequencing in the late 1990s and early 2000s (Han & She, 2017).

CRISPR repeats were first discovered by chance in 1987. It was detected in Escherichia coli during the analysis of the gene responsible for the isozyme conversion of alkaline phosphatase (Ishino et al., 2018). The repeat sequence contains 5 repeats of the same 29-nt sequence interspersed with different 32-nt sequences that are part of the 12 repeat loci clustered by the CRISPR-Cas system in *E. coli*. Subsequently, similar repetitive sequences were identified in other *E. coli* strains as well as in closely related enterobacteria, including Shigella dysenteriae and Salmonella enterica. Similarly, multiple 36-bp direct repeats (DRs) spanning 35 to 41 bp specific callators have been found in *Mycobacterium tuberculosis*. In fact, the DRs polymorphic feature in M. tuberculosis strains has been found to be useful in strain typing. This has revealed the prevalence of the DNA element in bacteria as well as extreme diversity due to the different repeat and spacer units in very closely related strains (Han & She, 2017).

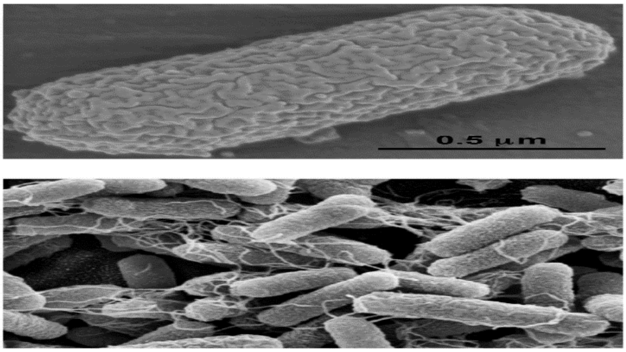

Figure 3. Image of *E. coli* under the microscope (Blount, 2015)

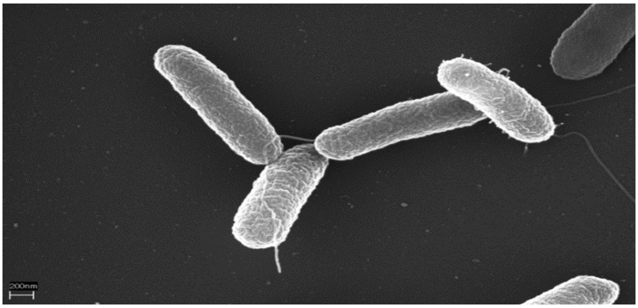

Figure 4. Image of Salmonella under the Microscope (Salmonella, 2024).

The history of CRISPR/Cas9 began with a microbiologist named Francisco Mojica, who worked at the University of Alicante in Spain. His research centered on a microbe called *Haloferax mediterranei*, a species in the family of archaea known for its resistance to extreme salt levels. This resistance was observed because restriction enzymes cut the microbe's genome in a manner dependent on environmental salt levels. In 1993, Mojica and colleagues discovered repeat sequences of 30 base pairs (bp) separated by “intermediate” sequences of approximately 36 bp in the genome of this *H. mediterranei* (Xu & Tsang, 2022).

Upon further investigation, these interesting repeat sequences were discovered by Mojica and colleagues to be conserved in other species, such as *Haloferax volcanii* and the more distantly related Halophilic archaea. Over the next 20 years, Mojica and colleagues found loci belonging to "short regularly interspaced repeats," later renamed CRISPR, in more than twenty different microbial species.

The Basic Local Alignment Search Tool program was used to analyze spacers from different species and determine whether they had genetic similarity to any known DNA sequences (Xu & Tsang, 2022).

Figure 5. Francis Mojica

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Almost two-thirds of the 88 microbial inserts examined were found to match viruses or microbe-related conjugative plasmids. In line with these data, it was hypothesized that CRISPR loci may be involved in the microbial adaptive immune system as a protective mechanism against pathogens (Mojica et al., 2005). CRISPR was introduced by Jansen and colleagues in 2002, and this abbreviation was quickly adopted by the research community, probably because it better reflects the characteristic structures of repeats (Jansen et al., 2002).

In the same article, Jansen and colleagues reported that CRISPR loci are linked to a group of genes, and that several of them are well conserved in organisms containing CRISPR but not in those lacking any CRISPR elements. These genes were identified as CRISPR-associates (cas) genes, and the first four cas genes (encoding Cas1-4 proteins) were found to be distributed in gene clusters in the immediate vicinity of the CRISPR loci (Jansen et al., 2002).

In 2007, it was experimentally determined by Barrangou and her colleagues that prokaryotes can acquire new intermediate pieces from the extrachromosomal genetic element as a part of the immune defense system (Barrangou et al., 2007).

They tested susceptible *S. thermophilus* strains with two phages and found that the survivors acquired new spacers alongside old spacers at the phage-derived sequences CRISPR loci. When re-exposed to phages, survivors showed resistance, confirming that spacers matching the phage genome conferred immunity (Barrangou et al., 2007).

In a study published in the journal Science in 2008, Brouns and colleagues proposed that five Cas proteins are required for the cleavage of a long precursor RNA strand derived from the CRISPR locus into a compact CRISPR RNA (crRNA) strand. Additionally, it was determined that palindromic repeats support the process of forming the secondary structure of crRNA. Additionally, CRISPR was programmed by targeting genes in the lambda phage, making CRISPR successfully programmed for the first time in history. The study resulted in the assumption that CRISPR targets DNA rather than RNA (Brouns et al., 2008).

Figure 6. Rodolphe Barrangou

In 2012, Jinek and colleagues proposed that a single guide RNA (sgRNA), i.e., a single engineered RNA chimera, could perform the same function as the tracrRNA: crRNA complex. This discovery will change the future of CRISPR by making it more efficient and programmable for research purposes (Jinek et al., 2012).

In 2013, successful CRISPR gene editing was performed in mammalian cells in many laboratories. Cong and colleagues published an article in the journal Science reporting tracrRNA and pre-crRNA models used to target the human EMX1 locus in mammalian cells along with S. pyogenes Cas9 (SpCas9). Additionally, this team of researchers developed a crRNA: tracrRNA duplex with chimeric RNA and targeted identified genomic locations in human and mouse cells (human PVALB and mouse Th) (Cong et al., 2013).

George Church's laboratory has investigated the utility of CRISPR in both non-homologous end joining (NHEJ) and homologous recombination in mammalian cells (Mali et al., 2013). Jennifer Doudna's laboratory has published findings of CRISPR gene editing in human cells using sgRNA (Jinek et al., 2013). Keith Joung's laboratory reported using CRISPR to target zebrafish embryos in vivo and introduce deletions into the germline (Maeder et al., 2013).

In recent studies, two women, Emmanuelle Charpentier and Jennifer Doudna, who made important discoveries in the field of DNA manipulation with the CRISPR-Cas9 system, called "genetic scissors", in 2020, a few years after the development of a genome editing method based on CRISPR-Cas9 technology and the discovery of all the basic molecular components of the system. , was awarded the Nobel Prize in Chemistry (Gostimskaya, 2022).

Figure 7. Jennifer Doudna and Emmanuelle Charpentier (Mansfiels, 2023).

TOOLS AND EQUIPMENT USED IN CRISPR APPLICATIONS

The protocol used in CRISPR application can be divided into prokaryotic and eukaryotic, due to reasons such as differences in cellular structures and genetic material (Hillary & Ceasar, 2022).

Tools and Equipment for Prokaryote Protocell:

1. Components That Make Up the CRISPR-Cas System (Hillary & Ceasar, 2022):

a. **Plasmids**
   - Cas Proteins: Enzyme endonuclease used to cut DNA fragments.
   - Guide RNA (gRNA): Guide RNA will direct the Cas protein to the matching DNA sequence.
   - Promoter: It will direct the expression of Cas protein and gRNA in bacterial cells.

b. Donor DNA (optional) (Richardson et al., 2016): The template created for homologous recombination is used if specific mutations or insertions are desired.

2. Bacterial Strain (Arroyo-Olarte et al., 2021):

a.Competent Cells

• These are bacterial cells that can take in foreign DNA. These can be chemical or electrocompetent cells.

3. Feedlot (Shabbir et al., 2016):

a) LB Broth or Agar: Used to grow bacterial cultures. Antibiotics may be added for selection.

4. Conversion Reagents (Lemay et al., 2018):

a) Calcium Chloride ($CaCl_2$): Used to develop chemically competitive cells.

b) Electroporation Cuvettes and Electroporator: Allows to introduce plasmids into electrocompetent cells.

5. Molecular Biology Reagents (Ramlee et al., 2015):

PCR Reagents: The system that allows amplifying DNA fragments is as follows

• Taq Polymerase or High Fidelity Polymerase

• dNTPs

• Target Sequence Specific Primers

a) DNA Gel Electrophoresis Equipment:

• Agarose

• Ethidium Bromide or GelRed: To stain DNA.

• DNA Ladder

• Gel Imaging System

6. Selection and Screening (Evans et al., 2018):

a) Screening Reagents: Preferred to confirm successful CRISPR modifications.

- Restriction Enzymes: To control regulations and digest PCR products.
- Sequencing Applications: To verify exact genetic changes.

7. Laboratory Equipment (Yang et al., 2014):

a) Centrifuge: To purify DNA and separate cells into pellets.

b) Incubator: Provides a suitable environment for the growth of bacterial cultures.

c) Micropipettes and Tips: For accurate measurement and transfer of liquids to different media.

d) Vortex Mixer: To mix reagents and samples.

e) Heat Block or Water Bath: To ensure incubation thermal balance during transformation and PCR.

Tools and Equipment Required for the Eukaryote Protocol:

1. CRISPR-Cas System Components (Hillary & Ceasar, 2022):

a) Plasmids:

- Cas Proteins: Endonuclease that cuts DNA.
- Guide RNA (gRNA): Designed to match the target DNA sequence. It directs the Cas protein to the correct location.
- Promoter: Directs the expression of Cas protein and gRNA in eukaryotic cells.

b) Donor DNA (optional) (Richardson et al., 2016):

A template for homologous recombination is used to make specific mutations or insertions.

2. Cell Culture (Wang et al., 2016):

a) Eukaryotic Cell Line: The specific cell line that is desired to be edited.

b) Cell Culture Medium: Suitable environment for growing the cell line.

c) Antibiotics: To ensure selection and prevent contamination.

3. Transfection Reagents (Cheng et al., 2021):

a) Lipofectamine or Similar Reagents (Yu et al., 2016): It is the reagent used to introduce plasmids into eukaryotic cells.

b) Electroporation Cuvettes and Electroporator: It is an alternative method used to introduce plasmids into cells.

c) Nucleofector Kits: To enable electroporation of cells that are difficult to transfect.

4. Molecular Biology Reagents (Santos et al., 2016):

a) **PCR Reagents**: Enables amplification of DNA fragments.

- High Fidelity Polymerase

• dNTPs

• Primers: Specific to the target sequence.

• PCR Buffer

b) **DNA Gel Electrophoresis Equipment:** Analyzes PCR products.

• Agarose

• Ethidium Bromide or GelRed: To stain DNA.

• DNA Ladder

• Gel Imaging System

5. Selection and Screening (Song et al., 2017):

a) Antibiotics or Selection Markers: Allows to select cells that receive the CRISPR plasmid.

b) Screening Reagents: To confirm successful CRISPR edits.

- Restriction Enzymes: Allows to break down PCR products that control the regulations.
- Sequencing Applications: Allows to verify exact genetic changes.
- Surveyor or T7E1 Test (Vouillot et al., 2015): Detects insertions or deletions in the target area.
- Western Blot: Cas9 veya düzenlenmiş proteinlerin eskpresyonunu kontrol eder.

6. Laboratory Equipment (Fernández et al., 2020):

a) Centrifuge: It ensures the purification of DNA and the formation of a pellet of the cell.

b) Cell Culture Incubator: Used to grow eukaryotic tables.

c) Micropipettes and Tips: Provide accurate images and transfer of liquids to different environments.

d) Vortex Mixer: Allows mixing reagents and samples.

e) Heat Block or Water Bath: Used for incubation steps during transformation and PCR and provides thermal balance.

GENERAL METHODOLOGY IN CRISPR STUDIES

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful and versatile technology used to edit genomes. This technology allows scientists to alter DNA sequences and gene function. The general methodology of CRISPR includes the following steps:

1. Identification of Target DNA Sequence (White et al., 2015)

Target Selection: This allows selection of a specific DNA sequence that you want to change. This sequence is typically 20 base pairs long. The target sequence must be different within the genome to avoid off-target effects.

PAM Sequence: The target DNA sequence for Cas9 must be a Protospacer Adjacent Motif (PAM), usually "NGG" (N can be any nucleotide). The presence of PAM is required for binding and cutting of Cas

Guide RNA (gRNA) Design: Guide RNA is designed to be complementary to the target DNA sequence. gRNA consists of two parts: target sequence-compatible crRNA (CRISPR RNA) and Cas9-binding tracrRNA (trans-activating crRNA). These two fragments can be combined into a single guide RNA (sgRNA) for simplicity.

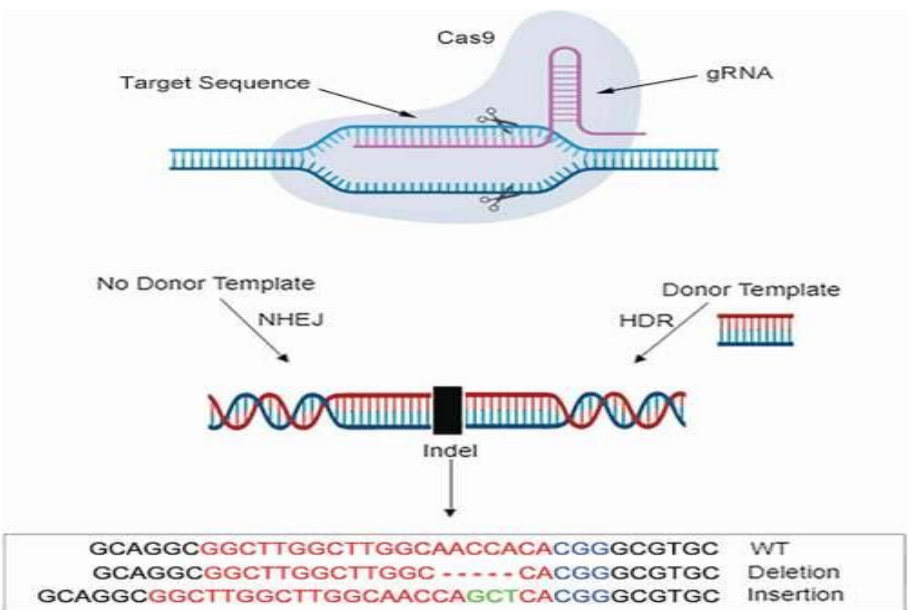

Figure 8. Schematic Representation of the Function of Donor DNA

2. Generation of CRISPR Components (Fuziwara et al., 2022)

Cas Proteins: Cas proteins are an endonuclease enzyme that causes double-strand breaks in DNA and can be designed for different properties.

Guide RNA (gRNA): gRNA can be synthesized chemically or transformed in vitro. gRNA is crucial for directing Cas9 to the correct side of the genome.

3. Delivery to Cells (Chandrasekaran et al., 2018)

Vector delivery: Plasmids, circular DNA molecules, can deliver genes coding for Cas9 and gRNA into cells. Viral vectors such as lentiviruses or adenoviruses can be used for transduction in cells that are particularly difficult to transfect.

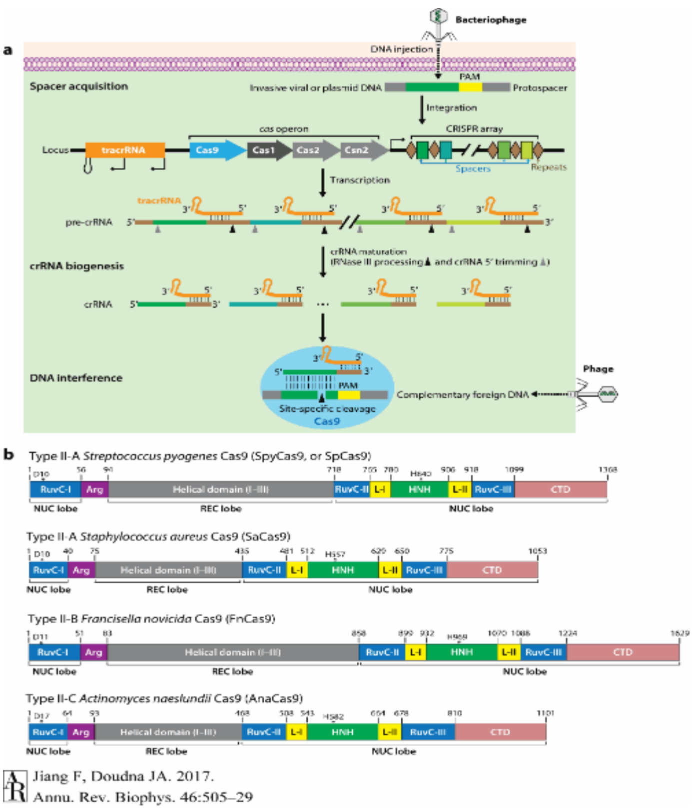

Figure 9. Schematic Illustration of CRISPR Cas9 Vector Delivery to Bacterial Immunity (Jiang & Doudna, 2017).

Electroporation and Microinjection: Electroporation uses an electric field to make cell membranes more permeable, allowing CRISPR components to enter cells. Microinjection involves injecting CRISPR components into cells using a fine needle. This is especially useful for single-celled organisms or embryos.

4. DNA Cleavage (Rivera-Torres & Kmiec, 2017)

Binding: Once inside the cell, the gRNA-Cas9 complex scans the DNA for a target sequence adjacent to a PAM site. The gRNA hybridizes with the complementary DNA strand and the Cas9 protein changes structurally.

Cut: Cas9 causes a double-strand break in the DNA at the target site. This break occurs three nucleotides upstream of the PAM sequence and has a blunt end.

5. DNA Repair (Babu et al., 2010)

Non-Homologous End Joining (NHEJ): NHEJ is a repair mechanism that can quickly rejoin the broken ends of DNA. This process is error-prone and often introduces insertions or deletions (indels) that disrupt the target gene, effectively disabling it.

Homology-Directed Repair (HDR): HDR is a precise mechanism that uses a homologous DNA template to accurately repair the break. Scientists can make specific mutations, corrections, or insertions using a donor template with the desired genetic structure flanked by sequences homologous to the break site.

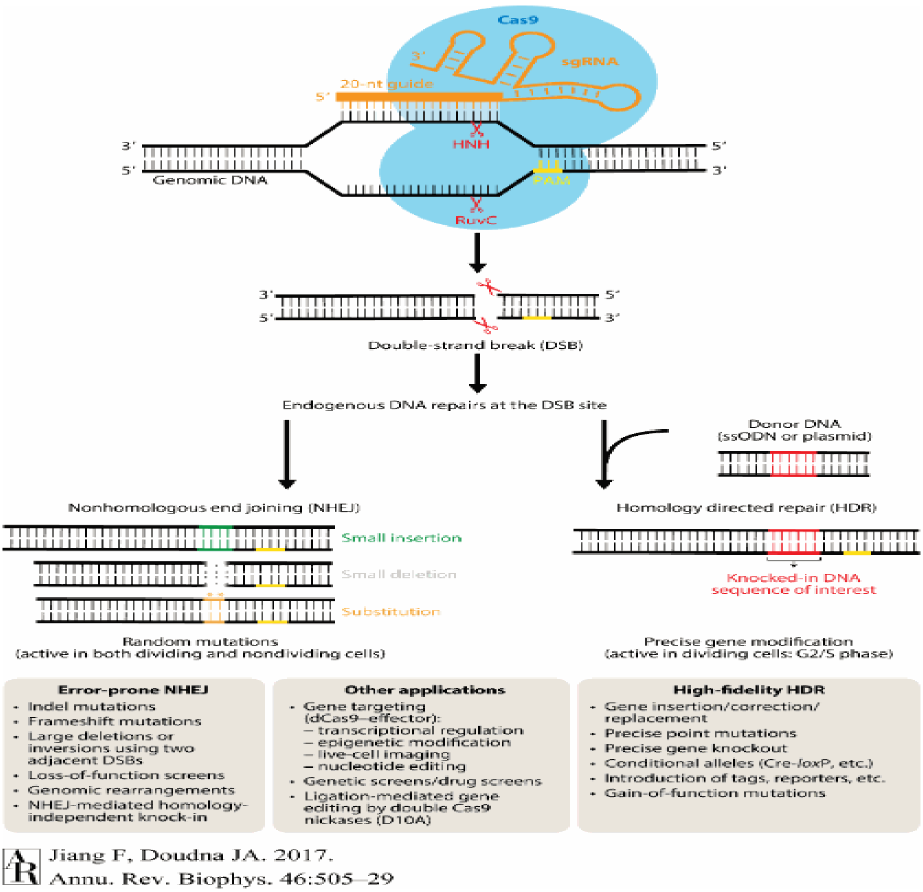

Figure 10. Schematic Representation of DNA Repair Forms (Jiang & Doudna, 2017).

6. Screening and Validation (Duan et al., 2019)

Screening: After CRISPR editing, cells are cultured and expanded. Different methods such as PCR, restriction enzyme digestion, or sequencing are used to identify successfully edited cells.

Verification: Edited cells are subjected to extensive validation to determine the existence and accuracy of the desired edits. Techniques such as Sanger or next-generation sequencing (NGS) are used to control for off-target effects and confirm edits. Functional assays can also be performed to determine whether gene editing has the intended biological effect.

CRISPR Cas Class Systems

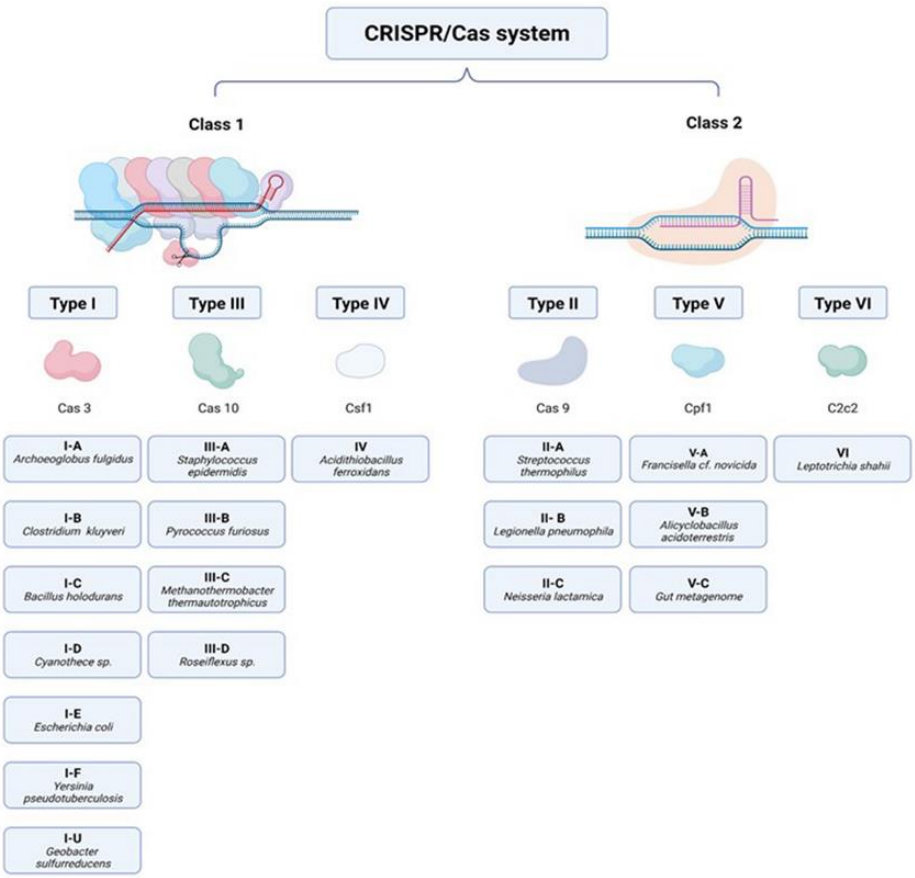

Figure 11. CRISPR Cas System Classes and Types (Aman Mohammadi et al., 2023)

Class 1 CRISPR-Cas Systems

Class 1 systems are characterized by multiprotein effector complexes adapted to target and degrade nucleic acids (Liu & Doudna, 2020).

Type I CRISPR-Cas System (Hidalgo-Cantabrana & Barrangou, 2020).

Key Components: Cascade complex (contains multiple Cas proteins) and Cas3.

Mechanism: The crRNA-driven Cascade complex binds to complementary DNA sequences and a PAM sequence must be present.

Once bound, the complex recruits Cas3, which has helicase and nuclease activities. Cas3, on the other hand, unwinds and degrades DNA, typically causing the target DNA to be destroyed.

Subtypes: I-A to I-F, differences in protein composition and PAM recognition.

Applications: First studied in bacteria and archaea for immune functions; less common in genome editing due to complexity.

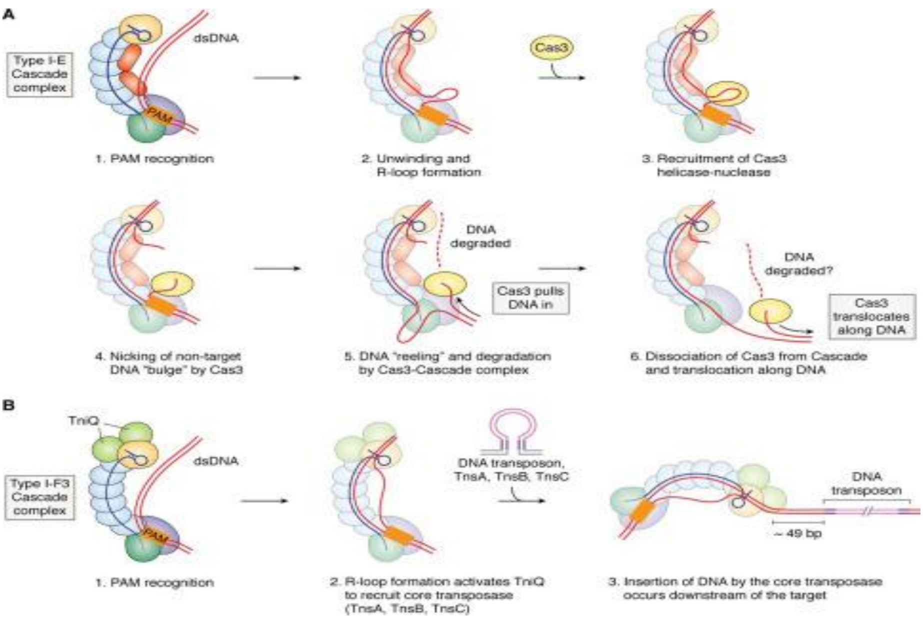

Figure 12. Effector Complex Structure and crRNA Biogenesis in Class 1 CRISPR-Cas Systems (Liu & Doudna, 2020).

Tip III CRISPR-Cas Sistemi (Stella & Marraffini, 2023)

Key Components: Csm (targeting DNA) or Cmr (targeting RNA) complex containing multiple Cas proteins and Csm6 or Csx1.

Mechanism: The complex can target both DNA and RNA. It uses crRNA to direct the complex to complementary **sequences.**

Once bound, it can directly degrade nucleic acids or nonspecifically cleave RNA by activating other nucleases such as Csm6.

Subtypes: III-A, III-B, etc., which vary in their targeting preferences and complex structures**.**

Applications: Mainly used in studying immune responses in microorganisms; They are potential applications in RNA targeting.

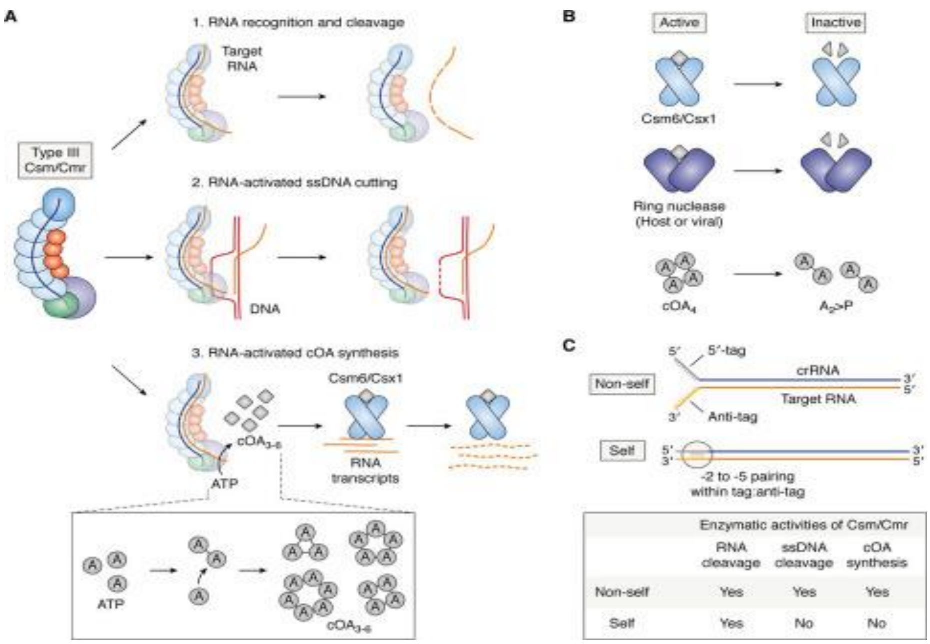

Figure 13. Schematic Representation of Class 1 Applications (Liu & Doudna, 2020)

Type IV CRISPR-Cas System (Zhou et al., 2021)

Key Components: Although it contains a simpler complex than other Class 1 systems, it has been characterized in fewer numbers.

Mechanism: The exact mechanisms and components are less known than in other species. It is thought to have RNA targeting capabilities.

Applications: Research is ongoing to understand their functions and potential uses.

CRISPR methods are divided into two main categories, Class 1 and Class 2, and these are further divided into themselves: Type1, Type 3, Type 4; It consists of Type 2, Type 5 and Type 6 (Yoshimi & Mashimo, 2022).

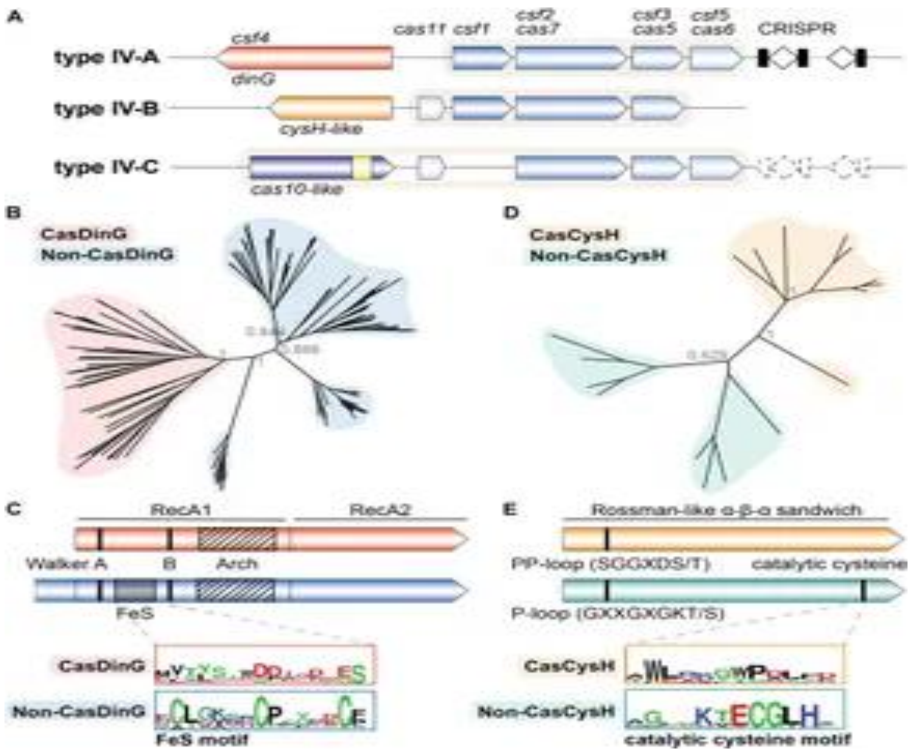

Figure 14. Schematic Representation of the Working Mechanisms of Type IV Cas Auxiliary Proteins (Taylor et al., 2021).

Class 2 CRISPR-Cas Systems

Class 2 systems are distinguished by using a single, large protein for interference, thus making them simpler and more versatile for genome editing applications (Makarova et al., 2019).

Type II CRISPR-Cas System (Cas9) (Chylinski et al., 2014)

Key Components: Cas9 protein and sgRNA (single guide RNA).

Mechanism: Cas9 is directed by sgRNA to a specific DNA sequence adjacent to a PAM. The most common PAM for Cas9 is “NGG”. Cas9 causes a double-strand break (DSB) at the target site, typically creating blunt ends.

Applications: Widely used for gene deletion, correction and insertion in genome editing.

Type V CRISPR-Cas System (Cas12) (Yan et al., 2019)

Key Components: Cas12 protein (formerly Cpf1) and crRNA.

Mechanism: Cas12 recognizes a T-rich PAM (usually TTTV) and is directed to the DNA targeted by the crRNA. Cas12 causes a gradual double-strand break that ends in sticky ends. Moreover, it can nonspecifically degrade single-stranded DNA after activation.

Applications: Used for precise genome editing and DNA detection experiments with sticky inserts.

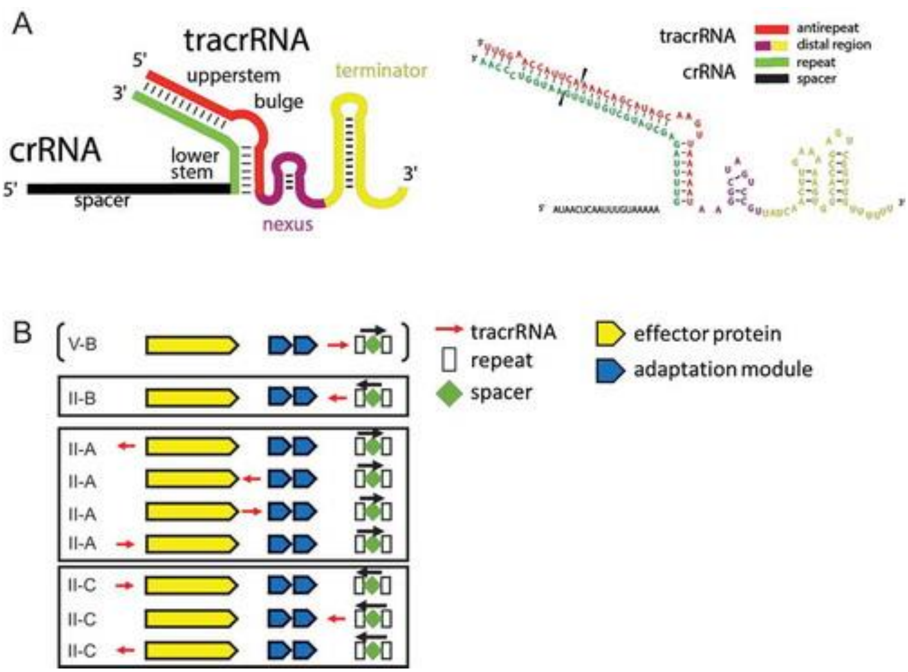

Figure 15. Comparison of Linkage Structures of Type II and Type V gRNAs (Faure et al., 2018).

Type VI CRISPR-Cas System (Cas13) (Nakagawa et al., 2022).

Key Components: Cas13 protein and crRNA.

Mechanism: Cas13 targets RNA instead of DNA. It is not necessarily a PAM, but it binds to a specific RNA sequence. Upon activation, Cas13 can cleave RNA at positions determined by crRNA. It also exerts secondary cleavage activity by nonspecifically cleaving nearby RNA molecules.

Applications: RNA editing, RNA degradation, diagnostics

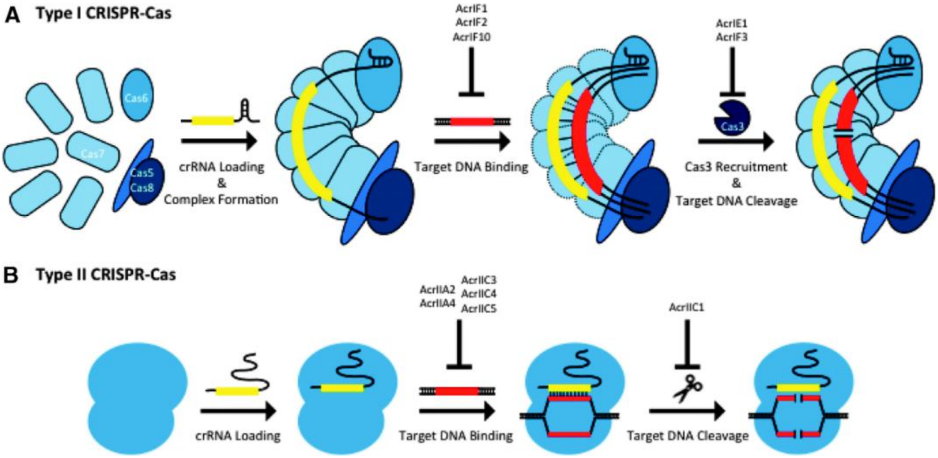

Figure 16. Schematic Representations of CRISPR Type I and Type II (Hwang & Maxwell, 2019).

The general methodology of CRISPR may include other Cas proteins besides Cas9. Each Cas protein has its own properties and applications.

Cas12 (Cpf1)

Targeting and Cutting: Cas12a (previously known as Cpf1) recognizes a different PAM sequence (usually TTTV, where V is A, C, or G) than Cas9. Cas12a makes gradual cuts in DNA, complete with sticky ends rather than the blunt ends produced by Cas9

Applications: Cas12a's unique cutting pattern may be advantageous for certain genome editing applications, such as creating specific overhangs for precise insertions. It is also used in CRISPR identification due to its secondary cleavage activity, which can nonspecifically degrade single-stranded DNA once activated.

Cas13

RNA Targeting: Besides Cas9 and Cas12 targeting DNA, Cas13 targets RNA. It does not require a PAM sequence, instead binding to RNA molecules and cleaving them.

Applications: Cas13 is used for RNA editing and RNA functional diagnostics. The ability to specifically cleave RNA can be used to knock down gene expression at the RNA level or to detect viral RNA in diagnostic tests.

Cas14

Small and Versatile: Cas14 proteins are smaller than Cas9 and Cas12 and do not require a PAM sequence. Therefore, it can target single-stranded DNA (ssDNA).

Applications: Cas14 is useful for applications that enable targeting of ssDNA, such as detecting single-stranded viral genomes or specific ssDNA sites in genomic DNA.

Cas3

DNA Degradation: Cas3 is involved in DNA degradation rather than precise regulation. It binds to target DNA and operationally degrades it.

Applications: Cas3 is less used for precision genome editing but can be used for large-scale deletions or complete removal of genomic regions.

CasX (Csm1)

Compact Size: CasX is another small Cas protein that shares similarities with Cas9 and Cas12, but is even more compact than these, which may be advantageous for delivery into cells.

Applications: CasX is still under investigation, but its small size and efficiency make it a promising candidate for different genome editing applications, especially in contexts where delivery size is a constraint.

CRISPR Base Editing

Description: Modifies DNA bases without creating double-strand breaks using a catalytically inactive Cas protein (dCas9 or dCas12) fused to a deaminase enzyme**.**

Applications: Point mutations, correction of single nucleotide polymorphisms.

CRISPR Prime Editing

Description: A more precise editing method combining reverse transcriptase with a modified guide RNA and a nickase Cas9.

Applications: Introducing exact additions, deletions, and all 12 possible base-to-base conversions.

CRISPR Intervention (CRISPRi)

Identification: Uses an inactive Cas9 (dCas9) to block transcription without binding to and cutting specific DNA sequences.

Applications: Gene silencing, transcriptional repression.

CRISPR Activation (CRISPRa)

Description: Uses dCas9 fused to transcriptional activators to enhance gene expression.

Applications: Gene activation, study of gene function.

CRISPR Epigenome Editing

Description: dCas9 fused to epigenetic modifiers is used to alter DNA methylation or histone modifications**.**

Applications: Examination and modification of epigenetic states.

CRISPR Diagnostic Methods

SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking):

Description: Uses Cas13 to detect specific RNA sequences with high sensitivity.

Applications: Viral detection, pathogen identification.

DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter):

Description: Uses Cas12 to detect DNA sequences and specifically for pathogen detection.

Applications: Viral and bacterial diagnosis.

Multiplexed CRISPR Editing

Description: Simultaneous targeting of multiple genomic regions using multiple gRNAs.

Applications: Complex trait studies, synthetic biology.

MADE WITH CRISPR TECHNOLOGY STUDIES IN THE FIELD OF FEATURES

In aquaculture, CRISPR can be used to improve preferred traits, increase disease resistance, and study gene functions in aquatic creatures (Roy et al., 2022).

In 2014, Edvardsen and his colleagues published their study to show that it was possible to use CRISPR/Cas9 technology to edit genes in Atlantic salmon (*Salmo salar L.*). Researchers specifically aimed to observe the phenotypic differences that occur by targeting and disrupting a selected gene, thus validating the use of this technology in salmonids (Edvardsen et al., 2014).

The researchers singled out a gene called tyrosinase (tyr), which plays a role in the production of melanin, a pigment responsible for coloration in multiple organisms. This gene is a strategic choice because changes in pigmentation can be observed quickly, allowing direct assessment of gene editing success. In the study, specific guide RNAs (gRNA) were designed to target the tyrosinase gene. The CRISPR/Cas9 system was then used to introduce double-strand breaks at targeted locations within the gene.

CRISPR/Cas9 components were injected into fertilized Atlantic salmon eggs at the single-cell stage.This method allowed gene editing to occur before embryos began to develop, allowing for potential gene disruption in all cells of the resulting fish. After the fish developed, the researchers examined the tyrosinase gene for mutations. Specifically, they sought to discover insertions or deletions (indels) in the target region that would indicate successful gene editing. The study successfully revealed mutations in the tyrosinase gene. The resulting mutations caused an impairment in melanin production, leading to observable phenotypic changes in pigmentation. Specifically, the fish showed a reduction in melanophores (pigment cells), resulting in lighter skin color. This research demonstrated that CRISPR/Cas9 can efficiently and specifically target and regulate genes found in Atlantic salmon. A common concern in gene editing, besides the occurrence of off-target effects, has not been comprehensively detailed, but the study has highlighted the potential to achieve specific gene modifications.

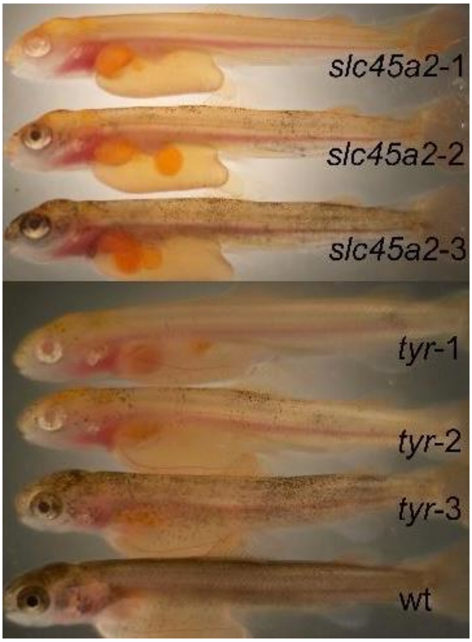

Figure 17. Graded phenotype levels induced by CRISPRslc45a2/Cas9 (slc45a2-1 to slc45a2-3) and CRISPRtyr/Cas9 (tyr-1 to tyr-3) (Edvardsen et al., 2014).

In a different study in 2014, Minghui Li and colleagues focused on investigating gene function in the Nile tilapia (*Oreochromis niloticus*), especially in the context of sexual differentiation and reproduction, using CRISPR/Cas9 genome editing technology. The study successfully demonstrated the use of CRISPR/Cas9 to create targeted mutations in some genes such as nanos2, nanos3, dmrt1 and foxl2, which play important roles in gonad development and sex differentiation (Li et al., 2014).

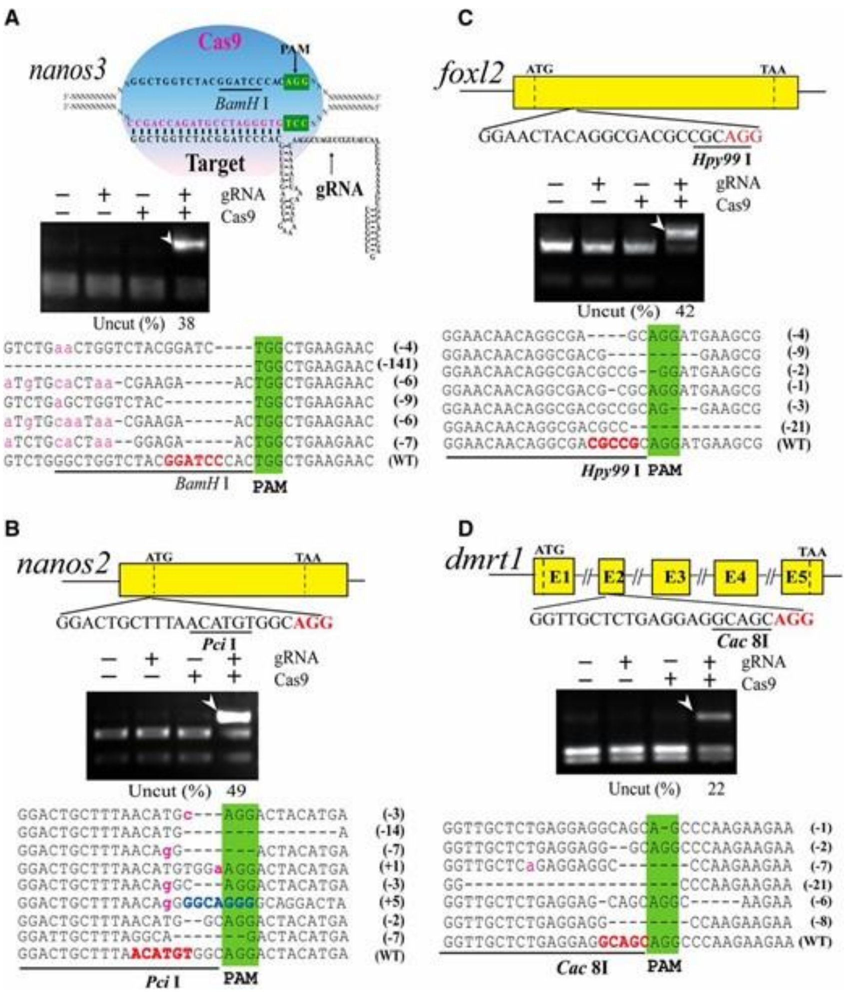

Figure 18. Efficient Disruption of Tilapia Genes with CRISPR/Cas9: nanos3 (A), nanos2 (B), foxl2 (C), and dmrt1 (D) were Selected as Targets to Demonstrate the Feasibility of CRISPR/Cas9-Mediated Mutagenesis (Li et al. , 2014).

The researchers used CRISPR/Cas9 to target and disrupt genes nanos2 and nanos3, which are important for germ cell development, and dmrt1 and foxl2, which are involved in sex differentiation. They achieved high mutation efficiency with a 95% success rate in gene disruptions.

Mutations in nanos2 and nanos3 resulted in germ cell-deficient gonads in both male (XY) and female (XX) tilapia, when confirmed by GFP labeling and Vasa staining.

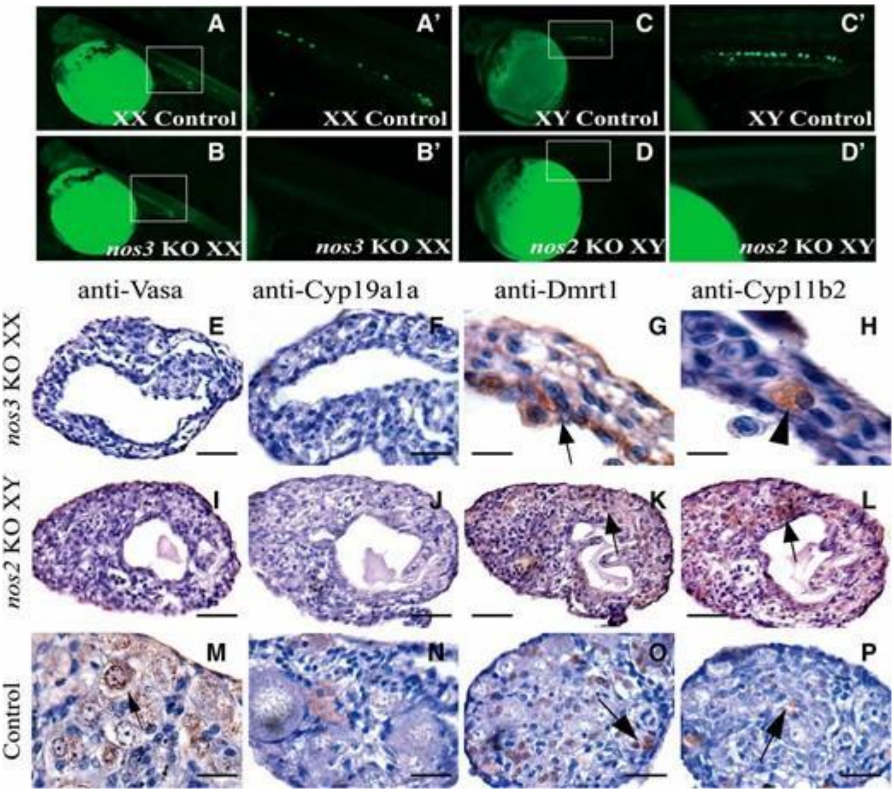

Figure 19. Mutation of nanos2 and nanos3 with CRISPR/Cas9 Resulted in Germ Cell-Deficient Gonads (Li et al., 2014).

Loss of the Dmrt1 and foxl2 genes triggered significant changes in the gonads. For example, masculinization has been observed in fish with both XY and XX chromosomes, with increased expression of androgen-producing enzymes and changes in serum androgen levels. In a study published in 2018 by Sheng-Han Wu and colleagues, they examined the role of a type of peptide that increases the disease resistance of zebrafish through immune modulation.

The study aimed to examine the effects of a granulin peptide secreted by tilapia on the survival of transgenetic zebrafish infected with Vibrio vulnificus. In particular, researchers sought to understand how this peptide affects potential immunity to increase the fish's chances of survival (Wu et al., 2018). First, they isolated and identified the granulin peptide secreted by tilapia. They tested its potential immune-modulating effects in zebrafish. Transgenetic zebrafish expressing specific genes related to immune responses were used as a model system. These fish were exposed to Vibrio vulnificus to stimulate bacterial infection. Tilapia was administered to zebrafish infected with granulin peptide, and various tests were performed to evaluate its effect on survival rates, immune system responses, and general health status.

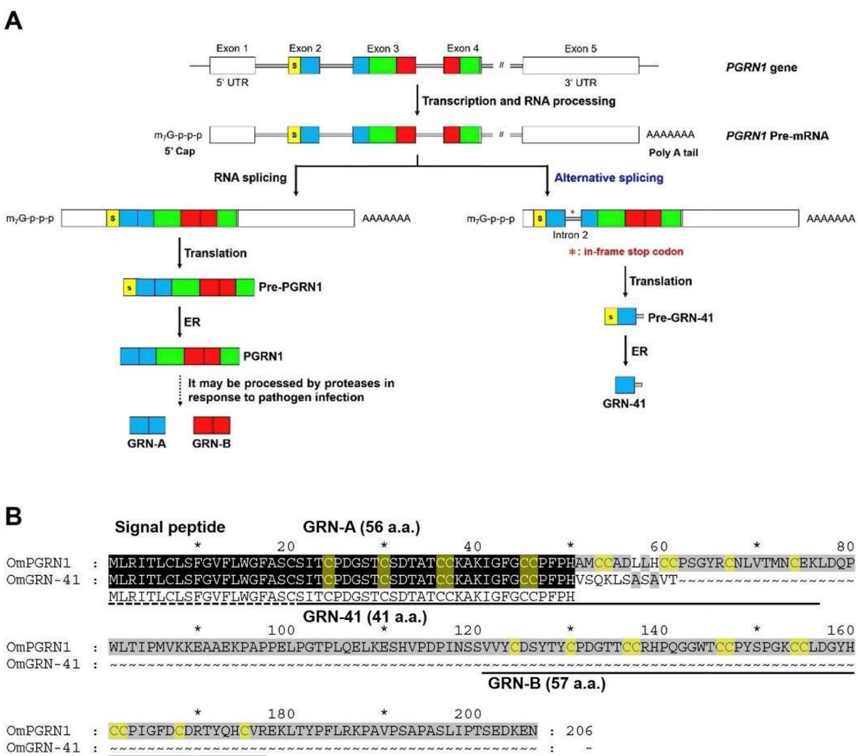

Figure 20. PGRN1 protein and GRN peptides derived from the Tilapia PGRN1 gene (Wu et al., 2018).

Researchers measured survival rates, bacterial load and immune-related gene expression. The study revealed that tilapia granulin peptide significantly increased the survival of transgenic zebrafish infected with Vibrio vulnificus. This shows that the peptide has a strong protective effect against bacterial infection. The peptide's mechanism of action involves modulating potential immune responses. This may include increasing the activity of immune cells, increasing the production of antimicrobial peptides, and improving the overall inflammatory response.

In a study published in 2019, Thawatchai Chaijarasphong and colleagues aimed to develop a rapid and sensitive diagnostic method to detect WSSV (White Spot Syndrome Virus) in shrimps. White spot syndrome is an important disease that causes serious losses in aquaculture (Sánchez-Paz, 2010). The study aimed to use a CRISPR/Cas12a-based system to obtain a reliable and efficient detection method that could potentially be used in field conditions.

The CRISPR-Cas12a (Cpf1) system, which differs from other methods with its secondary cleavage activity when bound to target DNA, was used in the research. This feature allows it to randomly cleave nearby single-stranded DNA (ssDNA) after it recognizes and binds to the target DNA. combined the CRISPR-Cas12a system with recombinase polymerase amplification (RPA) for rapid DNA amplification. Once WSSV's DNA is present, it will be amplified and the CRISPR-Cas12a complex will cleave a fluorescently labeled reporter molecule, producing a detectable signal. The test was designed to be extremely sensitive, detecting even low levels of viral DNA. Its sensitivity was achieved by designing guide RNAs (gRNAs) that specifically recognize the WSSV genome.

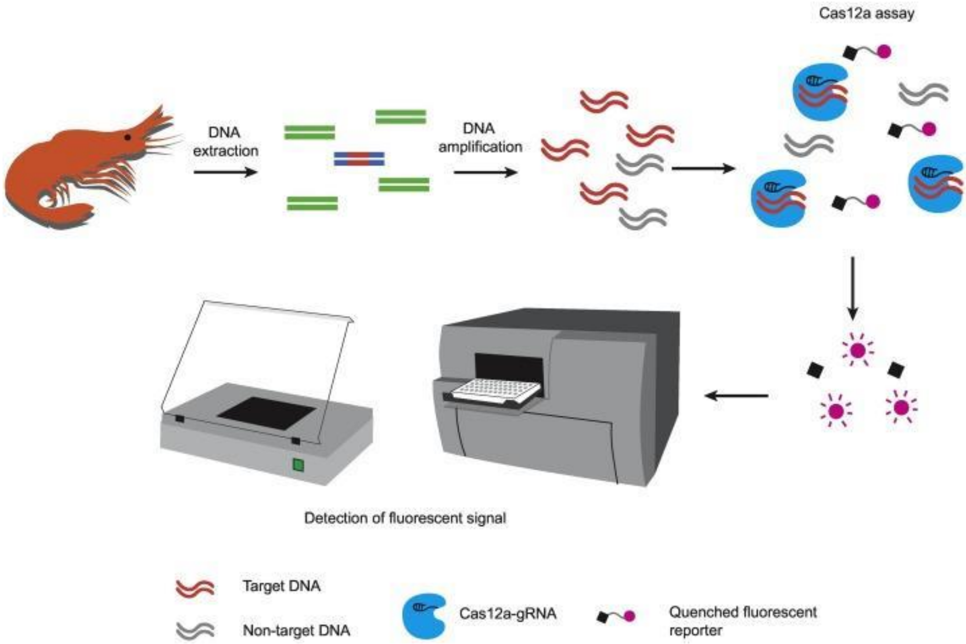

Figure 21. Scheme of WSSV Detection by Nucleic Acid Amplification Combined with Cas12a Nuclease Assay (Chaijarasphong et al., 2019).

This CRISPR-Cas12a-based diagnostic method worked with high sensitivity, being able to detect WSSV even at very low concentrations, making it suitable for early detection of infections. Its analysis provides much faster results than traditional PCR methods. The simplicity and portability of the setup means that it can also be used in field conditions and is a practical solution for in situ testing in aquaculture environments. The creation of this diagnostic tool has important implications for the aquaculture industry. Early and accurate diagnosis of WSSV can help manage and control outbreaks in shrimp farming, minimize financial losses, and improve biosecurity measures. In a research study conducted in 2022, Michael Coogan and his team aimed to increase the growth rates and nutritional value of channel catfish (Ictalurus punctatus) using Crisr Cas9 technology. The main aim was to increase muscle growth and increase omega-3 fatty acid levels in fish (Coogan et al., 2022).

The researchers targeted the myostatin gene, a regulator of which has a negative effect on muscle growth. They aimed to increase muscle development by silencing this gene. Additionally, the team thought that they could increase omega-3 levels by inserting the elongase gene from Japanese salmon into the melanocortin-4 receptor (mc4r) gene locus (this gene plays a role in the synthesis of omega-3 fatty acids, which are beneficial for human health (Scorletti & Byrne, 2018)). CRISPR Cas9 components, including specialized guide RNAs (gRNAs) and the Cas9 enzyme, were injected into fertilized catfish embryos. In this way, target genes could be accurately regulated at an early developmental stage. The study evaluated the success of gene editing by examining targeted genes for mutations or successful knock-on events. They also observed the phenotypic changes occurring in the catfish, focusing on muscle growth and fatty acid profiles. Silencing the myostatin gene led to increased muscle mass in catfish. This conclusion was reached because there was an increase in the size and weight of the gene-edited fish compared to the non-edited control fish. While successful integration of the elongase gene has been observed, detailed results regarding the actual increase in omega-3 levels have not been extensively reported.

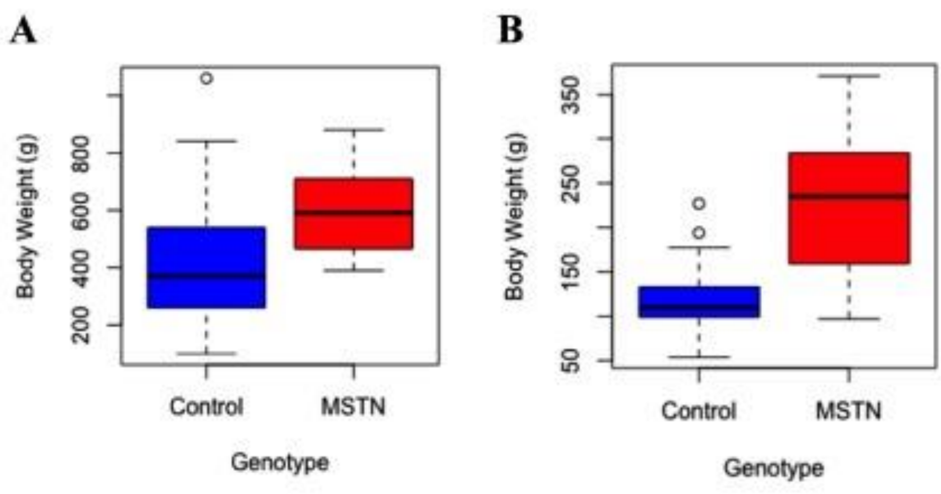

Figure 22. Graphs Showing the Masses of Catfish in Control and Experimental Groups (A. 2017, 18 Months After Hatching B. 2018 18 Months After Hatching) (Coogan et al., 2022).

In 2024, Toshiya Nishimura and colleagues conducted a study focusing on knocking out the dnd1 gene using the CRISPR Cas13d system to sterilize fish. This gene plays a crucial role in germ cell development, and its disruption can lead to infertility, making it an important target for managing reproduction in aquaculture species (Kim Joana Westerich et al., 2023).

The researchers identified guide RNAs (gRNAs) that target conserved sequences of the dnd1 gene in several species of teleost fish, including the Japanese rice fish (Oryzias latipes), a species frequently used in experiments, and the commercially attractive rainbow trout (*Oncorhynchus mykiss*). they designed. Their goal was to create a broadly applicable sterilization technique that could prevent the proliferation of genetically modified or artificially selected fish from escaping into natural ecosystems (Nishimura et al., 2024). Experiments have shown that CRISPR Cas13d-mediated knockout of dnd1 causes sterility in both Japanese rice fish and rainbow trout. The team performed successful sterilization by observing that functional germ cells did not develop in fish subjected to this method. This research provides a promising solution for the aquaculture industry that provides reproductive control and reduces the risks associated with accidental escape of farmed fish. Moreover, the interchangeability of gRNAs from species to species suggests that this method can be extended to other species and offers a comprehensive solution to control fish populations in aquaculture environments.

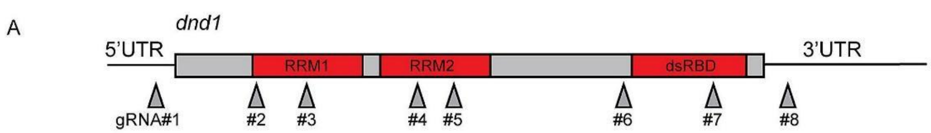

Figure 23. Schematic Representation of gRNA Target Regions (Nishimura et al., 2024).

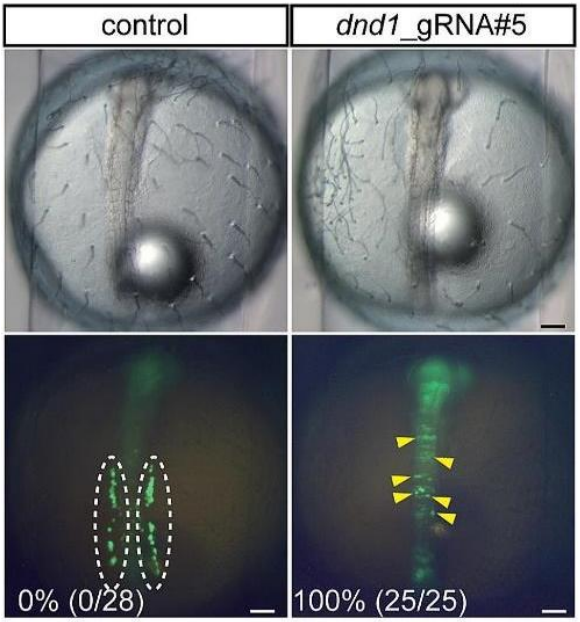

Figure 24. Images of Control and Experimental Groups (Nishimura et al., 2024).

In the same issue of the journal in which this study was published, Xiao-Lei Su and her colleagues aimed to use CRISPR-Cas9 genome editing technology to develop desired characteristics in the blunt-nosed sea bream (*Megalobrama amblycephala*), an important aquaculture species. The primary focus was to increase the species' tolerance to low oxygen (hypoxic) levels by targeting specific genes involved in the hypoxia response pathway (Su et al., 2024).The researchers focused on the erythropoietin (EPO) gene, an important gene in the Hypoxia Inducible Factor (HIF) signaling pathway. This gene is crucial for red blood cell production and adaptation to low-oxygen conditions. Using CRISPR-Cas9, the researchers aimed to induce mutations in the EPO gene, examining its role and potentially increasing the fish's tolerance to hypoxia. CRISPR-Cas9 components, including specific guide RNAs (gRNAs) and Cas9 protein, were introduced into fertilized eggs or early embryos of blunt-nosed sea bream. Genetic modifications used sequencing techniques to confirm successful editing of the EPO gene. The study evaluated the physiological and behavioral responses of fish arranged in controlled hypoxic conditions. Important measurements such as survival rates, growth performance, and overall health have been made. Silencing or modification of the EPO gene has resulted in fish with better tolerance to low oxygen conditions. This is evidenced by better survival possibilities and more stable physiological responses under hypoxic stress. The edited fish demonstrated better growth performance, suggesting that the genetic modifications may also have affected other metabolic pathways beneficial to growth under aquaculture conditions.

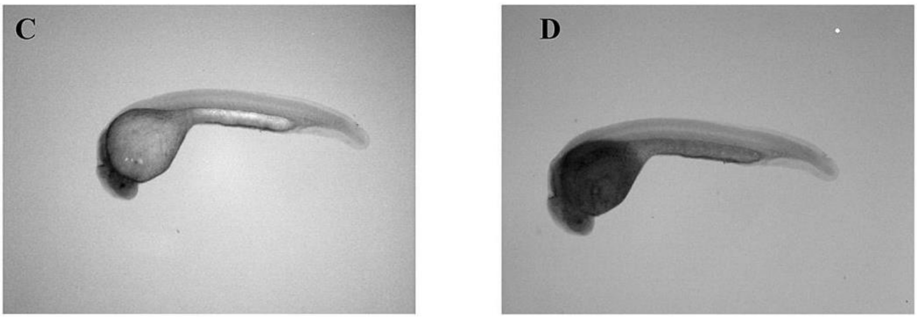

Figure 25. Photographs of Experimental(C) and Control(D) Groups (Su et al., 2024).

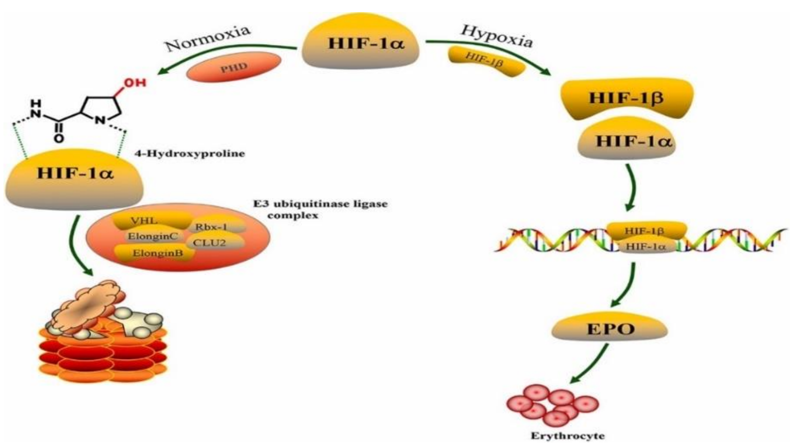

Figure 26. Full-Length cDNA Sequence and Amino Acid Sequences of EPO from Blunt-nosed Bream (Red Frame are Silenced Target Position Markers) (Su et al., 2024).

ADVANTAGES AND DISADVANTAGES OF CRISPR TECHNOLOGY

CRISPR gene editing technology has led to various developments in many different fields. We can list some of its important advantages as follows:

Precision Gene Editing: CRISPR has revolutionized the field of genetic engineering by providing a precise and effective method for altering specific genes. This level of precision allows scientists to edit DNA at an unprecedented level (Hsu et al., 2014).

Treatment of Genetic Diseases: CRISPR has significant potential for the treatment of genetic disorders. Researchers have made headway in using CRISPR to eliminate or alleviate the effects of diseases such as sickle cell anemia, cystic fibrosis, and muscular dystrophy by editing underlying genetic mutations (Hussain et al., 2019).

Cancer Therapies: The use of CRISPR as a tool to develop cancer treatments is still under investigation. Scientists are exploring ways to use CRISPR to target and eliminate cancer cells by altering certain genes that cause cancer progression (Katti et al., 2022).

Agricultural Developments: CRISPR applications have led to the development of genetically modified crops with properties such as resistance to agricultural pests, diseases and environmental stress. This is an important step towards food security and sustainability (Camerlengo et al., 2022).

Developing Disease-Resistant Animals: CRISPR has been used to create genetically modified animals that are more resistant to diseases. Thanks to farm animals designed with CRISPR, antibiotic consumption of animals can be reduced and they can be more resistant to infectious diseases (Wani et al., 2022).

Infectious Disease Research: CRISPR has played an important role in the study of infectious diseases. Researchers are using CRISPR to modify genes in pathogens, learn about their virulence, and develop potential strategies to combat infectious diseases (Jolany vangah et al., 2020).

Despite all these good aspects, there are concerns about the applications of this technology (Taning et al., 2017). We can list the main concerns as follows:

Ethical Concerns: One of the major drawbacks revolves around the ethical implications of altering the human gene line. If the regulation of genes in embryos leads to hereditary changes, the emergence of a concept of "designer babies" and the emergence of undesirable consequences brings the morality of the application of this technology into question (Brokowski & Adli, 2019).

Environmental Impact: Genetic modifications in agriculture can have unpredictable consequences on ecosystems and biodiversity. The release of genetically modified organisms into the environment creates uncertainties regarding the potential ecological impact (Mondal et al., 2022).

Regulatory Challenges: CRISPR technology has evolved faster than the process of establishing definitive legislation on what areas its applications should be limited to. Establishing guidelines and ethical standards for the responsible use of CRISPR technology is crucial to prevent misuse and unintended consequences (Mir et al., 2022).

Dual Use Concerns: CRISPR technology may be used for bona fide purposes. However, its dual-use nature raises concerns about it turning into a weapon. Their ability to engineer pathogens and create genetically modified organisms for malicious purposes poses a security risk (DiEuliis & Giordano, 2017).

CRISPR gene editing holds great promise for significant advances in fields such as medicine, agriculture and biology. However, it brings uncertainty and uncertainty regarding ethics, security and legislation that need to be carefully evaluated. As one of the most recent developments in this regard, the Chinese biophysicist Hei Jiankui's announcement of twin babies immunized against HIV with CRISPR Cas9 in 2018 and his receiving a three-year prison sentence in 2019 can be cited as an example ("The World's First Time Changing the Genes of Embryos Chinese Scientist Sentenced to 3 Years in Prison," 2019).

REFERENCES

Aman Mohammadi, M., Maximiano, M. R., Hosseini, S. M., & Franco, O. L. (2023). CRISPR-Cas engineering in food science and sustainable agriculture: recent advancements and applications. *Bioprocess and Biosystems Engineering*, *46*(4), 483–497. https://doi.org/10.1007/s00449-022-02842-5.

Arroyo-Olarte, R. D., Bravo Rodríguez, R., & Morales-Ríos, E. (2021). Genome Editing in Bacteria: CRISPR-Cas and Beyond. *Microorganisms*, *9*(4), 844. https://doi.org/10.3390/microorganisms9040844.

Babu, M., Beloglazova, N., Flick, R., Graham, C., Skarina, T., Nocek, B., Gagarinova, A., Pogoutse, O., Brown, G., Binkowski, A., Phanse, S., Joachimiak, A., Koonin, E. V., Savchenko, A., Emili, A., Greenblatt, J., Edwards, A. M., & Yakunin, A. F. (2010). A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair. *Molecular Microbiology*, *79*(2), 484–502. https://doi.org/10.1111/j.1365-2958.2010.07465.x.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science (New York, N.Y.)*, *315*(5819), 1709–1712. https://doi.org/10.1126/science.1138140.

Blount, Z. D. (2015). The unexhausted potential of E. coli. *ELife*, *4*(e05826). https://doi.org/10.7554/elife.05826.

Brokowski, C., & Adli, M. (2019). CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. *Journal of Molecular Biology*, *431*(1), 88–101. Science Direct. https://doi.org/10.1016/j.jmb.2018.05.044.

Brouns, S. J. J., Jore, M. M., Lundgren, M., Westra, E. R., Slijkhuis, R. J. H., Snijders, A. P. L., Dickman, M. J., Makarova, K. S., Koonin, E. V., & van der Oost, J. (2008). Small CRISPR RNAs Guide Antiviral Defense in Prokaryotes. *Science*, *321*(5891), 960–964. https://doi.org/10.1126/science.1159689.

Camerlengo, F., Frittelli, A., & Pagliarello, R. (2022). CRISPR towards a Sustainable Agriculture. *Encyclopedia*, *2*(1), 538–558. https://doi.org/10.3390/encyclopedia2010036.

Chaijarasphong, T., Thammachai, T., Itsathitphaisarn, O., Sritunyalucksana, K., & Suebsing, R. (2019). Potential application of CRISPR-Cas12a fluorescence assay coupled with rapid nucleic acid amplification for detection of white spot syndrome virus in shrimp. *Aquaculture*, *512*, 734340. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2019.734340.

Chandrasekaran, A. P., Song, M., Kim, K.-S., & Ramakrishna , S. (2018). Chapter Six - Different Methods of Delivering CRISPR/Cas9 Into Cells. *Progress in Molecular Biology and Translational Science*, *159*, 157–176. ScienceDirect. https://doi.org/10.1016/bs.pmbts.2018.05.001.

Cheng, L., Cai, Y., & Hou, W. (2021). Generation of Knockout and Fragment Deletion Mutants in Soybean by CRISPR-Cas9. In *CRISPR-Cas Methods* (pp. 123–136). Humana.

Chylinski, K., Makarova, K. S., Charpentier, E., & Koonin, E. V. (2014). Classification and evolution of type II CRISPR-Cas systems. *Nucleic Acids Research*, *42*(10), 6091–6105. https://doi.org/10.1093/nar/gku241.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science*, *339*(6121), 819–823. https://doi.org/10.1126/science.1231143.

Coogan, M., Alston, V., Su, B., Khalil, K., Elaswad, A., Khan, M., Simora, R. M. C., Johnson, A., Xing, D., Li, S., Wang, J., Lu, C., Wang, W., Hettiarachchi, D., Hasin, T., Terhune, J., Butts, I. A. E., & Dunham, R. A. (2022). CRISPR/Cas-9 induced knockout of myostatin gene improves growth and disease resistance in channel catfish (Ictalurus punctatus). *Aquaculture*, *557*, 738290. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2022.738290.

Coogan, M., Alston, V., Su, B., Khalil, K., Elaswad, A., Khan, M., Simora, R. M. C., Johnson, A., Xing, D., Li, S., Wang, J., Lu, C., Wang, W., Hettiarachchi, D., Hasin, T., Terhune, J., Butts, I. A. E., & Dunham, R. A. (2022). CRISPR/Cas-9 induced knockout of myostatin gene improves growth and disease resistance in channel catfish (Ictalurus punctatus). *Aquaculture*, *557*, 738290. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2022.738290.

Danna, K., & Nathans, D. (1971). Specific cleavage of simian virus 40 DNA by restriction endonuclease of Hemophilus influenzae. *Proceedings of the National Academy of Sciences*, *68*(12), 2913–2917. https://doi.org/10.1073/pnas.68.12.2913.

DiEuliis, D., & Giordano, J. (2017). Gene editing using CRISPR/Cas9: implications for dual-use and biosecurity. *Protein & Cell*, *9*(3), 239–240. https://doi.org/10.1007/s13238-017-0493-4.

Duan, B., Zhou, C., Zhu, C., Yu, Y., Li, G., Zhang, S., Zhang, C., Ye, X., Ma, H., Qu, S., Zhang, Z., Wang, P., Sun, S., & Liu, Q. (2019). Model-based understanding of single-cell CRISPR screening. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10216-x.

Dünyada ilk kez embriyoların genlerini değiştiren Çinli bilim insanına 3 yıl hapis cezası. (2019, December 30). *BBC News Türkçe*. https://www.bbc.com/turkce/haberler-dunya-.50945821#:~:text=D%C3%BCnyada%20ilk%20kez%20embriyolar%C4%B1n%20genlerini%20de%C4%9Fi%C5%9Ftiren%20%C3%87inli%20bilim%20insan%C4%B1na%203%20y%C4%B1l%20hapis%20cezas%C4%B1

Dünyada ilk kez embriyoların genlerini değiştiren Çinli bilim insanına 3 yıl hapis cezası. (2019, December 30). *BBC News Türkçe*. https://www.bbc.com/turkce/haberler-dunya-50945821#:~:text=D%C3%BCnyada%20ilk%20kez%20embriyolar%C4%B1n%20genlerini%20de%C4%9Fi%C5%9Ftiren%20%C3%87inli%20bilim%20insan%C4%B1na%203%20y%C4%B1l%20hapis%20cezas%C4%B1

Edvardsen, R. B., Leininger, S., Kleppe, L., Skaftnesmo, K. O., & Wargelius, A. (2014). Targeted Mutagenesis in Atlantic Salmon (Salmo salar L.) Using the CRISPR/Cas9 System Induces Complete Knockout Individuals in the F0 Generation. *PLoS ONE*, *9*(9), e108622. https://doi.org/10.1371/journal.pone.0108622

Evans, B. A., Smith, O. L., Pickerill, E. S., York, M. K., Buenconsejo, K. J. P., Chambers, A. E., & Bernstein, D. A. (2018). Restriction digest screening facilitates efficient detection of site-directed mutations introduced by CRISPR in C. albicans UME6. *PeerJ*, *6*, e4920. https://doi.org/10.7717/peerj.4920

Faure, G., Shmakov, S. A., Makarova, K. S., Wolf, Y. I., Crawley, A. B., Barrangou, R., & Koonin, E. V. (2018). Comparative genomics and evolution of trans-activating RNAs in Class 2 CRISPR-Cas systems. *RNA Biology*, *16*(4), 1–14. https://doi.org/10.1080/15476286.2018.1493331

Fernández, A., Morín, M., Muñoz-Santos, D., Josa, S., Montero, A., Rubio-Fernández, M., Cantero, M., Fernández, J., Hierro, del, Castrillo, M., Miguel Ángel Moreno-Pelayo, & Lluís Montoliu. (2020). Simple Protocol for Generating and Genotyping Genome-Edited Mice With CRISPR-Cas9 Reagents. *Current Protocols in Mouse Biology*, *10*(1). https://doi.org/10.1002/cpmo.69

Fernández, C. R. (2019, April 8). *Francis Mojica, the Spanish Scientist Who Discovered CRISPR*. Labiotech.eu. https://www.labiotech.eu/interview/francis-mojica-crispr-interview

Fuziwara, C. S., Mello, D. C. de , & Kimura, E. T. K. (2022). Gene Editing with CRISPR/Cas Methodology and Thyroid Cancer: Where Are We? *ProQuest*, *14*(3), 844. https://doi.org/10.3390/cancers14030844

Gostimskaya, I. (2022). CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Moscow)*, *87*(8), 777–788. https://doi.org/10.1134/s0006297922080090

Han, W., & She, Q. (2017, January 1). *Chapter One - CRISPR History: Discovery, Characterization, and Prosperity* (R. Torres-Ruiz & S. Rodriguez-Perales, Eds.). ScienceDirect; Academic Press. https://www.sciencedirect.com/science/article/pii/S1877117317301382?casa_token=2qVn8wc9mP0AAAAA:mpC3Vjz92XjMb2eEEV1Hk2Mv2IbOLxDCvWzSXoT45iqmov8Asxprz2sHrczxMsvEphqRmSChbt0

Hidalgo-Cantabrana, C., & Barrangou, R. (2020). Characterization and applications of Type I CRISPR-Cas systems. *Biochemical Society Transactions*, *48*(1), 15–23. https://doi.org/10.1042/bst20190119

Hillary, V. E., & Ceasar, S. A. (2022). A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. *Molecular Biotechnology*, *65*(3). https://doi.org/10.1007/s12033-022-00567-0

Hsu, Patrick D., Lander, Eric S., & Zhang, F. (2014). Development and Applications of CRISPR-Cas9 for Genome Engineering. *Cell*, *157*(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

Hussain, W., Mahmood, T., Hussain, J., Ali, N., Shah, T., Qayyum, S., & Khan, I. (2019). CRISPR/Cas system: A game changing genome editing technology, to treat human genetic diseases. *Gene*, *685*, 70–75. https://doi.org/10.1016/j.gene.2018.10.072

Hussain, W., Mahmood, T., Hussain, J., Ali, N., Shah, T., Qayyum, S., & Khan, I. (2019). CRISPR/Cas system: A game changing genome editing technology, to treat human genetic diseases. *Gene*, *685*, 70–75. https://doi.org/10.1016/j.gene.2018.10.072

Hwang, S., & Maxwell, K. L. (2019). Meet the Anti-CRISPRs: Widespread Protein Inhibitors of CRISPR-Cas Systems. *The CRISPR Journal*, *2*(1), 23–30. https://doi.org/10.1089/crispr.2018.0052

Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *Journal of Bacteriology*, *200*(7), e00580-17. https://doi.org/10.1128/JB.00580-17

Jansen, Ruud., Embden, Jan. D. A. van, Gaastra, Wim., & Schouls, Leo. M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology*, *43*(6), 1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x

Jiang, F., & Doudna, J. A. (2017). CRISPR–Cas9 structures and mechanisms. *Annual Review of Biophysics*, *46*(1), 505–529. https://doi.org/10.1146/annurev-biophys-062215-010822

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science*, *337*(6096), 816–821. https://doi.org/10.1126/science.1225829

Jinek, M., East, A., Cheng, A., Lin, S., Ma, E., & Doudna, J. (2013). RNA-programmed genome editing in human cells. *ELife*, *2013*(2), e00471. https://doi.org/10.7554/eLife.00471

Jolany vangah, S., Katalani, C., Booneh, H. A., Hajizade, A., Sijercic, A., & Ahmadian, G. (2020). CRISPR-Based Diagnosis of Infectious and Noninfectious Diseases. *Biological Procedures Online*, *22*(1). https://doi.org/10.1186/s12575-020-00135-3

Katti, A., Diaz, B. J., Caragine, C. M., Sanjana, N. E., & Dow, L. E. (2022). CRISPR in cancer biology and therapy. *Nature Reviews Cancer*, *22*(22).

https://doi.org/10.1038/s41568-022-00441-w

Kim Joana Westerich, Reinecke, S., Emich, J., Margot Julia Wyrwoll, Stallmeyer, B., Meyer, M., Oud, M. S., Fietz, D., Pilatz, A., Kliesch, S., Reichman-Fried, M., Katsiaryna Tarbashevich, Limon, T., Stehling, M., Friedrich, C., Tüttelmann, F., & Raz, E. (2023). Linking human Dead end 1 (DND1) variants to male infertility employing zebrafish embryos. *Human Reproduction*, *38*(4), 655–670. https://doi.org/10.1093/humrep/dead031

Klyashtornyy, N. (2023). *Re: Which are the equipment and software required to establish small lab of Crispr for Plant breeding?*. ResearchGate. https://www.researchgate.net/post/Which_are_the_equipment_and_software_required_to_establish_small_lab_of_Crispr_for_Plant_breeding/6454b854c226b5eabb0f9397/citation/download.

Komor, A. C., Badran, A. H., & Liu, D. R. (2017). CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. *Cell*, *168*(1-2), 20–36. https://doi.org/10.1016/j.cell.2016.10.044

Lemay, M.-L., Renaud, A., Rousseau, G., & Moineau, S. (2018). Targeted Genome Editing of Virulent Phages Using CRISPR-Cas9. *BIO-PROTOCOL*, *8*(1). https://doi.org/10.21769/bioprotoc.2674

Leung, C., & Jia, Z. (2018). Molecular Techniques Used to Explore Glutamate Receptors in Synaptic Plasticity and Memory. In *Molecular-Genetic and Statistical Techniques for Behavioral and Neural Research* (pp. 419–443). Academic Press.

Li, M., Yang, H., Zhao, J., Fang, L., Shi, H., Li, M., Sun, Y., Zhang, X., Jiang, D., Zhou, L., & Wang, D. (2014). Efficient and Heritable Gene Targeting in Tilapia by CRISPR/Cas9. *Genetics*, *197*(2), 591–599. https://doi.org/10.1534/genetics.114.163667

Liu, T. Y., & Doudna, J. A. (2020). Chemistry of Class 1 CRISPR-Cas effectors: binding, editing, and regulation. *The Journal of Biological Chemistry*, *295*(42). https://doi.org/10.1074/jbc.REV120.007034

Liu, T. Y., & Doudna, J. A. (2020). Chemistry of Class 1 CRISPR-Cas effectors: binding, editing, and regulation. *The Journal of Biological Chemistry*, *295*(42). https://doi.org/10.1074/jbc.REV120.007034

Maeder, M. L., Linder, S. J., Cascio, V. M., Fu, Y., Ho, Q. H., & Joung, J. K. (2013). CRISPR RNA–guided activation of endogenous human genes.

Nature Methods, *10*(10), 977–979. https://doi.org/10.1038/nmeth.2598

Makarova, K. S., Wolf, Y. I., Alkhnbashi, O. S., Costa, F., Shah, S. A., Saunders, S. J., Barrangou, R., Brouns, S. J. J., Charpentier, E., Haft, D. H., Horvath, P., Moineau, S., Mojica, F. J. M., Terns, R. M., Terns, M. P., White, M. F., Yakunin, A. F., Garrett, R. A., van der Oost, J., & Backofen, R. (2015). An updated evolutionary classification of CRISPR–Cas systems. *Nature Reviews Microbiology*, *13*(11), 722–736. https://doi.org/10.1038/nrmicro3569

Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J. J., Charpentier, E., Cheng, D., Haft, D. H., Horvath, P., Moineau, S., Mojica, F. J. M., Scott, D., Shah, S. A., Siksnys, V., Terns, M. P., Venclovas, Č., White, M. F., Yakunin, A. F., & Yan, W. (2019). Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. *Nature Reviews Microbiology*, *18*(2), 67–83. https://doi.org/10.1038/s41579-019-0299-x

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-Guided Human Genome Engineering via Cas9. *Science*, *339*(6121), 823–826. https://doi.org/10.1126/science.1232033

Mansfiels, Z. (2023, December 1). *İki kadının Nobel'e uzanan yolu…* . TurcoMoney. https://www.turcomoney.com/iki-kadinin-nobele-uzanan-yolu.html

Mir, T. U. G., Wani, A. K., Akhtar, N., & Shukla, S. (2022). CRISPR/Cas9: Regulations and challenges for law enforcement to combat its dual-use. *Forensic Science International*, *334*(111274), 111274. https://doi.org/10.1016/j.forsciint.2022.111274

Mojica, F. J. M., Díez-Villaseñor, C., García-Martínez, J., & Soria, E. (2005). Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. *Journal of Molecular Evolution*, *60*(2), 174–182. https://doi.org/10.1007/s00239-004-0046-3

Mondal, S., Singh, S. P., & Lahir, Y. K. (2022). Emerging Trends in Environmental Biotechnology. In *Emerging Trends in Environmental Biotechnology* (pp. 131–142). CRC Press.

Nakagawa, R., Kannan, S., Altae-Tran, H., Takeda, S. N., Tomita, A., Hirano, H., Kusakizako, T., Nishizawa, T., Yamashita, K., Zhang, F., Nishimasu, H., & Nureki, O. (2022). Structure and engineering of the minimal type VI CRISPR-Cas13bt3. *Molecular Cell*, *82*(17), 3178-3192.e5. https://doi.org/10.1016/j.molcel.2022.08.001

Nishimura, T., Takahashi, E., & Fujimoto, T. (2024). Sterilization of fish through adaptable gRNAs targeting dnd1 using CRISPR-Cas13d system. *Aquaculture*, *593*, 741269–741269. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2024.741269

Nishimura, T., Takahashi, E., & Fujimoto, T. (2024). Sterilization of fish through adaptable gRNAs targeting dnd1 using CRISPR-Cas13d system. *Aquaculture*, *593*, 741269–741269. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2024.741269

Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR–Cas technologies and applications. *Nature Reviews Molecular Cell Biology*, *20*(8), 490–507. https://doi.org/10.1038/s41580-019-0131-5

Pourcel, C., Salvignol, G., & Vergnaud, G. (2005). CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology*, *151*(3), 653–663. https://doi.org/10.1099/mic.0.27437-0

Ramlee, M. K., Yan, T., Cheung, A. M. S., Chuah, C. T. H., & Li, S. (2015). High-throughput genotyping of CRISPR/Cas9-mediated mutants using fluorescent PCR-capillary gel electrophoresis. *Scientific Reports*, *5*(1), 1–13. https://doi.org/10.1038/srep15587

Redman, M., King, A., Watson, C., & King, D. (2016). What Is CRISPR/Cas9? *Archives of Disease in Childhood - Education & Practice Edition*, *101*(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459

Redman, M., King, A., Watson, C., & King, D. (2016). What Is CRISPR/Cas9? *Archives of Disease in Childhood - Education & Practice Edition*, *101*(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459

Richardson, C. D., Ray, G. J., DeWitt, M. A., Curie, G. L., & Corn, J. E. (2016). Enhancing homology-directed genome editing by catalytically active and inactive CRISPR-Cas9 using asymmetric donor DNA. *Nature Biotechnology*, *34*(3), 339–344. https://doi.org/10.1038/nbt.3481

Rivera-Torres, N., & Kmiec, E. B. (2017). A Standard Methodology to Examine On-site Mutagenicity As a Function of Point Mutation Repair

Catalyzed by CRISPR/Cas9 and SsODN in Human Cells. *Journal of Visualized Experiments*, *126*. https://doi.org/10.3791/56195

Rodolphe Barrangou. (n.d.). Food, Bioprocessing and Nutrition Sciences. Retrieved August 1, 2024, from https://cals.ncsu.edu/food-bioprocessing-and-nutrition-sciences/people/rbarran/

Roy, S., Kumar, V., Behera, B. K., Parhi, J., Mohapatra, S., Chakraborty, T., & Das, B. K. (2022). CRISPR/Cas Genome Editing—Can It Become a Game Changer in Future Fisheries Sector? *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.924475

Salmonella. (2024, March 24). Wiktionary. https://en.wiktionary.org/wiki/Salmonella

Sánchez-Paz, A. (2010). White spot syndrome virus: an overview on an emergent concern. *Veterinary Research*, *41*(6), 43. https://doi.org/10.1051/vetres/2010015

Sankaran, S. M., Smith, J. D., & Roy, K. R. (2021). CRISPR-Cas9 Gene Editing in Yeast: A Molecular Biology and Bioinformatics Laboratory Module for Undergraduate and High School Students. *Journal of Microbiology & Biology Education*, *22*(2). https://doi.org/10.1128/jmbe.00106-21

Santos, D. P., Kiskinis, E., Eggan, K., & Merkle, F. T. (2016). Comprehensive protocols for CRISPR/Cas9-based gene editing in human pluripotent stem cells. *Current Protocols in Stem Cell Biology*, *38*, 5B.6.1–5B.6.60. https://doi.org/10.1002/cpsc.15

Scorletti, E., & Byrne, C. D. (2018). Omega-3 fatty acids and non-alcoholic fatty liver disease: Evidence of efficacy and mechanism of action. *Molecular Aspects of Medicine*, *64*, 135–146. ScienceDirect. https://doi.org/10.1016/j.mam.2018.03.001

Shabbir, M. A. B., Hao, H., Shabbir, M. Z., Hussain, H. I., Iqbal, Z., Ahmed, S., Sattar, A., Iqbal, M., Li, J., & Yuan, Z. (2016). Survival and Evolution of CRISPR–Cas System in Prokaryotes and Its Applications. *Frontiers in Immunology*, *7*(375). https://doi.org/10.3389/fimmu.2016.00375

Song, C.-Q., Li, Y., Mou, H., Moore, J., Park, A., Pomyen, Y., Hough, S., Kennedy, Z., Fischer, A., Yin, H., Anderson, D. G., Conte, D., Zender, L., Wang, X. W., Thorgeirsson, S., Weng, Z., & Xue, W. (2017). Genome-Wide CRISPR Screen Identifies Regulators of Mitogen-Activated Protein Kinase as Suppressors of Liver Tumors in Mice.

Gastroenterology, *152*(5), 1161-1173.e1. https://doi.org/10.1053/j.gastro.2016.12.002

Stella, G., & Marraffini, L. (2023). Type III CRISPR-Cas: beyond the Cas10 effector complex. *Trends in Biochemical Sciences*, *49*(1), 28–37. Cell Press. https://doi.org/10.1016/j.tibs.2023.10.006

Su, X.-L., Zheng, G.-D., & Zou, S.-M. (2024). Knockout of EPO gene in blunt snout bream (Megalobrama amblycephala) by CRISPR/Cas9 reveals its roles in hypoxia-tolerance. *Aquaculture*, *592*, 741227–741227. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2024.741227

Su, X.-L., Zheng, G.-D., & Zou, S.-M. (2024). Knockout of EPO gene in blunt snout bream (Megalobrama amblycephala) by CRISPR/Cas9 reveals its roles in hypoxia-tolerance. *Aquaculture*, *592*, 741227–741227. ScienceDirect. https://doi.org/10.1016/j.aquaculture.2024.741227

Taning, C. N. T., Van Eynde, B., Yu, N., Ma, S., & Smagghe, G. (2017). CRISPR/Cas9 in insects: Applications, best practices and biosafety concerns. *Journal of Insect Physiology*, *98*, 245–257. ScienceDirect. https://doi.org/10.1016/j.jinsphys.2017.01.007

Taylor, H. N., Laderman, E., Armbrust, M., Hallmark, T., Keiser, D., Bondy-Denomy, J., & Jackson, R. N. (2021). Positioning Diverse Type IV Structures and Functions Within Class 1 CRISPR-Cas Systems. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.671522

Vouillot, L., Thélie, A., & Pollet, N. (2015). Comparison of T7E1 and Surveyor Mismatch Cleavage Assays to Detect Mutations Triggered by Engineered Nucleases. *G3: Genes, Genomes, Genetics*, *5*(3), 407–415. https://doi.org/10.1534/g3.114.015834

Wang, J., Zhang, X., Cheng, L., & Luo, Y. (2019). An overview and metanalysis of machine and deep learning-based CRISPR gRNA design tools. *RNA Biology*, *17*(1), 13–22. https://doi.org/10.1080/15476286.2019.1669406

Wang, Q., Zhang, B., Xu, X., Long, F., & Wang, J. (2018). CRISPR-typing PCR (ctPCR), a new Cas9-based DNA detection method. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-32329-x

Wang, T., Lander, E. S., & Sabatini, D. M. (2016). Viral Packaging and Cell Culture for CRISPR-Based Screens. *Cold Spring Harbor Protocols*, *2016*(3), pdb.prot090811. https://doi.org/10.1101/pdb.prot090811

Wani, A. K., Akhtar, N., Singh, R., Prakash, A., Raza, S. H. A., Cavalu, S., Chopra, C., Madkour, M., Elolimy, A., & Hashem, N. M. (2022). Genome centric engineering using ZFNs, TALENs and CRISPR-Cas9 systems for trait improvement and disease control in Animals. *Veterinary Research Communications*, *47*(1), 1–16. https://doi.org/10.1007/s11259-022-09967-8

Westra, E. R., Buckling, A., & Fineran, P. C. (2014). CRISPR–Cas systems: beyond adaptive immunity. *Nature Reviews Microbiology*, *12*(5), 317–326. https://doi.org/10.1038/nrmicro3241

White, M. K., Hu, W., & Khalili, K. (2015). The CRISPR/Cas9 genome editing methodology as a weapon against human viruses. *Discovery Medicine*, *19*(105), 255–262. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445958/

Wu, S.-H., Lin, H.-J., Lin, W.-F., Wu, J.-L., & Gong, H.-Y. (2018). A potent tilapia secreted granulin peptide enhances the survival of transgenic zebrafish infected by Vibrio vulnificus via modulation of innate immunity. *Fish & Shellfish Immunology*, *75*, 74–90. ScienceDirect. https://doi.org/10.1016/j.fsi.2018.01.044

Wu, S.-H., Lin, H.-J., Lin, W.-F., Wu, J.-L., & Gong, H.-Y. (2018). A potent tilapia secreted granulin peptide enhances the survival of transgenic zebrafish infected by Vibrio vulnificus via modulation of innate immunity. *Fish & Shellfish Immunology*, *75*, 74–90. ScienceDirect. https://doi.org/10.1016/j.fsi.2018.01.044

Xu, C. L., & Tsang, S. H. (2022, January 1). *Chapter 1 - The history of CRISPR: from discovery to the present* (S. H. Tsang, Ed.). ScienceDirect; Academic Press. https://www.sciencedirect.com/science/article/pii/B9780128178768000073?via=ihub

Yan, W. X., Hunnewell, P., Alfonse, L. E., Carte, J. M., Keston-Smith, E., Sothiselvam, S., Garrity, A. J., Chong, S., Makarova, K. S., Koonin, E. V., Cheng, D. R., & Scott, D. A. (2019). Functionally diverse type V CRISPR-Cas systems. *Science*, *363*(6422), 88–91. https://doi.org/10.1126/science.aav7271

Yang, L., Yang, J. L., Byrne, S., Pan, J., & Church, G. M. (2014).

CRISPR/Cas9-Directed Genome Editing of Cultured Cells. *Current Protocols in Molecular Biology*, *107*(1). https://doi.org/10.1002/0471142727.mb3101s107

Yoshimi, K., & Mashimo, T. (2022). Genome editing technology and applications with the type I CRISPR system. *Gene and Genome Editing*, *3-4*, 100013. https://doi.org/10.1016/j.ggedit.2022.100013

Yu, X., Liang, X., Xie, H., Kumar, S., Ravinder, N., Potter, J., de Mollerat du Jeu, X., & Chesnut, J. D. (2016). Improved delivery of Cas9 protein/gRNA complexes using lipofectamine CRISPRMAX. *Biotechnology Letters*, *38*(6), 919–929. https://doi.org/10.1007/s10529-016-2064-9

Zhou, Y., Bravo, J. P. K., Taylor, H. N., Steens, J. A., Jackson, R. N., Staals, R. H. J., & Taylor, D. W. (2021). Structure of a type IV CRISPR-Cas ribonucleoprotein complex. *IScience*, *24*(3), 102201. Cell Press. https://doi.org/10.1016/j.isci.2021.102201

CHAPTER 12

PHENOLIC COMPOUNDS AS FUNCTIONAL ADDITIVES IN FISH FEED: BENEFITS, BIOCHEMICAL ROLES, MECHANISMS, CHALLENGES, AND FUTURE INNOVATIONS IN AQUACULTURE

Assist. Prof. Dr. Boran KARATAŞ[1]
Prof. Dr. Aysun KOP[2]
Prof. Dr. Önder YILDIRIM[3]

[1] Van Yüzüncü Yıl University, Faculty of Fisheries, Department of Aquaculture, Van, Türkiye. borankaratas@yyu.edu.tr, Orcid ID: 0000-0003-4353-1293
[2] Ege University, Faculty of Fisheries, Aquaculture Department, İzmir, Türkiye, aysun.kop.firat@ege.edu.tr, Orcid ID: 0000-0003-1724-5672
[3] Mugla Sıtkı Kocman University, Faculty of Fisheries, Department of Aquaculture, Muğla, Turkey onderyildirim@mu.edu.tr, Orcid ID 0000-0003-2591-0310

INTRODUCTION

In the rapidly expanding aquaculture industry, the need for sustainable and effective dietary interventions is paramount to meet rising global seafood demands while ensuring fish health and welfare. One promising avenue involves the use of phenolic compounds, naturally occurring bioactive substances prevalent in a wide array of plants, algae, and agricultural by-products. Phenolic compounds are primarily valued for their diverse biological activities, including antioxidative, anti-inflammatory, antimicrobial, and immunomodulatory effects, which collectively contribute to improved fish growth, health, and resilience to environmental and pathogenic stresses (Maqsood et al., 2013; Ahmadifar et al., 2021; Naiel et al., 2021). By integrating phenolic compounds into fish feeds, aquaculture practitioners can potentially reduce reliance on synthetic additives, antibiotics, and other chemicals, moving towards more eco-friendly and health-conscious feeding strategies (Chakraborty et al., 2014; Albuquerque et al., 2021).

The antioxidative properties of phenolic compounds are particularly significant in fish diets, as oxidative stress is a common challenge in intensive aquaculture systems. Fish exposed to high-density farming conditions often encounter environmental and physiological stressors that lead to the production of reactive oxygen species (ROS), which can damage cellular structures and impair immune function (Herrera et al., 2019; Ciji and Akhtar 2021). Antioxidant-rich phenolics, such as those found in herbs, seaweed, and other plant materials, can mitigate oxidative damage, thereby enhancing fish vitality and growth rates (Tufarelli et al., 2017; Beltrán & Esteban, 2022). Furthermore, these compounds have been shown to improve immune responses by stimulating key immune-related genes and enhancing disease resistance, a crucial factor given the high risk of infectious diseases in aquaculture environments (Firmino et al., 2021; Naiel et al., 2023).

Recent studies have documented the antimicrobial activities of various phenolic compounds, which can support gut health and reduce the prevalence of bacterial pathogens without the overuse of antibiotics (Chakraborty & Hancz, 2011; Chen et al., 2023). These effects are especially beneficial as regulatory restrictions on antibiotic use continue to tighten in many countries, pushing the aquaculture industry to seek alternative solutions for managing health and preventing infections (Galina et al., 2009). For example, polyphenols from plants and algae have been shown to inhibit the growth of specific bacterial strains, supporting a balanced gut microbiome and promoting better nutrient utilization, which in turn contributes to more efficient growth and feed conversion ratios (Ahmadifar et al., 2021; Andriopoulos et al., 2022).

In addition to these benefits, phenolic compounds have shown potential as growth enhancers and metabolic modulators, influencing pathways related to growth, digestion, and lipid metabolism (Chakraborty et al., 2014; Ahmadifar et al., 2021; Faheem et al., 2022; Abdel-Latif et al., 2023; Siddik et al., 2023). Research on seaweed-derived phenolics, for instance, has highlighted their role in modulating lipid profiles and energy utilization, which are critical for achieving desired growth performance in various fish species (Naiel et al., 2021; Subbiah et al., 2023). Beyond growth effects, phenolic-rich additives may contribute to stress resistance by stabilizing hormonal responses and providing anti-stress benefits during handling, transport, and environmental fluctuations, which are common stressors in aquaculture (Bulfon et al., 2015; Jakobek & Blesso, 2023).

Despite these numerous advantages, the practical application of phenolic compounds in fish feeds is not without challenges. Key issues include variability in bioavailability, possible adverse effects at high dosages, and interactions with other dietary components that can impact their efficacy (Balasundram et al., 2006; Albuquerque et al., 2021). Bioavailability of phenolics can be influenced by fish species, digestive physiology, and the type of feed formulation used, raising questions about optimal dosing and delivery mechanisms. To address these limitations, ongoing research is investigating the structural diversity, metabolism, and stability of phenolic compounds, aiming to maximize their potential in aquafeeds through encapsulation technologies, bioactive synergies, and tailored formulations (Martins et al., 2016; Leyva-López et al., 2020).

This review provides an extensive examination of phenolic compounds as functional feed additives in aquaculture, focusing on their biochemical properties, mechanisms of action, and benefits for fish health and productivity. It addresses practical challenges in their incorporation into aquafeeds, including stability and bioavailability issues, while highlighting the need for innovative strategies to enhance their effectiveness. The review also discusses future directions for research, emphasizing the potential of synergistic formulations to maximize health benefits. Ultimately, it underscores the importance of optimizing phenolic compounds in aquaculture nutrition to promote sustainable practices that meet the increasing demand for high-quality, eco-friendly aquafeeds.

BIOCHEMICAL PROPERTIES OF PHENOLIC COMPOUNDS IN AQUACULTURE

Phenolic compounds, abundant in plants, represent a diverse group of secondary metabolites characterized by one or more hydroxyl groups attached to an aromatic ring (Crozier et al., 2006; Tsimogiannis and Oreopoulou 2019; Vuolo et al., 2019). Their biochemical properties make them well-suited as functional feed additives, particularly due to their antioxidant, anti-inflammatory, and antimicrobial attributes (Balasundram et al., 2006; Maqsood et al., 2013). These compounds can be classified broadly into phenolic acids, flavonoids, tannins, and lignans, with each subclass exhibiting distinct properties and bioactivities (Vuolo et al., 2019). The structure of phenolic compounds determines their ability to scavenge free radicals, an action primarily attributed to the hydroxyl groups, which donate hydrogen atoms and stabilize reactive oxygen species (ROS) (Albuquerque et al., 2021). This ROS-scavenging potential is crucial in aquaculture, where oxidative stress can significantly impact fish health and growth.

Plant-based phenolics exhibit a range of antioxidant mechanisms, including direct scavenging of ROS, inhibition of pro-oxidative enzymes, and enhancement of the endogenous antioxidant defense system in fish. These mechanisms have attracted attention in aquaculture, where oxidative stress is a common challenge due to environmental fluctuations and intensive farming practices (Andriopoulos et al., 2022). In addition to antioxidant properties, phenolic compounds influence immune responses, digestion, and metabolic regulation in fish (Chakraborty et al., 2014). For example, tannins and flavonoids can modulate inflammatory cytokine production, reducing tissue inflammation and strengthening the immune system (Leyva-López et al., 2020).

A significant challenge in using phenolics is their bioavailability, which varies due to differences in molecular structure, solubility, and metabolism. Structural differences among phenolic compounds affect their intestinal absorption, metabolic pathways, and excretion rates, influencing their efficacy as bioactive feed additives (Tufarelli et al., 2017). Studies have shown that certain plant extracts, such as those from macroalgae, possess bioavailable phenolics with high antioxidant capacities, making them promising candidates for aquaculture applications (Naiel et al., 2021).

MECHANISMS OF ACTION IN FISH HEALTH AND PRODUCTIVITY

Phenolic compounds function through various mechanisms that support fish health and productivity, making them suitable as natural feed additives in aquaculture (Ahmadifar et al., 2021). A primary mechanism is their antioxidant activity, which mitigates oxidative stress by neutralizing ROS and enhancing the activity of endogenous antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase (Mariappan et al., 2023). This action is crucial in preventing cellular damage, a common issue in high-density farming environments that can lead to decreased growth rates and increased susceptibility to diseases (Subbiah et al., 2023).

In addition to antioxidant effects, phenolic compounds exhibit immunomodulatory functions by enhancing immune cell proliferation, cytokine production, and antibody responses (Firmino et al., 2021). These immunomodulatory effects are linked to the ability of phenolics to interact with receptors and signaling pathways involved in the innate and adaptive immune responses (Chen et al., 2023). Certain phenolics also act as anti-inflammatory agents, reducing the release of pro-inflammatory mediators like nitric oxide and tumor necrosis factor-alpha (TNF-α), thus minimizing tissue inflammation in fish (Awad & Awaad, 2017).

Furthermore, phenolics support digestive health and nutrient utilization. By modulating gut microbiota composition, phenolics enhance the population of beneficial bacteria, aiding in nutrient absorption and reducing gut pathogens (Bulfon et al., 2015). They can also increase digestive enzyme activity, enhancing feed efficiency and growth rates (Chakraborty & Hancz, 2011). These mechanisms collectively improve fish resilience, growth, and overall productivity, underscoring the value of phenolic compounds in aquaculture.

BENEFITS IN FISH HEALTH AND PRODUCTIVITY

The incorporation of phenolic compounds as feed additives in aquaculture has demonstrated significant benefits in enhancing fish health and boosting productivity. Derived mainly from plant-based sources, phenolic compounds have been linked to improved growth performance, immune function, antioxidant capacity, and digestive health in various fish species. With the growing demand for sustainable and health-oriented aquaculture practices, phenolic compounds offer a natural and effective solution for promoting overall fish well-being while reducing dependency on antibiotics and synthetic additives (Ahmadifar et al., 2021; Mariappan et al., 2023). Understanding these benefits is crucial for optimizing phenolic use in aquafeeds and aligning aquaculture practices with modern standards of environmental stewardship and fish welfare.

Enhanced Growth Performance

One of the most tangible benefits of phenolic compounds in aquaculture is their positive impact on fish growth performance. The presence of specific phenolics, such as flavonoids, tannins, and phenolic acids, has been associated with enhanced growth rates, often evidenced by improvements in weight gain, feed conversion ratios (FCRs), and protein utilization efficiency (Makkar et al., 2007; Ahmadifar et al., 2021; Taştan and Salem 2021; Formato et al., 2022). Phenolic compounds can stimulate growth by influencing metabolic processes and enhancing nutrient absorption, allowing fish to allocate more energy toward tissue development and less toward managing oxidative stress or fighting infections (Yang et al., 2015; Akbari et al., 2022; Subbiah et al., 2023). Certain phenolic compounds promote the release of growth-related hormones, such as insulin-like growth factor (IGF), which is pivotal for growth and tissue development in fish (Midhun et al., 2016; Safari et al., 2020; Ahmadifar et al., 2021). Additionally, phenolics improve feed efficiency, an important factor considering feed costs represent a substantial portion of operational expenses in aquaculture. By improving FCRs, phenolic additives not only enhance productivity but also contribute to a more cost-effective and sustainable feeding approach.

Immune Modulation and Disease Resistance

Another critical benefit of phenolic compounds in aquaculture lies in their role as immunostimulants, enhancing disease resistance and reducing the need for antibiotics. Phenolic compounds strengthen both innate and adaptive immune responses in fish, bolstering defenses against pathogens commonly encountered in intensive farming systems (Ahmadifar et al., 2021; Alam et al., 2024; Gheorghe et al., 2024). For instance, phenolics have been shown to upregulate immune-related genes, stimulate the activity of phagocytes (e.g., macrophages), and increase the production of cytokines—proteins essential for signaling immune responses (Mariappan et al., 2023). By activating and reinforcing immune responses, phenolics help fish withstand bacterial, viral, and parasitic infections more effectively (Chakraborty et al., 2011; Dawood et al., 2018; Tadese et al., 2022). Moreover, phenolic compounds can reduce the severity of infections by modulating the inflammatory response, minimizing tissue damage, and promoting faster recovery (Gao et al., 2021; Saleh et al., 2022; Zhang et al., 2022). This immunomodulatory effect is particularly valuable in reducing mortality rates in aquaculture systems, thus contributing to stable and predictable production outcomes (Ahmadifar et al., 2021).

The reduced reliance on antibiotics is another significant benefit related to the immune-supporting effects of phenolic compounds. As antibiotic resistance becomes a growing concern in both aquaculture and global health, phenolic compounds offer an attractive alternative by providing natural, plant-based immunity support (Bhanja et al., 2023; Hossain et al., 2024). Their ability to strengthen immune function without contributing to antibiotic resistance aligns with the principles of sustainable aquaculture and food safety.

Antioxidant Capacity and Cellular Protection

The antioxidant properties of phenolic compounds play a crucial role in maintaining cellular health and resilience in fish, which is vital under the conditions of modern aquaculture where stress levels are often high. Phenolic compounds act as potent antioxidants by neutralizing reactive oxygen species (ROS) and protecting cells from oxidative damage (Zhang and Tsao 2016; Kaurinovic and Vastag 2019; Lv et al., 2021). Oxidative stress, if left unmanaged, can lead to lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing growth, reproduction, and overall vitality in fish

(Subbiah et al., 2023). By countering oxidative stress, phenolic compounds help maintain cellular integrity and support the longevity of various tissues, including the gills, liver, and muscles, which are frequently exposed to high oxygen turnover rates in intensive aquaculture environments.

Moreover, phenolic compounds can upregulate endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), further enhancing the fish's intrinsic defense against oxidative damage (Jahazi et al., 2020; Lv et al., 2021; Mohammady et al., 2022). This dual antioxidant mechanism both direct scavenging of free radicals and enhancement of natural antioxidant defenses creates a comprehensive protection system that safeguards fish health (Ahmadifar et al., 2021). Antioxidant support also improves overall stress tolerance, making fish more resilient to environmental challenges such as fluctuating water temperatures, pH changes, and hypoxia. Consequently, phenolic compounds not only promote growth by reducing the metabolic costs associated with cellular repair but also extend fish survival rates in suboptimal conditions, contributing to more reliable production outcomes.

Improved Digestive Health and Nutrient Utilization

Phenolic compounds also contribute to enhanced digestive health, which directly translates into better nutrient utilization and growth in fish. Some phenolic compounds support the health of the gut microbiota, which plays a fundamental role in the digestion and assimilation of nutrients (Valdés et al., 2015; Aravind et al., 2021; Domínguez-Avila et al., 2021). For instance, flavonoids and tannins have been found to foster the growth of beneficial bacteria, such as Lactobacillus and Bifidobacterium, while inhibiting harmful pathogens, leading to a more balanced intestinal microbiome (Chakraborty & Hancz, 2011). A well-balanced gut microbiota promotes efficient digestion and nutrient absorption, thus improving feed conversion efficiency.

Additionally, phenolic compounds can enhance the secretion of digestive enzymes, such as amylase, protease, and lipase, which aid in the breakdown of carbohydrates, proteins, and fats, respectively (Martinez-Gonzalez et al., 2017; Melzig 2023). Enhanced enzymatic activity ensures that nutrients are more readily available for absorption, supporting optimal growth. Tannins, despite their occasional reputation as anti-nutritional factors in some contexts, can have beneficial effects in aquaculture at appropriate doses by binding to harmful compounds in the gut, thereby preventing their absorption and allowing more efficient nutrient processing (Omnes et al., 2017; Yang et al., 2024). Improved nutrient utilization not only leads to better growth rates but also reduces feed waste and environmental impact by minimizing uneaten feed and nutrient excretion (Ahmadifar et al., 2021).

Stress Reduction and Adaptation

Aquaculture fish are often subject to various stressors, including handling, stocking density, and changes in water quality. Stress can compromise fish immune function, growth, and reproductive success, leading to lower productivity and higher susceptibility to disease (Pankhurst and Van Der Kraak 1997; Mateus et al., 2017; Liu et al., 2022). Phenolic compounds play a role in alleviating stress responses in fish by modulating the release of stress hormones such as cortisol, which is a primary mediator of stress-related responses in fish (Subbiah et al., 2023; Alam et al., 2024; Sánchez-Velázquez et al., 2024). By reducing cortisol levels and buffering against oxidative stress, phenolics can lower the physiological impact of stress, promoting better health and welfare outcomes for fish in intensive aquaculture settings.

In addition to cortisol modulation, phenolic compounds may also aid in the fish’s ability to adapt to environmental challenges. For instance, phenolics have been shown to improve osmoregulation in fish under salinity stress, helping them maintain ion balance and homeostasis (Moghadam et al., 2022; Salem et al., 2024). Improved stress resilience translates to reduced mortality rates and improved growth and health metrics, making phenolic compounds particularly valuable in systems where stress is a common challenge.

Environmental and Economic Sustainability

The use of phenolic compounds also has broader implications for the sustainability and economic viability of aquaculture. By improving feed efficiency, health, and survival rates, phenolic compounds contribute to a reduction in resource use and operational costs. Improved feed conversion ratios mean less feed is needed to achieve target growth rates, which reduces feed costs and minimizes the environmental footprint associated with feed production. Furthermore, by promoting health and immunity, phenolic compounds reduce the incidence of disease outbreaks, leading to lower mortality rates and reduced dependency on costly treatments (Ahmadifar et al., 2021). This dual benefit of economic and environmental sustainability aligns with the global movement toward more responsible and eco-friendly aquaculture practices.

PRACTICAL CONSIDERATIONS AND CHALLENGES IN APPLICATION

While phenolic compounds offer promising benefits in aquaculture, several practical considerations and challenges must be addressed for effective application. Bioavailability remains one of the primary concerns, as many phenolics are metabolized rapidly and may not reach effective concentrations in target tissues (Bešlo et al., 2023). Factors like molecular structure, gut pH, and interactions with other feed components can impact their absorption and utilization. Techniques such as nano-encapsulation and use of liposomal carriers have shown potential to enhance the stability and bioavailability of phenolics in aquaculture feeds (Mariappan et al., 2023).

Another challenge lies in the variability of phenolic content in plant sources, influenced by factors such as plant species, harvesting season, and extraction method (Makkar et al., 2007). This variability complicates standardization and dosage consistency, which are essential for reproducible effects. Additionally, the potential toxicity of certain phenolics at high concentrations requires careful formulation and dosage control (Chakraborty & Hancz, 2011).

Cost is another consideration; high-quality plant extracts can be expensive, making large-scale use in aquaculture challenging. Identifying cost-effective sources of phenolics, such as agro-industrial by-products, could mitigate this issue (Leyva-López et al., 2020). Furthermore, while phenolics are generally recognized as safe, long-term studies on their effects at various dosages are limited, warranting further research to establish optimal inclusion levels and avoid any adverse effects on fish health (Albuquerque et al., 2021). Addressing these challenges is essential to fully harness the potential of phenolics in aquafeeds.

FUTURE DIRECTIONS: INNOVATIONS AND RESEARCH GAPS

Future research on phenolic compounds in aquaculture should prioritize strategies to overcome bioavailability issues, possibly through advanced delivery systems and synthetic analogs (Jakobek & Blesso, 2023). Encapsulation techniques, for instance, could protect phenolics from degradation in the gastrointestinal tract, allowing for better absorption and targeted delivery (Tufarelli et al., 2017). Additionally, exploring synergistic combinations of phenolics with other bioactive compounds could amplify their benefits, enhancing their effectiveness against oxidative stress and disease (Freile-Pelegrín & Robledo, 2013).

There is also a need for more in vivo studies to validate the efficacy and safety of specific phenolic compounds, especially under diverse aquaculture conditions. Research should focus on long-term effects, optimal dosages, and potential interactions with conventional feed ingredients (Subbiah et al., 2023). Developing standardized extraction methods and quality controls will be critical to ensuring consistency in phenolic content and efficacy in commercial feeds (Naiel et al., 2021).

Exploring underutilized sources of phenolics, such as marine algae and agro-industrial by-products, could provide sustainable and cost-effective alternatives for aquafeed formulation (Andriopoulos et al., 2022). Such innovations could not only expand the availability of phenolic sources but also support eco-friendly practices in line with sustainable aquaculture goals. By addressing these research gaps, the industry can enhance the functional value of phenolics and contribute to the resilience and sustainability of global aquaculture practices.

CONCLUSION

In conclusion, phenolic compounds present a promising avenue for enhancing fish health, growth, and resilience in aquaculture through their diverse biochemical properties and multifaceted mechanisms of action. Their ability to act as antioxidants, immune modulators, and growth enhancers highlights their potential as functional feed additives, providing a sustainable alternative to conventional antibiotics and growth promoters. However, practical challenges such as limited bioavailability, stability issues, and regulatory constraints must be addressed to fully realize the benefits of phenolics in aquafeeds. Ongoing research into advanced delivery systems, standardization of phenolic sources, and exploration of synergistic formulations offers encouraging prospects for overcoming these limitations. As aquaculture continues to expand, integrating phenolic compounds in fish diets aligns well with the industry's goal to produce eco-friendly and health-promoting feeds. Future research should prioritize optimizing phenolic compound formulations, improving cost-effectiveness, and assessing long-term impacts to unlock their full potential in sustainable aquaculture practices.

REFERENCES

Abdel-Latif, H. M., Yilmaz, S., & Kucharczyk, D. (2023). Functionality and applications of phytochemicals in aquaculture nutrition. *Frontiers in Veterinary Science*, *10*, 1218542.

Ahmadifar, E., Pourmohammadi Fallah, H., Yousefi, M., Dawood, M. A., Hoseinifar, S. H., Adineh, H., ... & Doan, H. V. (2021). The gene regulatory roles of herbal extracts on the growth, immune system, and reproduction of fish. Animals, 11(8), 2167.

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., ... & Abdel-Latif, H. M. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. *Reviews in Fisheries Science & Aquaculture*, *29*(4), 478-511.

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611-633.

Alam S, Urva-til-wusqa, Iqbal Z, Qamar MR, Afzal M, Mustafa G, Ali HM & Hussain R, (2024). Immunostimulation and stress reduction in farmed fish fed herbal extracts. In: Abbas RZ, Khan AMA, Qamar W, Arshad J and Mehnaz S (eds), Complementary and Alternative Medicine: Botanicals/Homeopathy/Herbal Medicine. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 422-430.

Albuquerque, B. R., Heleno, S. A., Oliveira, M. B. P., Barros, L., & Ferreira, I. C. (2021). Phenolic compounds: Current industrial applications, limitations and future challenges. *Food & function*, *12*(1), 14-29.

Andriopoulos, V., Gkioni, M. D., Koutra, E., Mastropetros, S. G., Lamari, F. N., Hatziantoniou, S., & Kornaros, M. (2022). Total phenolic content, biomass composition, and antioxidant activity of selected marine microalgal species with potential as aquaculture feed. *Antioxidants*, *11*(7), 1320.

Aravind, S. M., Wichienchot, S., Tsao, R., Ramakrishnan, S., & Chakkaravarthi, S. J. F. R. I. (2021). Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. *Food Research International*, *142*, 110189.

Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & shellfish immunology*, *67*, 40-54.

Bhanja, A., Payr, P., & Mandal, B. (2023). Phytobiotics: Response to Aquaculture as Substitute of Antibiotics and other Chemical Additives. *South Asian Journal of Experimental Biology*, *13*(5).

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chemistry*, *99*(1), 191-203.

Beltrán, J. M. G., & Esteban, M. Á. (2022). Nature-identical compounds as feed additives in aquaculture. *Fish & Shellfish Immunology*, *123*, 409-416.

Bešlo, D., Golubić, N., Rastija, V., Agić, D., Karnaš, M., Šubarić, D., & Lučić, B. (2023). Antioxidant activity, metabolism, and bioavailability of polyphenols in the diet of animals. *Antioxidants, 12*(6), 1141.

Bulfon, C., Volpatti, D., & Galeotti, M. (2015). Current research on the use of plant-derived products in farmed fish. *Aquaculture Research*, *46*(3), 513-551.

Chakraborty, S. B., & Hancz, C. (2011). Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, *3*(3), 103-119.

Chakraborty, S. B., Horn, P., & Hancz, C. (2014). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. *Reviews in Aquaculture*, *6*(1), 1-19.

Chen, X., Lan, W., & Xie, J. (2023). Natural phenolic compounds: Antimicrobial properties, antimicrobial mechanisms, and potential utilization in the preservation of aquatic products. *Food Chemistry*, 138198.

Ciji, A., & Akhtar, M. S. (2021). Stress management in aquaculture: A review of dietary interventions. *Reviews in Aquaculture*, *13*(4), 2190-2247.

Crozier, A., Clifford, M. N., & Ashihara, H. (2006). Plant secondary metabolites. *Occurrence, Structure and Role in the Human Diet*, Blackwell-Publishers.

Dawood, M. A., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture*, *10*(4), 950-974.

Domínguez-Avila, J. A., Villa-Rodriguez, J. A., Montiel-Herrera, M., Pacheco-Ordaz, R., Roopchand, D. E., Venema, K., & González-Aguilar, G. A. (2021). Phenolic compounds promote diversity of gut microbiota and maintain colonic health. *Digestive diseases and sciences*, *66*(10), 3270-3289.

Faheem, M., Abbas, R. Z., Liaqat, I., Hoseinifar, S. H., Maneepitaksanti, W., & Van Doan, H. (2022). Bio-active components in medicinal plants: A mechanistic review of their effects on fish growth and physiological parameters–A review. *Annals of Animal Science*, *22*(4), 1127-1149.

Firmino, J. P., Galindo-Villegas, J., Reyes-López, F. E., & Gisbert, E. (2021). Phytogenic bioactive compounds shape fish mucosal immunity. *Frontiers in immunology*, *12*, 695973.

Formato, M., Cimmino, G., Brahmi-Chendouh, N., Piccolella, S., & Pacifico, S. (2022). Polyphenols for livestock feed: sustainable perspectives for animal husbandry?. *Molecules*, *27*(22), 7752.

Freile-Pelegrín, Y., & Robledo, D. (2013). Bioactive phenolic compounds from algae. *Bioactive compounds from marine foods: plant and animal sources*, 113-129.

Galina, J., Yin, G., Ardo, L., & Jeney, Z. (2009). The use of immunostimulating herbs in fish. An overview of research. *Fish physiology and biochemistry*, *35*, 669-676.

Gao, X., Xu, Z., Liu, G., & Wu, J. (2021). Polyphenols as a versatile component in tissue engineering. *Acta biomaterialia*, *119,* 57-74.

Gheorghe, R. A. I., Tăpăloagă, D., Șonea, C., Ghimpețeanu, O. M., Gurău, M. R., & Tăpăloagă, P. R. (2024). Reviewing the Role of Functional Ingredients in Improving Immune Health and Disease Management in Breeding Animals: Current Perspectives and Emerging Trends. *Annals of" Valahia" University of Târgovişte. Agriculture*, *16*(1), 43-50.

Herrera, M., Mancera, J. M., & Costas, B. (2019). The use of dietary additives in fish stress mitigation: comparative endocrine and physiological responses. *Frontiers in endocrinology*, *10*, 447.

Hossain, M. S., Small, B. C., Kumar, V., & Hardy, R. (2024). Utilization of functional feed additives to produce cost-effective, ecofriendly aquafeeds high in plant-based ingredients. *Reviews in Aquaculture*, *16*(1), 121-153.

Jahazi, M. A., Hoseinifar, S. H., Jafari, V., Hajimoradloo, A., Van Doan, H., & Paolucci, M. (2020). Dietary supplementation of polyphenols positively affects the innate immune response, oxidative status, and growth performance of common carp, Cyprinus carpio L. *Aquaculture*, *517*, 734709.

Jakobek, L., & Blesso, C. (2023). Beneficial effects of phenolic compounds: native phenolic compounds vs metabolites and catabolites. *Critical reviews in food science and nutrition*, 1-19.

Kaurinovic, B., & Vastag, D. (2019). *Flavonoids and phenolic acids as potential natural antioxidants* (pp. 1-20). London, UK: IntechOpen.

Leyva-López, N., Lizárraga-Velázquez, C. E., Hernández, C., & Sánchez-Gutiérrez, E. Y. (2020). Exploitation of agro-industrial waste as potential source of bioactive compounds for aquaculture. *Foods*, *9*(7), 843.

Liu, Z., Zhou, T., & Gao, D. (2022). Genetic and epigenetic regulation of growth, reproduction, disease resistance and stress responses in aquaculture. *Frontiers in Genetics*, *13*, 994471.

López-Pedrouso, M., Lorenzo, J. M., Cantalapiedra, J., Zapata, C., Franco, J. M., & Franco, D. (2020). Aquaculture and by-products: Challenges and opportunities in the use of alternative protein sources and bioactive compounds. *Advances in food and nutrition research*, *92*, 127-185.

Lv, Q. Z., Long, J. T., Gong, Z. F., Nong, K. Y., Liang, X. M., Qin, T., ... & Yang, L. (2021). Current state of knowledge on the antioxidant effects and mechanisms of action of polyphenolic compounds. *Natural Product Communications*, *16*(7), 1934578X211027745.

Makkar, H. P. S., Francis, G., & Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *animal*, *1*(9), 1371-1391.

Maqsood, S., Benjakul, S., & Shahidi, F. (2013). Emerging role of phenolic compounds as natural food additives in fish and fish products. *Critical reviews in food science and nutrition*, *53*(2), 162-179.

Mariappan, B., Kaliyamurthi, V., & Binesh, A. (2023). Medicinal plants or plant derived compounds used in aquaculture. In *Recent advances in aquaculture microbial technology* (pp. 153-207). Academic Press.

Martinez-Gonzalez, A. I., Díaz-Sánchez, Á. G., de la Rosa, L. A., Vargas-Requena, C. L., Bustos-Jaimes, I., & Alvarez-Parrilla, E. (2017). Polyphenolic compounds and digestive enzymes: In vitro non-covalent interactions. *Molecules*, *22*(4), 669.

Martins, N., Barros, L., & Ferreira, I. C. (2016). In vivo antioxidant activity of phenolic compounds: Facts and gaps. *Trends in Food Science & Technology*, *48*, 1-12.

Mateus, A. P., Power, D. M., & Canário, A. V. (2017). Stress and disease in fish. In *Fish diseases* (pp. 187-220). Academic Press.

Melzig, M. F. (2023). Plant polyphenols as inhibitors of hydrolases are regulators of digestion. *Complementary Medicine Research*, *30*(5), 453-459.

Midhun, S. J., Arun, D., Edatt, L., Sruthi, M. V., Thushara, V. V., Oommen, O. V., ... & Divya, L. (2016). Modulation of digestive enzymes, GH, IGF-1 and IGF-2 genes in the teleost, Tilapia (Oreochromis mossambicus) by dietary curcumin. *Aquaculture international*, *24*, 1277-1286.

Moghadam, H., Sourinejad, I., & Johari, S. A. (2022). Dietary turmeric, curcumin and nanoencapsulated curcumin can differently fight against salinity stress in Pacific white shrimp Penaeus vannamei Boone, 1931. *Aquaculture Research*, *53*(8), 3127-3139.

Mohammady, E. Y., Soaudy, M. R., Mohamed, A. E., EL-Erian, M. M. A., Farag, A., Badr, A. M., ... & Hassaan, M. S. (2022). Can dietary phytogenic mixture improve performance for growth, digestive enzyme activity, blood parameters, and antioxidant and related gene expressions of Nile tilapia, Oreochromis niloticus?. *Animal Feed Science and Technology*, *290*, 115369.

Naiel, M. A., Alagawany, M., Patra, A. K., El-Kholy, A. I., Amer, M. S., & Abd El-Hack, M. E. (2021). Beneficial impacts and health benefits of macroalgae phenolic molecules on fish production. *Aquaculture*, *534*, 736186.

Naiel, M. A., El-Kholy, A. I., Negm, S. S., Ghazanfar, S., Shukry, M., Zhang, Z., ... & Abdel-Latif, H. M. (2023). A mini-review on plant-derived phenolic compounds with particular emphasis on their possible applications and beneficial uses in aquaculture. *Annals of Animal Science*, *23*(4), 971-977.

Omnes, M. H., Le Goasduff, J., Le Delliou, H., Le Bayon, N., Quazuguel, P., & Robin, J. H. (2017). Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (Dicentrarchus labrax L.). *Aquaculture Reports*, *6*, 21-27.

Pankhurst, N. W., & Van Der Kraak, G. (1997). Effects of stress on reproduction and growth of fish. *Fish stress and health in aquaculture*, *1*, 73-93.

Safari, R., Hoseinifar, S. H., Imanpour, M. R., Mazandarani, M., Sanchouli, H., & Paolucci, M. (2020). Effects of dietary polyphenols on mucosal and humoral immune responses, antioxidant defense and growth gene expression in beluga sturgeon (Huso huso). *Aquaculture*, *528*, 735494.

Saleh, M., Essawy, E., Shaalan, M., Osman, S., Ahmed, F., & El-Matbouli, M. (2022). Therapeutic intervention with dietary chitosan nanoparticles alleviates fish pathological and molecular systemic inflammatory responses against infections. *Marine drugs*, *20*(7), 425.

Salem, M. E., Almisherfi, H. M., El-Sayed, A. F. M., Makled, S. O., & Abdel-Ghany, H. M. (2024). Modulatory effects of dietary prickly pear (Opuntia ficus-indica) peel on high salinity tolerance, growth rate, immunity and antioxidant capacity of Nile tilapia (Oreochromis niloticus). *Fish Physiology and Biochemistry*, *50*(2), 543-556.

Sánchez-Velázquez, J., Peña-Herrejón, G. A., & Aguirre-Becerra, H. (2024). Fish Responses to Alternative Feeding Ingredients under Abiotic Chronic Stress. *Animals*, *14*(5), 765.

Siddik, M. A., Francis, P., Rohani, M. F., Azam, M. S., Mock, T. S., & Francis, D. S. (2023). Seaweed and Seaweed-Based Functional Metabolites as Potential Modulators of Growth, Immune and Antioxidant Responses, and Gut Microbiota in Fish. *Antioxidants*, *12*(12), 2066.

Subbiah, V., Xie, C., Dunshea, F. R., Barrow, C. J., & Suleria, H. A. (2023). The quest for phenolic compounds from seaweed: Nutrition, biological activities and applications. *Food Reviews International*, *39*(8), 5786-5813.

Tadese, D. A., Song, C., Sun, C., Liu, B., Liu, B., Zhou, Q., ... & Kevin, N. T. (2022). The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. *Reviews in Aquaculture, 14*(2), 816-847.

Taştan, Y., & Salem, M. O. A. (2021). Use of phytochemicals as feed supplements in aquaculture: A review on their effects on growth, immune response, and antioxidant status of finfish. *Journal of Agricultural Production*, *2*(1), 32-43.

Tsimogiannis, D., & Oreopoulou, V. (2019). Classification of phenolic compounds in plants. In *Polyphenols in plants* (pp. 263-284). Academic Press.

Tufarelli, V., Casalino, E., D'Alessandro, A. G., & Laudadio, V. (2017). Dietary phenolic compounds: biochemistry, metabolism and significance in animal and human health. *Current drug metabolism*, *18*(10), 905-913.

Valdés, L., Cuervo, A., Salazar, N., Ruas-Madiedo, P., Gueimonde, M., & González, S. (2015). The relationship between phenolic compounds from diet and microbiota: impact on human health. *Food & function*, *6*(8), 2424-2439.

Vuolo, M. M., Lima, V. S., & Junior, M. R. M. (2019). Phenolic compounds: Structure, classification, and antioxidant power. In *Bioactive compounds* (pp. 33-50). Woodhead Publishing.

Yang, C., Chowdhury, M. K., Hou, Y., & Gong, J. (2015). Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. *Pathogens*, *4*(1), 137-156.

Yang, M., Jiang, D., Lai, W., Chen, K., Zhang, L., Lu, L., ... & Jiang, J. (2024). Hydrolysable tannin improves growth performance and liver health of largemouth bass (Micropterus salmoides) fed high soybean meal diets. *International Journal of Biological Macromolecules*, *276*, 133773.

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science*, *8*, 33-42.

Zhang, W., Zhao, J., Ma, Y., Li, J., & Chen, X. (2022). The effective components of herbal medicines used for prevention and control of fish diseases. *Fish & shellfish immunology*, *126*, 73-83.

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