# ADVANCEMENTS IN AQUACULTURE: SUSTAINABLE PRACTICES, INNOVATIONS, AND APPLICATIONS

**Editors**
**Prof. Dr. Aysun KOP**
**Assist. Prof. Dr. Boran KARATAŞ**

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# PREFACE

Aquaculture, the practice of cultivating aquatic organisms, stands as a pivotal cornerstone in our pursuit to confront the pressing challenges of feeding a rapidly growing global population while safeguarding our delicate ecosystems. In a world grappling with increasing concerns about food security, sustainability, and the impacts of climate change, the role of aquaculture has transcended its historical subsistence roots to become a foundational pillar of modern food production. Beyond revolutionizing our approach to food consumption, aquaculture has ignited innovation and transformation across a myriad of domains in our society, spanning economics, ecology, health, and technology.

This book, comprised of thirteen in-depth chapters, stands as a testament to the indispensable role that aquaculture plays in the ever-evolving landscape of agriculture and fisheries. Our goal is to provide a comprehensive and accessible resource for those already deeply involved in the aquaculture sector and for newcomers eager to explore its vast potential. Consequently, we have endeavored to ensure that this book caters to a diverse readership, ranging from students and educators to researchers, industry professionals, and policymakers. As editors, we express our profound gratitude to the contributors who have generously shared their expertise and insights, thus making this book a reflection of the collective knowledge and unwavering dedication within the field of aquaculture. It is our hope that this book will stand as a valuable resource for all those intrigued by the dynamic world of aquaculture.

**EDITORS**

# CHAPTER 1

## ASSESSING ELEMENT CONCENTRATIONS IN SEAWEEDS WITHIN GLOBAL AQUACULTURE: IMPLICATIONS AND PERSPECTIVES

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## INTRODUCTION

Algae, one of the essential communities of aquatic ecosystems, play a vital role in marine and freshwater environments. They provide organic food for heterotrophic organisms and oxygenate the environment through photosynthesis, thereby maintaining ecological balance (Cirik and Cirik, 2017). These diverse organisms are divided into two primary size categories: microalgae and macroalgae. Microalgae are microscopic, often unicellular organisms that can be found in both freshwater and marine environments (Abdel-Latif et al., 2022). They lack differentiated tissues, yet their role in primary production is indispensable. As the base of many aquatic food chains, microalgae contribute significantly to the sustenance of various marine species (Wei et al., 2022). Macroalgae, on the other hand, are larger, multicellular organisms primarily found in marine environments. Unlike microalgae, they possess differentiated structures resembling roots, stems, and leaves. Their sizes vary widely, ranging from 1-2 cm to 40-50 m, depending on the species (Ak, 2015; Cirik and Cirik, 2017). These macroalgae contribute to the ecosystem's productivity and provide food, shelter, and breeding environments for various aquatic organisms (Cirik and Cirik, 2017). Based on the pigments they contain, macroalgae can be further divided into three distinct classes: Brown (Phaeophyceae), Red (Rhodophyceae), and Green (Chlorophyceae). This classification is not merely taxonomic but also indicative of their unique biochemical compositions (Salamanca et al., 2005; Costa et al., 2021).

As macroalgae are commonly known, seaweeds have been utilized for centuries in many parts of the world, including Japan, Korea, Ireland, Iceland, and Spain (Dawczynski et al., 2007; The Heritage Council, 2008). Their primary application has been for human consumption, a tradition thriving today. Rich in protein, fatty acids, vitamins, secondary metabolites, and minerals, seaweeds are widely recognized as functional foods (Miˇsurcova, 2011; Wells et al., 2017). They are also called 'sea vegetables' when used for human nutrition, reflecting their nutritional value (FAO, 2018; Morrissey et al., 2001). Worldwide, of the 221 species of seaweed, 145 are directly consumed as food. These include 79 Rhodophyta, 38 Phaeophyceae, and 28 Chlorophyta (Pereira, 2011). The growing interest in seaweed's nutritional and functional properties has led to increased research and development in this field, further expanding their potential applications (Burtin, 2003). This burgeoning interest underscores the importance of understanding seaweeds' biology, ecology, and nutritional aspects, as they continue to emerge as a valuable resource for human well-being and environmental sustainability.

According to the Food and Agriculture Organization (FAO) report of 2022, the global fisheries and aquaculture sectors produced a combined total of 214 million tons in 2021, with fisheries accounting for 90.3 million tons and aquaculture contributing 122.6 million tons. This production is predominantly animal-based, comprising 83.18% of the total, including fish, shellfish, and mollusks. The remaining 16.82% is plant-based, with most of this category comprising seaweeds (FAO, 2022). Globally, seaweed production has reached 36 million tons, of which an overwhelming 97%, or approximately 35.1 million tons, is cultivated (Fig 1). This represents a significant growth trend in the industry, with a threefold increase in seaweed production over the last 20 years and a 1.7-fold increase in the last decade alone. The economic implications of this growth are equally noteworthy. The global market value for seaweed was USD 5.4 billion in 2011, nearly doubling to USD 9.9 billion in the last ten years. This upward trajectory is expected to continue, with projections indicating a 2.3% annual growth rate through 2030, (Grand View Research, 2022). The increasing demand for algae-based products in various sectors fuels this growth.

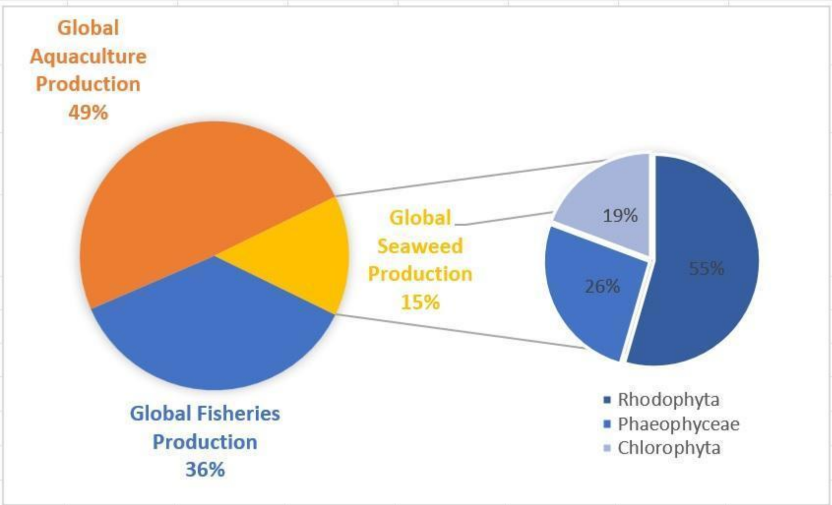


**Figure 1.** Global Fisheries and aquaculture production.

One emerging trend in the seaweed industry is humans' direct consumption of seaweed (Nitschke and Stengel, 2016). Although it is challenging to precisely estimate the quantities consumed, recent studies, such as the one conducted by Naylor et al. (2021), suggest that between 31-38% of global seaweed production is consumed directly by humans. This shift towards direct consumption reflects changing dietary patterns and the recognition of seaweed's nutritional benefits. However, the utilization of seaweed extends beyond direct human consumption. The remainder of the production is employed in diverse applications, including the extraction of phycocolloids (such as agar, carrageenan, and alginates), the creation of fertilizers, the formulation of feed ingredients, the development of health products, and emerging applications like bioplastics (Banach et al., 2020). Interestingly, a significant proportion of the products derived from these applications are also indirectly consumed by humans, further emphasizing the multifaceted role of seaweed in contemporary society.

With the growing demand for alternative food sources of seaweed, the need to determine the safety of seaweed used as food and feed sources (Van der Spiegel et al., 2013). It is known that seaweed can accumulate elements, so if they are found in the environment. For this reason, it is crucial to examine the presence of heavy metals in cultivating edible seaweed (Muñoz & Díaz, 2022) and Figure 2 illustrates this process. This review, therefore, seeks to address the elemental content of seaweeds grown as human food, focusing on both the nutritional benefits and potential risks. By evaluating the existing literature and current practices, it aims to provide a comprehensive understanding of the factors that influence the elemental composition of seaweed and their effects on human health. The findings of this review may contribute to developing guidelines and regulations that ensure the safe and sustainable utilization of seaweed in various applications, aligning with the global shift towards environmentally responsible consumption and production.

## 1. ELEMENTS AND HUMAN HEALTH

The human body requires a balanced intake of essential elements, macrominerals, and trace elements to function optimally. Essential elements such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and sodium (Na) are needed in large quantities and serve as the building blocks of organic molecules, including proteins, carbohydrates, and fats. These elements are fundamental to energy production, cell function, and other vital physiological processes (Briffa et al., 2020; Fu & Xi, 2020; Zhu & Costa, 2020). In addition to these essential elements, the body also requires specific minerals in smaller amounts, including calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), chlorine (Cl), and sulfur (S). These macrominerals support bone health, nervous transmission, water balance, muscle function, and metabolic responses. Furthermore, trace elements such as arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), vanadium (V), and zinc (Zn) are required in minimal amounts. They are essential for maintaining skeletal structure, regulating acid-base balance, and providing critical elements of the colloidal system.

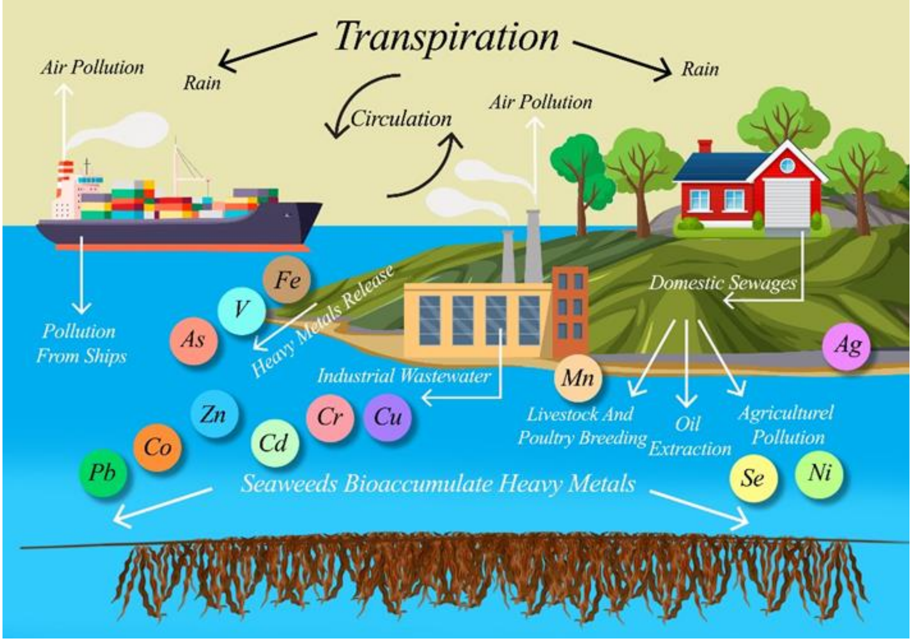


**Figure 2.** Sources of heavy metals and their bioaccumulation by seaweeds.

Trace elements also play a vital role in various biological processes, including enzymatic activity, immune function, hormonal production, and antioxidative defense. For example, zinc is a cofactor for many enzymes and is essential in metabolic processes such as DNA synthesis, protein metabolism, cell division, and immune and nervous system functions (Roohani et al., 2013; Sapkota & Knoell, 2018). Iron, another essential micronutrient, is a fundamental building block of proteins like hemoglobin and myoglobin, which transport oxygen. It also plays a vital role in ATP synthesis, which is required for energy production (Camaschella, 2015). A lack of copper can lead to decreased energy production, irregularities in glucose and cholesterol metabolism, heightened oxidative stress, accumulation of iron in tissues, changes in the structure and function of blood and immune cells, anomalies in neuropeptide creation and processing, irregular cardiac electrical activity, weakened heart muscle contractility, and lasting impacts on neurobehavioral and immune functions (Harris, 2003; Saari, 2000). However, the balance between beneficial and toxic levels of these elements can be delicate. Inadequate or excessive intake can result in health problems, such as zinc deficiency leading to impaired immunity and neural development disorders (Roohani et al., 2013; Yasuda and Tsutsui, 2016; Sapkota and Knoell, 2018), or iron deficiency causing anemia, particularly in young children and women of reproductive age (Camaschella, 2015).

Edible seaweeds, including *Ulva lactuca*, *Chondrus crispus*, and *Laminaria hyperborea*, have been studied for their potential health benefits, such as preventing inflammation, cardiovascular disease, and mental disorders. They are reported to be abundant sources of polyunsaturated fatty acids (Van Ginneken et al., 2011). Moreover, different groups of seaweeds can absorb various minerals, with brown seaweeds having higher absorption rates due to specific polysaccharides (Muñoz and Díaz, 2022). However, seaweeds can also accumulate toxic metals in the environment where they grow, posing potential health risks (Almela et al., 2006). Studies have shown that seaweeds may contain substances like iodine, alpha-naphthoflavones (ANFs), heavy metals, radioactive isotopes, ammonium, dioxins, and pesticides at safety-threatening levels (Van der Spiegel et al., 2013). Therefore, testing seaweed for metal contamination before consumption is essential, and the effects of seaweed foods on human metabolism should be studied in detail (Wells et al., 2017).

Environmental factors can also influence the concentration of mercury in seaweed, including the uptake capacity of the seaweed, seasonal variations, mercury levels in the aquatic environment, regional growth conditions, environmental pollution levels, water quality, and other factors. Whether post-harvest handling methods and processes of seaweed can reduce mercury levels remains an area for further investigation (FSAI, 2020). The complex interplay between essential elements, macrominerals, trace elements, and the potential for both beneficial and toxic effects underscore the need for a comprehensive understanding of these factors in human health. The role of seaweeds as both a source of valuable nutrients and a potential vector for toxic substances adds a layer of complexity that warrants careful consideration.

## 2. HAZARDOUS EFFECTS OF METALS

Heavy metals have specific mechanisms and different toxic effects on humans depending on the metal structure (Tırınk and Özkoç, 2021). This complexity arises because excessive amounts of metals can affect biological processes in organisms and cause health problems. Exposure to metal toxicity in humans can adversely affect a wide range of organs and systems, with toxicity occurring when metals accumulate in excessive quantities. Figure 3 summarizes the health impacts of heavy metals.

The primary mechanism of heavy metal toxicity is the increased production of reactive oxygen species (ROS) in biological systems, which results in oxidative damage and, ultimately, adverse health effects (Fu and Xi, 2020). The occurrence of oxidative stress can explain this due to an imbalance of oxidants/antioxidants in cells. Furthermore, high heavy metal exposure and essential metal deficiencies have been associated with oxidative stress (Singh et al., 2019). The toxicity of ROS is based on their ability to oxidize cellular and extracellular structures such as nucleic acids, lipids, and proteins (Solenkova et al., 2014), and Figure 2 illustrates this process.

The toxic effects and health problems associated with different metals are multifaceted and may vary significantly. Progressive physical, muscular, and neurological degenerative diseases can result from long-term exposure to toxic minerals, which have been linked to conditions like Alzheimer's and Parkinson's (Zahir et al. 2005). Many metals, particularly mercury, lead, and cadmium, are neurotoxic. The accumulation of or exposure to these metals can cause damage to nerve cells, leading to neurological disorders, reduced mental function, memory problems, and even seizures. This highlights the critical need for understanding and mitigating exposure to these elements.


**Figure 3.** Health impacts of heavy metals

Some metals, in particular arsenic and cadmium, can cause damage to the lungs. Exposure can lead to inflammation of the lungs, fibrosis (hardening of the tissue), and difficulty breathing. Long-term exposure may increase the risk of lung cancer, underscoring the importance of monitoring and controlling the environmental levels of these metals. Cadmium, mercury, and lead are

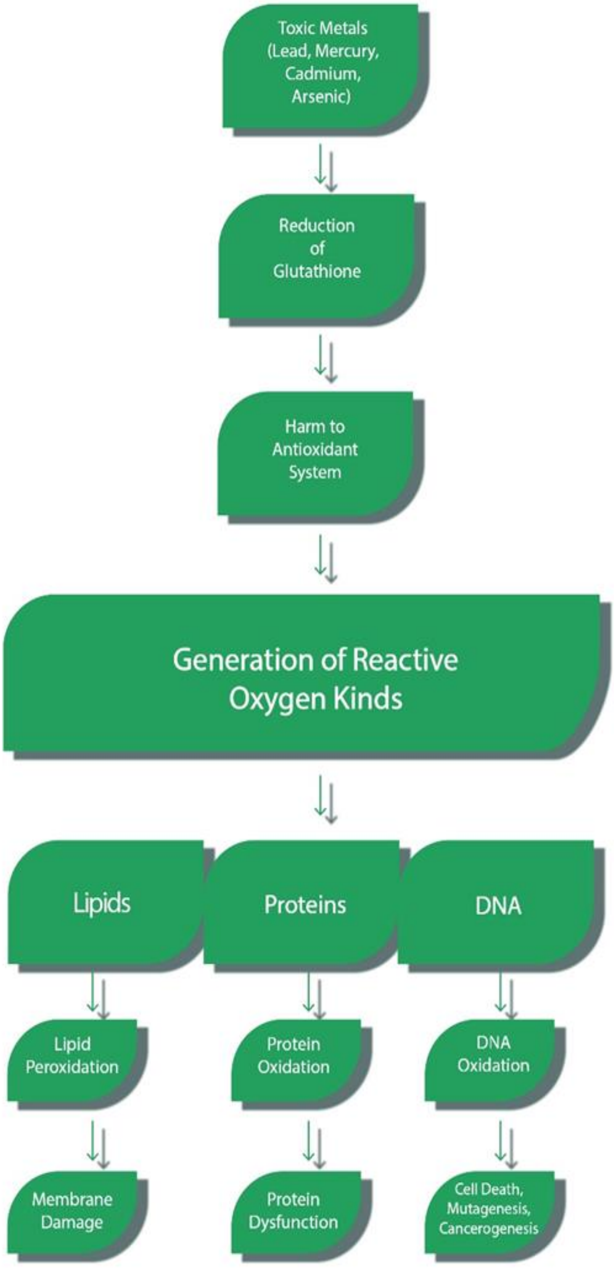


**Figure 4.** Systems of Metal Toxicity (Solenkova et al., 2014)

renal toxins. Accumulating these metals can impair kidney function, leading to kidney failure, kidney stones, and other kidney problems, as the kidneys are a major organ responsible for removing metals from the body. Recurrent long-term exposure to certain metals may even be linked to certain types of cancer (+). A recent study showed an association between cancer deaths and cadmium exposure in the Japanese population (Watanabe et al., 2020), further emphasizing the need for rigorous regulation and monitoring.

Some metals, in particular arsenic and cadmium, can cause damage to the lungs. Exposure can lead to inflammation of the lungs, fibrosis (hardening of the tissue), and difficulty breathing. Long-term exposure may increase the risk of lung cancer, underscoring the importance of monitoring and controlling the environmental levels of these metals. Cadmium, mercury, and lead are renal toxins. Accumulating these metals can impair kidney function, leading to kidney failure, kidney stones, and other kidney problems, as the kidneys are a major organ responsible for removing metals from the body. Recurrent long-term exposure to certain metals may even be linked to certain types of cancer (+). A recent study showed an association between cancer deaths and cadmium exposure in the Japanese population (Watanabe et al., 2020), further emphasizing the need for rigorous regulation and monitoring.

Lead poisoning damages the nervous and immune systems and can cause kidney, liver, and cardiovascular dysfunction (Tsuchiva et al., 1986; Garcia-Leston et al., 2012; Counter et al., 2009). Children, pregnant women, and the elderly are particularly vulnerable to the effects of lead exposure. Children are at particular risk from prenatal and postnatal Pb intake (Buchanan et al., 2011; Counter et al., 2009) and are one of the most vulnerable groups to the adverse effects of lead exposure, especially during periods of intense brain and neurological development (Zhang et al, 2012). Lead exposure is also linked to behavioral problems such as conduct disorder, hyperactivity, and attention deficit. It can cause health problems, including memory problems, neurological damage, and cardiovascular disease, making the control of lead exposure a public health priority.

Prolonged or high levels of mercury exposure can cause a wide range of health problems, including adverse effects on the central nervous system, effects on the gastrointestinal, renal, reproductive, and cardiovascular systems (FSAI, 2016). The nervous system is susceptible to mercury compounds, and research shows severe brain and renal damage (Güven et al., 2004), necessitating careful consideration of mercury levels in food and the environment.

Long-term excessive intake of zinc, an essential micronutrient, can lead to nausea, headaches, fever, abdominal pain, and deficiencies of copper and iron. Zn is also irritating to human skin (Saha et al. 2016). Pb and Al are non-essential for humans, and excessive intake can damage nervous, skeletal, cardiovascular, enzymatic, endocrine, and immune systems (Zhang et al. 2012; Kawahara et al. 2007). Al is toxic to most forms of life due to its unique chemical properties, which bind to the phosphate groups of DNA or RNA, affecting the topology of the DNA and influencing the transcription of genes (Muñoz and Díaz 2022). In humans, Al toxicity has been linked to neurodegenerative disorders (Kawahara et al. 2007), further emphasizing the importance of understanding the biological effects of these metals.

Arsenic is a concern for humans as it causes carcinogenesis in the liver, kidney, bladder, and skin cells (Tsai et al., 1998; Bernstam and Nriagu, 2000). Both compound forms (total As and inorganic As) are classified by IARC (2009) (International Agency for Research on Cancer) as Group 1 carcinogens for humans (Camurati and Salomone, 2019). Besides health risk assessment, quantifying arsenic in seaweeds is vital to elucidate marine arsenic cycling (Francesconi et al., 1994). Brown algae have been reported to have higher total arsenic content than other algal classes, especially *Cystoseira* sp. and *Sargassum* sp., belonging to this class (Cirik et al., 2018).

Copper has toxic effects. Copper toxicity can result in substantial oxidative stress and tissue damage (Uriu-Adams and Keen, 2005). The mechanisms behind the acute toxic effects of copper in humans are not thoroughly understood. However, it is reasonable to speculate that these effects likely stem from a combination of heightened oxidative stress at various points in the body, coupled with significant disruptions in several endocrine system components (Handy, 2003; Yang et al., 2004). Copper toxicity can emerge from chronic or prolonged exposure to elevated copper levels or due to certain genetic disorders such as Wilson's disease. Symptoms of copper toxicity encompass diarrhea, headaches, and, in severe cases, kidney failure. Additionally, it can contribute to other health problems like liver damage, neurological disorders, bone deterioration, skin discoloration, and immune system issues (Paz et al., 2019; Dawood et al., 2020).

## 3. ELEMENT UPTAKE IN SEAWEEDS

As integral components of aquatic ecosystems, seaweeds can take up metals in water through various mechanisms such as absorption, adsorption, or active transport, and a process collectively referred to as bioaccumulation. This unique characteristic renders seaweeds valuable bio-indicator organisms for identifying and monitoring bioaccumulation levels of toxic metals in aquatic environments (Sanchez-Rodriguez et al., 2001).

The ability of seaweeds to accumulate metals is multifaceted and depends on several factors, with the bioavailability of metals in the surrounding water and the uptake capacity of the algae being paramount. Metal uptake by algae can be achieved through two primary mechanisms: electrostatic attraction to the surface and active uptake. The former occurs when negatively charged polysaccharides on the seaweed surface attract positively charged metal ions, leading to a passive uptake of metal ions, as observed in the case of Zn (Besada et al. 2009). In contrast, active uptake involves a more complex process where specialized carrier proteins transport metal ions across the cell membrane into the cytoplasm. This metabolically driven mode of uptake is evident for metals such as Cu, Mn, Se, and Ni (Besada et al. 2009).

As macroalgae reside at the base of the aquatic food chain pyramid, their role in transporting pollution up the trophic chain in aquatic environments is crucial (Souza et al., 2012). The intake of metals accumulated by seaweeds has significant implications for human health, emphasizing the importance of producing seaweeds for various applications, including human consumption, animal feeds, and chemical extracts for medical and laboratory uses. With the natural occurrence of seaweeds being insufficient to meet industrial demands (Sultana et al., 2023), aquaculture emerges as a vital source to fulfill these requirements. However, due to seaweeds' ability to accumulate various toxic substances, the cultivation process must ensure a clean source, necessitating investigations into the presence of heavy metals in seaweed grown under specific conditions and environments (Muñoz and Díaz, 2022).

Furthermore, the influence of cultivation techniques on metal content cannot be overlooked. For instance, conventional agriculture has been found to yield significantly higher metal contents than organic agriculture, highlighting the role of cultivation methods in mitigating health risks (Rubio et al., 2017). This observation underscores the importance of adopting appropriate cultivation practices to minimize potential health hazards associated with metal accumulation.

It is also essential to recognize that some metals are vital micro-nutrients for organisms, influencing their metabolism. Although essential in small amounts for maintaining normal physiological functions, these trace elements can become toxic at specific concentrations. In some cases, the difference between beneficial and toxic levels may be slight (FSAI, 2020). This delicate balance emphasizes the importance of maintaining trace elements within a reasonable range of concentrations, as excessive levels can lead to harmful consequences (Hsu et al., 2001; Stern, 2010). The metal uptake by seaweeds is a complex yet vital area of research with far-reaching implications for environmental monitoring, human health, and industrial applications. The multifaceted nature of metal uptake mechanisms, coupled with the critical role of cultivation practices, necessitates a comprehensive and nuanced approach to ensure seaweeds' safe and sustainable utilization.

## 4. FOOD SAFETY REGULATIONS

Many edible algae species generally present no risk to human safety (Cheney, 2016). Nevertheless, some potential safety risks are associated with seaweed consumption due to chemical, biological, and physical contaminants that are naturally present in the aquatic environment. Food regulatory agencies play an essential role in public health and significantly contribute to the population's well-being in various ways (Burris et al., 2010). Calculating the health risk of contaminated seafood is essential to estimate the potential effects of consumption (Traina et al., 2019). Regarding toxicity and contaminant levels, safety is required for any raw material humans use (Hafting et al., 2015).

Each country has its regulatory framework to monitor heavy metal levels in food and set maximum acceptable levels. Seaweed consumption is precisely regulated, and details of its chemical composition and inter-species variation are required to obtain authorization for its use in human nutrition (Rubio et al., 2017). National regulatory authorities set maximum acceptable levels for certain heavy metals based on data from scientific research, population health, dietary habits, and industrial activities. In order to ensure the safe consumption of food and to protect public health, these maximum levels are usually set. Internationally, some health and agricultural organizations guide acceptable levels of heavy metals in food. For example, global food safety standards and guidelines are established by organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO).

However, these standards vary between countries, which may set national regulations. Regulation 1881/2006 of the European Commission is the legal regulation of the European Union that sets maximum levels for chemical contaminants in food and has been updated by Regulation 69/2008 (European Commission, 2013). Surprisingly, however, the European Union has little seaweed legislation, so there are few controls on potentially harmful metals (Besada et al., 2009). In the USA, levels are set for specific types of food through various regulations published by the FDA. Australia and New Zealand set maximum levels for heavy metals in a common regulation for both countries (Food Standards Australia New Zealand, FSANZ).

Seaweed species and their constituents need to meet specific consumer safety regulations, mainly as concentrations of heavy metals depend on the surrounding environment, and variations can be very localized (Holdt and Kraan, 2011). Nevertheless, seaweed can play a significant role in global food security by providing nutritious food when produced and consumed to safety standards (Leandro et al., 2020). Commission Regulation (EC) No 1881/2006 sets maximum limits (ML) for certain pollutants in various foodstuffs. This sets maximum levels for specific contaminants in food to maintain toxicologically acceptable levels for the protection of public health. Except for arsenic, cadmium, and lead for food supplements containing or derived from seaweed, no maximum levels have been set for metals in seaweed. In Ireland, Denmark, and the Netherlands, the use of seaweed is regulated by general food regulations, but in other European countries, there are no specific regulations (Holdt and Kraan, 2011). In Turkey, the Turkish Food Codex Communiqué on Dietary Supplements No. 28737 lists the vitamins and minerals permitted for use in dietary supplements following those listed in Annex I and Annex II. This requirement applies when the product is first manufactured and placed on the Turkish market. Only eight types of micro-algae and five types of seaweed are defined as safe sources in the regulation of the Turkish Codex Alimentarius. However, there is no regulation on cultivating or collecting seaweeds in coastal waters. These seaweeds have the potential to be consumed and distributed as food.

European Commission Regulation (EC) No 629/2008 (EC, 2008) and European Commission Regulation (EC) No 488/2014 (EC, 2014) limit the content of Cd in various food supplements consisting exclusively of dried seaweeds or products derived from seaweeds to a maximum of 3 mg/kg. France was the first European country to regulate seaweed as an unconventional food for human consumption (Besada et al. 2009). France has set limits for toxic metals, with upper limits for Cd (0.5mg/kg of water), Pb (5 mg/kg of water), Hg (0.1mg/kg of water), I (2000 mg/kg of water) and inorganic As (mg/kg of water) in edible seaweed (CEVA, 2014). Twenty-five edible red seaweed species were reported to contain trace elements (Al, As, Au, Ba, Br, Cd, Co, Cr, Cu, Fe, Hg, and I) (Pineiro, 2012). Pb and Al are not vital. Aluminum is reportedly highly toxic to humans (Kawahara et al. 2007). Regulations do not set maximum levels for the presence of Al in food. However, the WHO recommends a weekly intake of less than 7 mg/kg Al. For a 60 kg adult, this would be an intake of up to 60 mg daily (Kawahara et al. 2007). Only a small number of values for Al in edible seaweed have been reported so far. The data show its presence at low levels (mg/g w/w) and below WHO recommendations (Chlorophyceae: 0.095; Rhodophyceae: 0.004 - 1.55; Phaeophyceae: 0.0001 - <0.050). The complex interplay between regulations, environmental factors, and the intrinsic properties of seaweeds necessitates a comprehensive understanding of the potential risks and benefits associated with seaweed consumption. The ongoing development of international standards and national regulations reflects the growing recognition of seaweed's nutritional value and potential health risks, emphasizing the need for rigorous scientific research and responsible regulatory oversight.

## 5. CONCLUSION

In conclusion, seaweed's role as a primary producer in aquatic ecosystems and its diverse industrial applications underscore its global significance. The potential nutritional benefits are promising, but understanding toxic mineral composition in edible seaweeds remains limited. Clear guidelines on consumption and research into eco-friendly production are vital. The sustainable future of seaweed products hinges on responsible management, increased demand, and a focus on health and environmental stewardship. The balanced exploration of seaweed's benefits and risks will foster responsible utilization of this valuable resource.

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# CHAPTER 2

## PRODUCTION OF SEAWEED-BASED FOOD PRODUCTS IN THE FORMS OF DIETARY USE FOR HEALTHY LIFE

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## INTRODUCTION

The nutraceutical ingredients, functional foods and healthy beverages is growing rapidly due to the drastic change in lifestyle and the increasing prevalence of non-communicable diseases. Additionally, the increasing consumer exposure to health content is creating an opportunity for the functional food and nutraceutical ingredient industries (Subramanian and Anandharamakrishnan, 2023). In recent years, seaweeds are very popular food products, as well as they have a great potential as a healthy, sustainable, and natural foods (Govaerts and Olsen, 2023). They can be consumed directly, as well as in pill and powder forms, as a dietary supplement. It is also used in the production of functional products with high nutritional value by taking part in the formulations of bakery, dairy and meat products (Kılınç and Kılınç, 2022). Edible algae are a rich source of essential amino acids, antioxidants, polyunsaturated fatty acids, polysaccharides, minerals, and vitamins (Pasdar et al., 2024). For example, dietary consumption of iodine, through seaweed can contribute up to 33% of total iodine exposure, making seaweeds the strongest contributor to iodine in the diet (Ficheux et al., 2023). In addition, seaweeds are the main sources of commercially important agar, alginate, and carrageenan (Pradhan et al., 2022). Although they have previously only been used as thickening, and gelling agents in the food products or pharmaceutical industries, recent research has revealed their potential usage as complementary medicine (Mohamed et al., 2012). Also, seaweeds and their extracts have long been used as food, animal feed, fertilizers, soil improvers, and food or feed supplements. Additionally, they have rich in nutrients and bioactive molecules such as polysaccharides, polyphenolics, fluorotanins, pigments, fatty acids, sterols and plant hormones as well as the extracts of various seaweeds play an important role in the development of advanced plant and animal immune systems and antioxidant status (Fan and Critchley, 2024). Apart from this, the excellent bioactivity of seaweed proteins allows these proteins to be used in a variety of nutraceutical applications, including antimicrobial, antihypertension, diabetes preventive, anticoagulant, cancer preventive, antioxidant (Lu, 2024). Moreover, natural antioxidants have been extracted and identified from different seaweeds to develop new food-grade functional ingredients and medicines (Burgos-Diaz et al., 2022).

Seaweeds are an extremely versatile and valuable marine resource found around the world. The nutritional value and health benefits provided by the consumption of seaweed have made it an interesting raw material to be used in the development of seaweed-based products (Jayakody and Vanniarachchy, 2024). Additionally, they are also accepted as an alternative to animal proteins (Embling et al., 2022). Seaweeds in powder or extract form can increase the sensory, nutritional, and textural properties of food products. In addition, seaweed also influences the health properties of these food products. Also, the effect of seaweed differs significantly depending on the concentration used, and type, so commercial products based on seaweed need to be formulated in the most appropriate way (Pasdar et al., 2024). Therefore, future research may focus on improving the functional and sensory properties of alternative protein sources as well as developing new processing technologies to optimize their use in dairy products (Diaz- Bustamante et al., 2023). In one study, evaluation of phytochemical, nutritional and sensory properties of high fiber buns developed by using *Kappaphycus alvarezii* seaweed powder as a functional component (Sasue et al., 2023). The effect of seaweed powder on bacteria load of fresh fish was studied in another research (Kılınç et al., 2012). Furthermore, seaweed was used in the production of FCMs (food contact materials) such as bags, edible films edible coatings, active packaging, and smart packaging (Perera et al., 2024). Many foods and functional food products have also been produced using seaweeds such as *Ulva rigida* marinades (Kılınç et al., 2011), seaweed bread by using *Ulva rigida* (Turan et al., 2011), seaweed bread with *Lemna minor* (Tekoğul et al., 2011), different formulated seaweed soup (Kılınç et al., 2013a), vegetarian seafood meal (Kılınç et al., 2013b), seaweed bakery products (Griffin, 2015), sea beans (*Salicornia europaea*) bread (Kılınç, 2016), seaweed value-added functional food products (Kılınç and Kılınç, 2022), seaweed in used for producing milk-based products (Newton et al., 2023; Yuliarti et al., 2023) , seaweed in dairy protein replacement in yogurt and cheese (Diaz-Bustamante et al., 2023), seaweed powder as a functional ingredient (Sasue et al., 2023), fermented seaweeds (Reboleira et al., 2023), chocolates enriched with seaweed (Salgado et al., 2023), seaweed-based snacks (Jayakody and Vanniarachchy, 2024). On the importance of the subject in this study, it has been aimed to produce seaweed-based food products in the form of dietary use for healthy life.

## Seaweed Cultivation Environmental Conditions

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

Algae, in particular, produced in large quantities worldwide, helps meet the increasing food needs in some parts of the world. In particular, 99.5% of seaweed production is located in Asia. One of these cultivated species is gracilaria with 10.7% (Chopin, 2018).

Among the seaweeds collected from nature or cultured, gracilaria and ulva, which are naturally found in our country, are a very important source of use. Moreover, since they use organic substances in their environment, they ensure that the water is clean and of high quality.

*Gracilaria verrucosa* thallus branches in all directions in water and is 5-30 (maximum 60) cm tall. The salinity rate is between 0-15% and 0-50%, the optimum temperature is around 16°C, the optimum salinity range is 020% - 035%, and the temperature is between 4°C - 37°C (Santelices and Doty, 1989; Düsedau et al., 2023).

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

*Ulva lactuca var. rigida* C. Agardh, which loves eutrophic brackish waters, has a thick thallus and a lettuce-like structure. In their study in 1999, Malta et al. experimentally determined the optimum temperature and salinity values for growth. Due to the expected demand for snack seaweed products, salinity between 15% and 30% and temperature between 10°C and 35°C have been studied in large-scale ulva production in India. Optimum growth was found at 0.15% salinity and 10°C temperature (Malta and Kamermans, 1999; Mantri et, al., 2011; FAO, 2023).

**Seaweeds and Health Benefits**

Seaweeds, also known as marine macroalgae, are a diverse group of aquatic plants found in coastal waters and oceans all around the World (Hefft and Fornaciari, 2024). They include various types of red (eg. *Gracilaria* spp.), brown (eg, *Sargassum* spp.) and green algae (eg. *Ulva* spp.) (Kılınç et al., 2013c). They can prevent various chronic diseases because of including fucoxanthin, fucoidan, phlorotannin and astaxanthin. The components of seaweed provide protection against vascular endothelial dysfunction (Yamagata, 2021). Seaweeds are effective as therapeutic pharmacological assets for various disorders, including inflammatory, intestinal, cardiac, hypertension, cholesterol, obesity, dyslipidemia, cancer, rhinitis, wound-healing, osteoporosis, goitre, anaemia, gastrointestinal, thrombosis, asthma, gastric ulcers, blood coagulation, arthritis, detoxification, hormone, and diabetes. Additionally, seaweeds have an antiviral, antioxidant, antimicrobial and probiotic effects (Griffin, 2015; Pradhan et al., 2022). In addition to individualized health care, a regular diet rich in seaweed can increase the nutritional content of food products. The various bioactive components of seaweeds as well as their pharmacological, nutritional, biochemical, industrial, and clinical, applications for human well-being have been focused (Pradhan et al., 2022).

## Usages of Seaweeds for Food Applications

Seaweed production globally has increased rapidly in recent years (Webb et al., 2023). Besides this, the cost of seaweed cultivation is low, because it does not need any chemical fertilizers, soil, pesticides or other chemicals to facilitate growth. Therefore, this situation can be affected to decrease the cost of production of seaweed-based products (Jayakody and Vanniarachchy, 2024).

Seaweeds are used in salads, soup, sushi and other Japanese dishes, seaweed snacks, and other seafood-based foods. The fermentation of seaweeds improves new food products. In another words, fermented seaweeds cause to increase the nutritional profile of seaweeds, give products with enhanced health-promoting effects and develop new flavors, (Reboleira et al., 2024). For example, *Ulva* sp. have the salty flavor, an odour resembling lemon and fresh grass, the crispy, firm, and chewy texture with a lightness in colour green or dark yellow (Jonsson et al., 2023). Edible seaweeds such as *Ulva* sp., *Alaria esculenta*, *Saccharina latissima*, *and Palmaria palmata*. These species are rich in especially dietary fiber (29.8–34.1%), carbohydrates (29.6–54.6%), minerals (25.8–36.8%), but at lower levels lipids (1.3–2.4%), and dietary protein (6.7–11.4%). Their amino acid profiles almost exactly matched those of the proteins, but at lower levels histidine in *Ulva* sp. Apart from this, all species generally have a high salinity taste, moderate umami, low bitterness and sourness, and no sweetness (Jonsson et al., 2023). Various types of seaweed-based food products have been produced as follows. Three different species of red seaweed, *Gelidium corneum* and two species of Gracilaria (*Gracilariopsis longissima* and *Agarophyte chilensis*) were used to produce agar-based products. *Gracilaria spp. had reported to be a good potential for producing* agar hydrogels for food product applications (Cebrian-Lloret et al., 2024). The use of seaweed dietary fiber (SDF) was as a potential alternative to phosphates in the quality profiles of phosphate-free sausages (Yuan et al., 2023). Over the past few decades, increasing knowledge about the impact of seaweed applications and their positive benefits on the nutritional quality and storability of food products have been increased (Fan and Critchley, 2024). Seaweed polysaccharide-based films as well as coatings are suitable as a solid solution not only give rise to prevent quality loss, but also extend the shelf-life of meat and fishery foods. Moreover, these edible films and coatings provide a space for integrating a mixture of compounds that can be potentially improved the functionality of the matrix to induce beneficial effects (Chaudhary, 2023).

Consumers are becoming health conscious, it is predicted that seaweed-based food products would be an excellent potential for human consumption. It has been concluded that seaweeds could potentially contribute to future global security in nutraceuticals as well as functional foods (Raja et al., 2022). Furthermore, the results of conducted study emphasized that the inclusion of seaweed in future vegetarian burgers or as a salt substitute may have a positive impact on the diets (Slegers et al., 2021). Apart from meat products containing seaweeds, the bakery foods in which they are included are biscuits, noodles, bread, cookies, cakes, and others. Accordingly, in general, foods containing seaweed increase their capacity for antioxidants, protein, total polyphenols and dietary fiber (Quitral et al., 2022). The addition of seaweeds can not only develop the textural and nutritional properties of food products, but also low-fat food products with less saturated fatty acids and fewer calories can be prepared using seaweed. Thus, the consumption of food products enriched with algae has a positive effect on lifestyle diseases (Roohinejad et al., 2017). Therefore, the studies made about the aquaculture, and processing of seaweeds in a healthy way should be encouraged. In the future, it is predicted that seaweeds as well as food products enriched with seaweeds would be the solution to food shortages that may be occured (Kılınç and Kılınç, 2022).

## Produced Seaweed-Based Food Products in the Forms of Dietary Use for Healthy Life

Seaweed-based food products in the forms of dietary use for healthy life have been produced for people consumption such as seaweed drink, seaweed yoghurt, seaweed dessert, seaweed ice-cream, seaweed spice from green algae

(*Ulva rigida*) and red algae (*Gracilaria verrucosa*). These produced seaweed-based food products are given in the below.

**Picture 3.** *Ulva rigida* powder (Original)

**Picture 4.** *Gracilaria verrucosa* powder (Original)

**Picture 5.** *Ulva rigida* drink (Original)

**Picture 6.** *Gracilaria verrucosa* drink (Original)

**Picture 7.** *Ulva rigida* yoghurt (Original)

**Picture 8.** *Gracilaria verrucosa* yoghurt (Original)

**Picture 9.** *Ulva rigida* ice-cream (Original)

**Picture 10.** *Gracilaria verrucosa* İce-cream (Original)

**Picture 11.** *Ulva rigida* dessert (Original)

**Şekil 10.** Gracilaria dessert (Original)

**Picture 12**. *Ulva rigida* spice (Original)

**Picture 13.** *Gracilaria verrucosa* spice (Original)

## CONCLUSION

Seaweeds are of great importance in human nutrition in terms of the nutritional content they contain. For this reason, seaweed-based food products in the forms of dietary use for healthy life have been produced for people consumption such as seaweed drink, seaweed yoghurt, seaweed dessert, seaweed ice-cream, seaweed spice in this study. All of the formulations of seaweed-based food products have been determined as good taste, flavor, appearance, and odor properties. Therefore, it is advised that seaweeds should be used as an natural ingredient in all types of the food products for healty life. Additionally, it is necessary to produce various foods using seaweeds with high nutritional value and to support studies aimed at the development of new food products enriched with seaweeds. It is estimated that a large number of food products containing seaweed will be produced in the coming years, the cultivation and processing of seaweeds will become increasingly important, and in parallel, the consumption of seaweed-containing foods will increase more due to the health benefits of seeweeds.

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# CHAPTER 3

## STATISTICAL EVALUATION OF FEATURES SEAFOOD CONSUMPTION

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## INTRODUCTION

Nutrition is defined as taking the nutrients the body needs in sufficient quantities. Today, people have realized that not only satiety but also a balanced diet is an important issue. If nutrients are taken into the body in small or large amounts, growth and development are negatively affected, causing health deterioration. Consumption of animal foods is extremely important in a healthy diet.

Although the number of undernourished people has decreased in recent years, 821 million people remain malnourished and hunger remains a significant problem in many countries, especially in rural areas or developing regions (FAO 2018). While tackling the disproportionate impacts of climate change and environmental degradation on resources, human societies will also face the incredible challenge of providing food and livelihoods to a population of more than 9 billion by the mid-twentieth century.

The fisheries sector, which has an important place in human nutrition and health, has experienced significant changes and developments in the last 50 years. While fishing initially consisted of fishing from the sea and gathering in inland waters, aquaculture in inland waters later became widespread. Fishing made great progress and reached the largest fish production amount in the 19th and 20th centuries. Due to reaching the maximum sustainable production limit, climate change and similar reasons, there has not been a major change in the amount of fish obtained through fisheries after this date. However, fish farming has developed rapidly since the 1970s and today it has started to constitute almost half of the fish production transferred directly to human consumption. This change has caused significant developments and differentiations in the production, distribution, marketing and consumption structure of aquatic products. In the beginning, only the delivery of fishery products to consumers was important, in other words, supply security, today food safety has come to the fore. In this process, fishing, which is one of the oldest production activities in the world, has turned into an economic and complex supply chain. The Fisheries sector has become a sector that includes many economic actors, is multi-segmented and considers consumer preferences more. There has also been a change in marketing systems. Social security of Fisheries sector employees has started to be taken into account and working conditions have been relatively improved. Fisheries production technology has improved, aquatic products trade has accelerated, consumer preferences have differentiated and significant changes have occurred in aquatic products consumption patterns delivery of aquatic products to consumers by processing, storing and marketing them by quality standards, which is a valuable food in human nutrition, has become an important agenda item in all countries. Seafood is an indispensable element of an adequate and balanced nutrition plan and has an important role in providing approximately 20% of the protein intake of one-third of the world's population, especially in developing countries. The Fisheries Sector is a large sector that develops rapidly, increases product diversity and includes different products. Additionally, the recommended safe (normal) amount of protein to be taken to maintain energy balance in healthy adults is 0.8 - 1.2 g/kg/day (10-15% of total energy). And the tolerable (high) amount of protein has been determined as 1.4 – 2.2 g/kg/day (18-30% of total energy) (Weterterp-Plantenga et al. 2009; Hatunoğlu and Arıtıcı, 2023). Protein intake is 85.9g per person per day. It is enough to get 11.2g of this protein from fish. The daily protein intake per person, its share in all foods, was determined as 10 - 15% as a result of the evaluations at the WHO/FAO/UNU Joint Expert Advisory Meeting on Protein (Vasileska and Rechkoska, 2012).

Fish meat is an animal protein source with very important nutritional value and is known as an ideal food because it is low in energy value, easy to digest, healthy and cheap, and rich in unsaturated fatty acids (Bozbaş, 2023). Fish meat, which has a protein content of 17-20%, has an important place in eliminating the current animal protein deficit in parallel with the increasing world population (Dönmez and Tatar, 2001; Vasileska and Rechkoska, 2012). In this regard, fish and other aquatic products are generally considered a good source of protein. When the nutritional value of fish meat is compared with other animal foods; It is stated that it is high in minerals and protein, but low in fat, so it is in a much more advantageous position than other farm animals' meat. Aquatic products, especially fish; are products that can be prepared for quick and easy consumption, and are known as a good alternative source of animal protein and a source of omega-3 fatty acids to fill the protein gap.

The Fisheries sector, which has strategic importance with its importance in nutrition and contribution to employment, is developing rapidly in our country as well as in the world aquaculture is considered to be the indispensable sector of the future in meeting the animal protein needs of the ever-increasing world population.

## 1. STATUS OF FISHERIES PRODUCTION IN THE WORLD AND IN TURKEY

ASFIS (Aquatic Sciences and Fisheries Information System) has undertaken the task of compiling world capture and aquaculture production statistics at the species, genus, family or higher taxonomic levels in 3,169 statistical categories called species elements. The ASFIS species list includes 13,417 species items selected based on their interest or relationship to fisheries and aquaculture. It also provides information on the availability of fisheries production statistics for the species item in the FAO databases. According to the ASFIS classification, it can be stated that there are 31,708 species in the world. Of these species, 3807 species are obtained by aquaculture and 27,901 species are obtained by capture (Table 1). According to the ASFIS classification, it can be States that there are 140 species recorded in Turkey. Of these species, 30 species are obtained by aquaculture and 110 species are obtained by capture (Table 2). In 2021, China (55.15% aquaculture 14.44% Capture and total production 34.94%)) takes the first place in the world's highest production of aquaculture, both in fishing production and aquaculture production. Following China, the top three in cultivation are India (10.12%) and Indonesia (5.94%). Türkiye (0.51%) ranks 17th. In terms of fisheries production, Turkey ranks 45th in Capture production, following China (7.51%), and Peru (7.521%). In terms of total production, after China, India (7.61%) and Indonesia (6.72%) come respectively. Türkiye is in the 28th place. Turkey's share in world aquaculture production is 0.45%. Table 3 shows the aquatic products produced in the world and their contribution to production.

**Table 1.** Number of ASFIS Species by Production Areas in the World

| Production Area | ASFIS species Number |
|---|---|
| Africa - Inland waters | 29 |
| America, Nort, South - Inland waters | 11 |
| Asia - Inland waters | 12 |

| | |
|---|---|
| Atlantic, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 115 |
| Europe - Inland waters | 8 |
| Indian Ocean, Eastern, Western | 99 |
| Mediterranean and Black Sea | 104 |
| Oceania - Inland waters | 4 |
| Pacific, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 277 |
| **Aquaculture production (brackishwater)** | **659** |
| Africa - Inland waters | 281 |
| America, North, South - Inland waters | 349 |
| Asia - Inland waters | 615 |
| Atlantic, Northeast | 1 |
| Europe - Inland waters | 640 |
| Former USSR area - Inland waters | 11 |
| Oceania - Inland waters | 46 |
| **Aquaculture production (freshwater)** | **1943** |
| America, Nort, South - Inland waters | 2 |
| Asia - Inland waters | 2 |
| Atlantic, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 346 |
| Europe - Inland waters | 7 |
| Former USSR area - Inland waters | 1 |
| Indian Ocean, Eastern, Western | 123 |
| Mediterranean and Black Sea | 206 |
| Pacific, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 518 |
| **Aquaculture production (marine)** | **1205** |
| Africa - Inland waters | 374 |
| America, Nort, South - Inland waters | 439 |
| Antarctic areas | 56 |
| Arctic Sea | 18 |
| Asia - Inland waters | 451 |
| Atlantic, Antarctic, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 12754 |
| Europe - Inland waters | 851 |
| Former USSR area - Inland waters | 58 |

| | |
|---|---|
| Indian Ocean, Antarctic, Eastern, Western | 3697 |
| Marine areas outside the Antarctic | 415 |
| Mediterranean and Black Sea | 2931 |
| Oceania - Inland waters | 50 |
| Pacific, Antarctic, Eastern Central, Western Central, Northeast, Northwest, Southeast, Southwest | 5807 |
| **Capture production** | **27901** |
| **Genel Toplam** | **31708** |

**Table 2.** Numbers of ASFIS species according to Production Areas in Turkey

| **Production Area** | **ASFIS species Number** |
|---|---|
| Mediterranean and Black Sea | 1 |
| **Aquaculture production (brackishwater)** | **1** |
| Asia - Inland waters | 9 |
| **Aquaculture production (freshwater)** | **9** |
| Asia - Inland waters | 1 |
| Mediterranean and Black Sea | 19 |
| **Aquaculture production (marine)** | **20** |
| Asia - Inland waters | 25 |
| Marine areas outside the Antarctic | 2 |
| Mediterranean and Black Sea | 83 |
| **Capture production** | **110** |
| **Genel Toplam** | **140** |

### 1.1.Diversity of Aquatic Products

The variety of fish consumed as food is more than terrestrial animals. Aquatic products, one of the important foods of the meat group, vary according to the ecosystem they are in. Depending on the water they live in, fish can be classified as sea, brackish or freshwater fish, depending on the climate zone they live in; We can divide it into tropical, temperate and cold water fish. Fishery products are divided into 2 groups, fish and shellfish (Table 1), (Guillen, et al., 2019).

Fish are seafood that have fins and a backbone. Fish can be grouped as round, flat and round-fat fish in terms of appearance or physiognomy. In the round fish group; whiting, tuna, angler fish, cod fish, flat fish group; turbot and sole fish, and the round-oily fish group includes anchovy, salmon, trout, bonito, sardine and mackerel. Shellfish are marine creatures that have a shell on the outside but no backbone. Crustaceans can be grouped as cephalopods and molluscs. In the crustacean group; lobster, crab, shrimp, and sea crayfish, in the cephalopod group; octopus and squid, and in the mollusk group there are mussels, oysters and sea urchins (Garipağaoğlu, M.2019).

**Table 3.** Amount of aquatic products obtained from fisheries and aquaculture in the world (2021) *1000 tons

| Aquaculture ( *1000 Ton) | | | | Capture ( *1000 Ton) | | | | Total Production | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Country | 2021 | % | | Country | 2021 | % | | Country | 2021 | % |
| **1** | **China** | 51,221 | 55.15 | **1** | **China** | 13,226 | 14.44 | **1** | **China** | 64,447 | 34.94 |
| **2** | **India** | 9,403 | 10.12 | **2** | **Indonesia** | 6,876 | 7.51 | **2** | **India** | 14,033 | 7.61 |
| **3** | **Indonesia** | 5,515 | 5.94 | **3** | **Peru** | 5,627 | 6.14 | **3** | **Indonesia** | 12,391 | 6.72 |
| **4** | **Viet Nam** | 4,736 | 5.10 | **4** | **Russian Federation** | 5,072 | 5.54 | **4** | **Viet Nam** | 8,242 | 4.47 |
| **5** | **Bangladesh** | 2639 | 2.84 | **5** | **India** | 4,630 | 5.06 | **5** | **Peru** | 5,777 | 3.13 |
| **6** | **Norway** | 1,665 | 1.79 | **6** | **United States of America** | 4,261 | 4.65 | **6** | **Russian Federation** | 5,368 | 2.91 |
| **7** | **Egypt** | 1,576 | 1.70 | **7** | **Viet Nam** | 3,506 | 3.83 | **7** | **United States of America** | 4,709 | 2.55 |
| **8** | **Chile** | 1,427 | 1.54 | **8** | **Japan** | 3,179 | 3.47 | **8** | **Bangladesh** | 4,558 | 2.47 |
| **9** | **Thailand** | 990 | 1.07 | **9** | **Norway** | 2,473 | 2.70 | **9** | **Norway** | 4,137 | 2.24 |
| **10** | **Myanmar** | 929 | 1.00 | **10** | **Myanmar** | 1,977 | 2.16 | **10** | **Japan** | 3,800 | 2.06 |
| **11** | **Philippines** | 929 | 1.00 | **11** | **Bangladesh** | 1,920 | 2.10 | **11** | **Chile** | 3,200 | 1.74 |
| **12** | **Ecuador** | 896 | 0.97 | **12** | **Philippines** | 1,912 | 2.09 | **12** | **Myanmar** | 2,907 | 1.58 |
| **13** | **Brazil** | 649 | 0.70 | **13** | **Chile** | 1,774 | 1.94 | **13** | **Philippines** | 2,841 | 1.54 |
| **14** | **Japan** | 622 | 0.67 | **14** | **Thailand** | 1,589 | 1.74 | **14** | **Thailand** | 2,579 | 1.40 |
| **15** | **Korea, Republic of** | 582 | 0.63 | **15** | **Mexico** | 1,501 | 1.64 | **15** | **Egypt** | 1,995 | 1.08 |
| **16** | **Iran (Islamic Rep. of)** | 479 | 0.52 | **16** | **Malaysia** | 1,387 | 1.51 | **16** | **Korea, Republic of** | 1,951 | 1.06 |
| **17** | **Türkiye** | **472** | **0.51** | **17** | **Morocco** | 1,375 | 1.50 | **17** | **Mexico** | 1,748 | 0.95 |
| **18** | **United States of America** | 448 | 0.48 | **18** | **Korea, Republic of** | 1,369 | 1.49 | **18** | **Malaysia** | 1,625 | 0.88 |
| **19** | **Cambodia** | 347 | 0.37 | **19** | **Iceland** | 1,020 | 1.11 | **19** | **Ecuador** | 1,533 | 0.83 |
| **20** | **Russian Federation** | 295 | 0.32 | **20** | **Argentina** | 838 | 0.91 | **20** | **Brazil** | 1,374 | 0.74 |
| **21** | **Spain** | 280 | 0.30 | **21** | **Spain** | 802 | 0.88 | **21** | **Iran (Islamic Rep. of)** | 1,270 | 0.69 |
| **22** | **Nigeria** | 276 | 0.30 | **22** | **Oman** | 793 | 0.87 | **22** | **Spain** | 1,082 | 0.59 |
| **23** | **Taiwan Province of China** | 274 | 0.30 | **23** | **Iran (Islamic Rep. of)** | 791 | 0.86 | **23** | **Nigeria** | 1,059 | 0.57 |
| **24** | **Mexico** | 247 | 0.27 | **24** | **Nigeria** | 783 | 0.86 | **24** | **Canada** | 921 | 0.50 |
| **25** | **Malaysia** | 238 | 0.26 | **25** | **Denmark** | 733 | 0.80 | **25** | **Taiwan Province of China** | 898 | 0.49 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **26** | **United Kingdom** | 230 | 0.25 | **26** | **Canada** | 730 | 0.80 | **26** | **Cambodia** | 883 | 0.48 |
| **27** | **France** | 199 | 0.21 | **27** | **Brazil** | 725 | 0.79 | **27** | **United Kingdom** | 857 | 0.46 |
| **28** | **Colombia** | 193 | 0.21 | **28** | **Mauritania** | 678 | 0.74 | **28** | **Türkiye** | **836** | **0.45** |
| **29** | **Canada** | 191 | 0.21 | **29** | **Faroe Islands** | 646 | 0.71 | **29** | **Faroe Islands** | 762 | 0.41 |
| **30** | **Pakistan** | 165 | 0.18 | **30** | **Ecuador** | 636 | 0.69 | **30** | **Uganda** | 705 | 0.38 |
| **31** | **Peru** | 151 | 0.16 | **31** | **United Kingdom** | 626 | 0.68 | **31** | **Pakistan** | 657 | 0.36 |
| **Aquaculture ( *1000 Ton)** | | | | **Capture ( *1000 Ton)** | | | | **Total Production** | | | |
| **Country** | | **2021** | **%** | **Country** | | **2021** | **%** | **Country** | | **2021** | **%** |
| **32** | **Italy** | 146 | 0.16 | **32** | **Taiwan Province of China** | 624 | 0.68 | **32** | **France** | 628 | 0.34 |
| **33** | **Greece** | 144 | 0.15 | **33** | **South Africa** | 596 | 0.65 | **33** | **New Zealand** | 481 | 0.26 |
| **34** | **Uganda** | 139 | 0.15 | **34** | **Uganda** | 566 | 0.62 | **34** | **Ghana** | 460 | 0.25 |
| **35** | **Lao People's Dem. Rep.** | 135 | 0.15 | **35** | **Cambodia** | 536 | 0.59 | **35** | **Australia** | 301 | 0.16 |
| **36** | **Australia** | 126 | 0.14 | **36** | **Pakistan** | 493 | 0.54 | **36** | **Italy** | 286 | 0.16 |
| **37** | **Uzbekistan** | 119 | 0.13 | **37** | **Tanzania, United Rep. of** | 472 | 0.52 | **37** | **Colombia** | 284 | 0.15 |
| **38** | **New Zealand** | 117 | 0.13 | **38** | **Senegal** | 458 | 0.50 | **38** | **Korea, Dem. People's Rep** | 280 | 0.15 |
| **39** | **Faroe Islands** | 116 | 0.12 | **39** | **France** | 430 | 0.47 | **39** | **Greece** | 215 | 0.12 |
| **40** | **Saudi Arabia** | 114 | 0.12 | **40** | **Egypt** | 419 | 0.46 | **40** | **Lao People's Dem. Rep.** | 205 | 0.11 |
| **41** | **Nepal** | 101 | 0.11 | **41** | **Mozambique** | 395 | 0.43 | **41** | **Saudi Arabia** | 172 | 0.09 |
| **42** | **Ghana** | 89 | 0.10 | **42** | **Sri Lanka** | 387 | 0.42 | **42** | **Zambia** | 170 | 0.09 |
| **43** | **Korea, Dem. People's Rep** | 78 | 0.08 | **43** | **Angola** | 375 | 0.41 | **43** | **Uzbekistan** | 165 | 0.09 |
| **44** | **Zambia** | 63 | 0.07 | **44** | **Ghana** | 371 | 0.41 | **44** | **Nepal** | 122 | 0.07 |
| **45** | **Honduras** | 63 | 0.07 | **45** | **Türkiye** | **364** | **0.40** | **45** | **Honduras** | 79 | 0.04 |

Structurally, according to where body fat is stored; They are classified into 2 groups fish with white meat and dark muscle meat. According to this classification; White-meat fish are fish whose fat is stored in the liver, their muscle meat is lean, and they are very easy to digest. In general, trout, leer, baccalaureate, red mullet-tabbed, sea bream, sea bream, tongue, flounder, sea bream, sea bass, lantern, weasel, whiting, turbot, bream, mullet, swallow, kupez, lagos, bluefish, coral, purr, Grouper, sea bass, pike, pike and catfish are classified as white-fleshed fish. Fish with dark muscle meat store their fat in their muscles. These fish are more delicious than white meat fish. Anchovy, silverfish, eel, horse mackerel, butt, mackerel, chum, salmon, sardine, swordfish, bonito, bonito, shad, tuna, garfish and carp are examples of fish with dark muscle meat (Garipağaoğlu, M.2019; Guillen, et. . al., 2019 ).

The definition of scaled or scaleless is also frequently used in the classification of fish. According to this; Red mullet, berlam, sea bream, silver, anchovy, bream, mullet, kupez, lipsose, coral, minekop, sardine, carp, salmon, pike perch, pike, dengue, trance fish are scaly. Trout, leprechaun, bacalyaro, chinakop, sole, carpenter, weasel, horse mackerel, turbot, swordfish, red snapper, bigmouth, chum, grouper, sea bass, bluefish, whiting, purr, grouper, tuna, bonito, halibut, tuna, mackerel, garfish, are examples of scaleless fish (Garipağaoğlu, M.2019; Guillen, et. al., 2019).

### 1.2. Consumption

The daily energy contribution of seafood consumption to human nutrition is 34 calories per person. However, the contribution of fisheries to nutrition is important for high-quality, easily digestible animal proteins and especially for combating micronutrient deficiencies, apart from being an energy source. A serving of seafood weighing one hundred and fifty grams meets approximately 50-60% of an adult's daily protein needs. Aquatic products, which are generally consumed fresh, are now offered to consumers by being subjected to processing and preservation technologies such as cooling, freezing, salting, canning, smoking, drying and brining. 88% of the seafood produced in the world is offered directly to human consumption. 45% of these products are marketed as fresh, 34% are marketed as frozen, 10% are marketed as dried and 11% are marketed as canned. While consumption of frozen and processed seafood is widespread in developed countries, fresh consumption is dominant in developing countries. In the last 60 years, the average annual increase in world fisheries consumption (3.2%) has outstripped the population growth rate (1.6%). The annual increase rate of aquaculture consumption exceeded that of meat from all terrestrial animals (2.8%), after poultry (4.9%).

The average fish consumption per capita in Turkey is 6 kg. This consumption amount is well below the world average of 19.2 kg and the average of the European Union countries of 39 kg. Turkey is in the group of countries that consume very little in terms of seafood consumption (Fao, 2022).

While approximately 89% of the seafood produced in the world was 89% in 2019, 62% of the fishery products produced in Turkey are directly offered to domestic consumers. The proportion of aquatic products used in the production of fish meal and oil is approximately 25% (Table 4; Table 5).

Fish consumption varies from region to region due to transportation costs and consumption habits. While per capita fish consumption is 25 kg in the Black Sea region, it is 16 kg in big cities such as Istanbul and Ankara, and 0.5 kilograms in the Southeast and Eastern Anatolia Regions. While anchovies, horse mackerel and sardines are consumed a lot during the fishing season since they have affordable prices, turbot, sea bream and sea bass are consumed more in places with high-income levels and tourism centres. While the monthly fish consumption of low-income consumers is approximately 2.3 kilograms, it is around 8.5 kg for high-income consumers. The most important reason why seafood consumption is preferred in Turkey is the belief that it is healthy. This is followed by taste, habit and price variables. Half of the consumers in Turkey consume fish once or twice a month and their monthly fish consumption is less than 2 kilograms. Generally, more seafood is consumed in the winter months, and the rate of those who consume it all the time is quite low (20%). 82% of fish consumers in Turkey want to consume fresh fish. 78.5% of fish consumers prefer fisheries fish and 15.1% prefer fish obtained through aquaculture. The remaining consumers want to consume invertebrate aquatic products (mussels, squid, shrimp, etc.) (Anonymous, 2022).

**Table 4.** Total Fisheries and Aquaculture Production Quantity (Ton)*(The share of produced quantity in consumption)

| FAOStat Group Classification | 1961 | 1969 | 1970 | 1989 | 1999 | 2009 | 2019 |
|---|---|---|---|---|---|---|---|
| **Aquatic animals nei** | 41,426 | 70,411 | 168,939 | 362,134 | 775,332 | 1,274,492 | 1,562,293 |
| **Cephalopods** | 753,318 | 1,016,247 | 1,523,116 | 2,649,283 | 3,582,969 | 3,481,072 | 3,781,228 |
| **Crustaceans** | 1,363,420 | 1,932,335 | 3,073,756 | 4,581,880 | 7,471,050 | 11,136,518 | 16,661,717 |
| **Demersal fish** | 9,970,397 | 16,173,278 | 18,304,297 | 22,023,137 | 20,353,843 | 20,164,109 | 23,113,100 |
| **Freshwater & diadromous fish** | 4,824,419 | 6,228,846 | 7,682,487 | 14,607,431 | 27,520,338 | 44,711,574 | 65,930,856 |
| **Marine fish nei** | 3,325,017 | 5,464,789 | 6,850,943 | 9,014,184 | 10,302,194 | 9,404,231 | 10,851,682 |
| **Molluscs excl. cephalopods** | 1,915,898 | 2,540,164 | 3,738,933 | 6,543,166 | 13,196,654 | 1,6452,146 | 20,239,947 |
| **Pelagic fish** | 17,050,402 | 25,735,706 | 29,488,607 | 4,0861,027 | 39,254,493 | 37,586,417 | 35,693,236 |
| **Genel Toplam (%) *** | **39,244,297 (70.97)** | **59,161,776 (64.66)** | **70,831,078 (70.60)** | **100,642,242 (70.81)** | **122,456,873 (77.77)** | **144,210,557.10 (86.93)** | **177,834,058 (89.33)** |

**Table 5.** Total food supply Fisheries and Aquaculture Production Quantity (Ton)

| FAOStat Group Classification | 1961 | 1969 | 1970 | 1989 | 1999 | 2009 | 2019 |
|---|---|---|---|---|---|---|---|
| **Aquatic animals nei** | 37,527.00 | 118,474.04 | 354,743.39 | 262,989.00 | 684,627.12 | 1,303,344.22 | 1,531,161.00 |
| **Cephalopods** | 687,147.95 | 997,968.21 | 1,467,239.81 | 2,635,100.44 | 3,019,343.41 | 3,377,682.34 | 3,575,778.54 |
| **Crustaceans** | 1,336,101.67 | 1,925,991.14 | 3,240,840.69 | 4,884,709.86 | 8,088,869.85 | 11,751,705.75 | 17,401,291.77 |
| **Demersal fish** | 8,332,555.81 | 12,773,593.42 | 14,168,179.73 | 17,343,151.44 | 17,548,844.91 | 19,264,872.12 | 21,019,308.65 |
| **Freshwater & diadromous fish** | 4,704,748.27 | 6,143,618.22 | 7,600,888.66 | 14,522,414.10 | 26,090,138.75 | 43,389,911.82 | 63,412,548.88 |
| **Marine fish nei** | 3,232,429.67 | 4,833,866.21 | 5,980,830.71 | 8,566,320.56 | 8,708,302.60 | 7,821,523.33 | 8,069,962.52 |
| **Molluscs excl. cephalopods** | 1,829,143.74 | 2,221,054.20 | 3,971,223.57 | 6,605,287.05 | 12,783,135.42 | 15,754,144.04 | 19,630,274.63 |
| **Pelagic fish** | 7,690,548.12 | 9,239,960.81 | 13,221,748.48 | 16,448,018.16 | 18,306,134.71 | 22,705,721.47 | 24,211,236.11 |
| **Genel Toplam** | **27,850,202.23** | **38,254,526.25** | **50,005,695.04** | **71,267,990.61** | **95,229,396.77** | **125,368,905.10** | **158,851,562.10** |

Seafood is a valuable nutrient with high protein content, rich in amino acid content, vitamins and micronutrients, and also contains polyunsaturated fatty acids. It contains omega-3 fatty acids, which are polyunsaturated fatty acids that must be taken with the diet for human health, and is of great importance as it has low cholesterol and calorie value. In addition to their nutritional properties, seafood products are also known to be effective in protecting health and treating many diseases. Seafood is preferred in the diet of children and the elderly due to its high digestibility due to the low connective tissue it contains. However, the consumption of aquatic products in our country is at very low levels compared to the world average. Therefore, aquatic products are of great importance for a healthy and balanced diet, and these resources should be better utilized and their consumption should be expanded. Raising people's awareness about the consumption of aquatic products and emphasizing their importance in terms of taste and nutritional value will increase consumption amounts over time. Maldives is one of the countries that consume the most fish in the world (Table 6). Fish consumption in the world is 16.3kg/year-44g/day (1.5 meatball size); 23.8kg/year-63g/day (2 patties size) in developed countries; It is reported that it is up to 14.3kg/year-39g/day (~1.5 meatballs) in developing countries (Hecer, 2012).

The consumption of aquatic products in the world is increasing day by day. Fisheries consumption per capita has increased from 9.0 kg in 1961 to an annual average of 1.5 per cent and reached 19.2 kg by 2020 (FAO, 2022). Aquaculture consumption in Turkey is below the average recommended by the World Health Organization (11.7 kg/person), and there has been a fluctuating trend in our per capita consumption between 2000 and 2022.

Between these years, cultured fish production in Turkey increased from a minimum of 61 thousand tons in 2002 to a maximum of 514 thousand tons in 2022. Fisheries production increased from 302 thousand tons in 2014 to its highest value of 566 tons in 2002. Our total aquaculture production in Turkey was the lowest in 2014, 537 thousand tons, and reached the highest value, approximately 850 thousand tons in 2022 (TUIK, 2022). Despite this, there was no obvious increase in our fish consumption, and the highest fish consumption per capita was recorded as 8.6 kg in 2008 (Figure 1). According to the latest data obtained, the average consumption per capita in 2020 was 6.8 kg, and it varied between 5.5 kg and 8.6 kg between 2000 and 2022 (BSGM, 2023) (Figure 1).

**Table 6.** Countries Consuming the Most Fisheries in the World (FAO, 2020) *

| Country | Per Capita Consumption (kg) | Life Span (year) |
|---|---|---|
| **Maldives** | 87.30 | 84 |
| **Iceland** | 84.30 | 83 |
| **Macao** | 70.26 | 83 |
| **Hong Kong** | 65.79 | 83 |
| **Portugal** | 59.36 | 82 |
| **South Korea** | 54.66 | 83 |
| **Malaysia** | 53.33 | 83 |
| **New Zealand** | 52.15 | 82 |
| **Norway** | 50.57 | 83 |
| **Japan** | 46.65 | 84 |
| ***Türkiye*** | **6,20** | **79** |

*https://ourworldindata.org/grapher/fish-and-seafood-consumption-per-capita?tab=table&time=latest

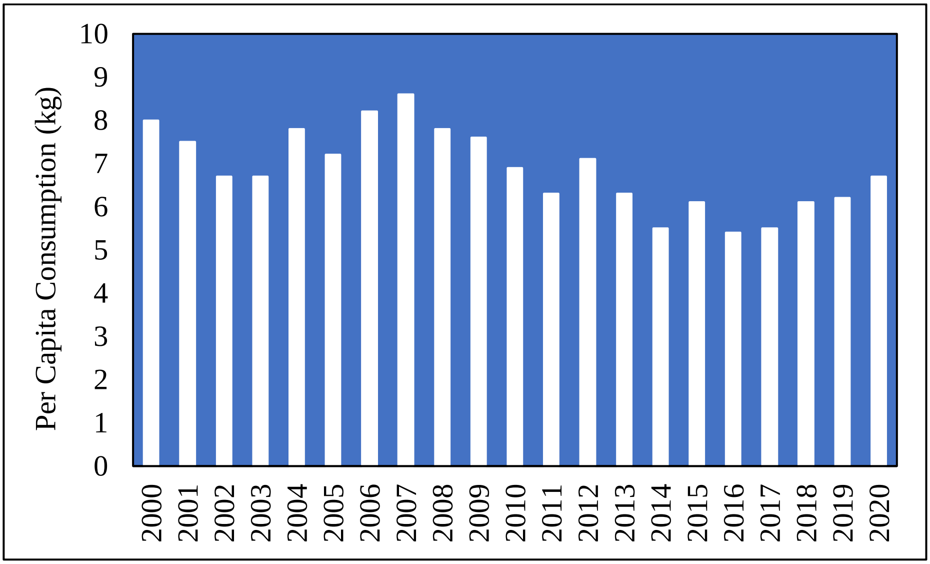


**Figure 1**. Distribution of Fishery Products Consumption in Turkey by Years (TUIK, 2022)

The increase in per capita consumption is not only due to the increase in production. In this rise; Many different factors such as increasing population, improvement in income and increase in demand due to urbanization, decrease in losses, better use of products and improvement of distribution channels are affected (FAO, 2018).

## 2. NUTRITIONAL VALUE OF FISH AND SEAFOOD

Inadequate, unbalanced or excessive nutrition not only negatively affects health; it also increases the health expenditures of individuals, families and countries. From this point of view, the World Health Organization and UNICEF, especially "Nutrition Improvement Approaches and Strategies", have been the focus of attention of health-related institutions and organizations, academics, researchers, associations, economists, managers and non-governmental organizations (Quaas, et al., 2016).

It is known that today's consumers are more interested in their health, they are looking for ways to protect their health, they aim to protect their health by eating right rather than using drugs, they make efforts in this direction and are in search of "healthy, high quality, affordable, safe food". Fish and other aquatic products are among the foods that have the characteristics that consumers are looking for (FAO, 2022).

According to this classification, changes can be observed in the taste and composition of the fish. Depending on the characteristics of the fish, its nutritional composition and therefore the processing method may also vary. While pelagic and oily fish are suitable for canning and smoking, white-fleshed fish may be suitable for surimi. Structurally, fish contain a very small amount of connective tissue. With this feature, fish meat is soft. It cooks easily even at low temperatures compared to other meats. It is also easy to digest (Baysal, 2023).

Fish is a food composed of water, protein, fat, vitamins and minerals. It is rich in vitamins and minerals such as Iodine, Vitamin D, Calcium, Phosphorus, Potassium, Selenium and Folic acid, and Omega Fatty acids, which are essential for the body. All nutrients it contains, including protein, have high bioavailability. With this content, fish is a functional food (Craig et al., 2017; Khalili Tilami and Sampels, 2018; Öksüz et al., 2018; Baysal, 2023).

Fish is one of the best sources of high-quality protein (Baysal, 2023).

Proteins and amino acids are the elements that build the body and life. Proteins, which are mainly involved in the development and repair of aging tissues, increase muscle strength and endurance and prevent age-related muscle loss (sarcopenia). It reduces fat mass, supports weight loss, and gives a feeling of satiety and satisfaction. With this feature, fish is the most popular food in slimming diets (Khalili Tilami and Sampels, 2018; Öksüz et al., 2018; Baysal, 2023). One meatball size (30g) of fish contains 6g of protein, and 100g of fish contains 20g of protein. Half of the daily protein requirement of an adult is met with 150g (about 5 meatballs) of fish consumed daily. It is recommended that 40-50% of the daily protein intake should be quality protein (Öksüz et al., 2018; TÜBER, 2022; Baysal 2023).

Fish contains a lower amount of fat than other types of meat. One hundred grams of fatty meat contains 18-20g of fat, half-fat meat contains 12-15g of fat, while fish contains half of these amounts, depending on the type of fish. While animal foods such as meat, milk, yoghurt, cheese, butter and egg yolk are the main source of saturated fatty acids, fish is the main source of very long chain unsaturated fatty acids (LC-PUFA), known as omega-3 (Baysal, 2023).

Omega-3 fatty acids are synthesized by marine algae. It is consumed by algae, plankton and other small marine animals. When fish consume phytoplankton, omega-3 fatty acids settle in the living body and join the food chain (Kudo et al., 2018; Baysal, 2023). Omega-3 fatty acids are obtained directly from fish oil and indirectly from α-Linolenic acid. Linoleic acid and α-Linolenic acid, polyunsaturated fatty acids, are essential fatty acids. Essential fatty acids cannot be synthesized in the human body. They must be taken externally through food (Liu et al., 2023). Linoleic acid, known as Omega-6, is found in sufficient amounts in vegetable oils and plants and is easily absorbed into the body; α-Linolenic acid, known as Omega-3, is found in limited amounts in flaxseed, rapeseed, purslane and walnuts and cannot be easily absorbed into the body. When essential fatty acids are taken into the body, Linoleic acid turns into Arachidonic Acid (AA), α-Linolenic acid turns into Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) (Baysal, 2023; Belury, 2023). Therefore, getting EPA and DHA from foods is the way to increase the levels of these omega-3 fatty acids in the body. Many fish species are sources of omega-3 fatty acids, especially sardines, mackerel and Pacific salmon.

Daily iodine requirement is 100-150µg for adults and 200-300µg for pregnant and lactating women (TÜBER, 2022, Baysal, 2023). Iodine is taken into the body mainly with water and food. Water, milk, meat, eggs, dark green leafy vegetables and legumes are foods that are good sources of iodine. However, the iodine content of foods varies depending on the iodine status of the soil and the region where it is grown or produced. The richest source of iodine is fish and other seafood growing in the seas and oceans (Baysal, 2023).

Iodine deficiency, one of the most common nutritional problems in the world, can be easily prevented by supplementing the diet with iodized salt. Species containing iodine in seafood can be listed as follows; Mussels, Shrimp, Crab, Lobster, Bonito, Anchovy and Samon. Nutrient composition of raw edible portion of fish species (per 100 g)

### 2.1. Aquatic Product in Human Nutrition

We need over 40 essential nutrients (which are vitamins, essential amino acids, minerals and omega fatty acids). The only food source that can provide almost all of these 40 essential nutrients is fish and aquaculture. Aquaculture is a unique food that contributes to food security because fish is a very effective and useful source of protein. 150 grams of fish provides 50-60 per cent of an adult's daily protein needs. In addition to protein, fish, seafood and seaweed provide a healthy diet containing essential omega-3 fatty acids and bioavailable micronutrients (vitamins A, B12 and D, as well as calcium, iron, iodine, zinc, selenium, phosphorus and zinc). (Figure 2), (FAO 2018).

**Tablo 7.** Nutrient composition of raw edible portion of fish species (per 100 g)

| Species | energy (kcal) | water (g) | protein (g) | fat (g) | cholesterol (mg) | calcium (mg) | iron (mg) | riboflavin (mg) | niacin (mg) |
|---|---|---|---|---|---|---|---|---|---|
| **Catfish, channel (farmed)** | 135 | 75.38 | 15.55 | 7.59 | 47 | 9 | 0.50 | 0.075 | 2.304 |
| **Cod, Atlantic** | 82 | 81.22 | 17.81 | **0.67** | 43 | 16 | 0.38 | 0.065 | 2.063 |
| **Grouper, mixed species** | 92 | 79.22 | 19.38 | 1.02 | 37 | 27 | 0.89 | 0.005 | 0.313 |
| **Haddock** | 87 | 79.92 | 18.91 | 0.72 | 57 | 33 | 1.05 | 0.037 | 3.803 |
| **Halibut, Atlantic or Pacific** | 110 | 77.92 | 20.81 | 2.29 | 32 | 47 | 0.84 | 0.075 | 5.848 |
| **Herring, Atlantic** | 158 | 72.05 | 17.96 | 9.04 | 60 | 57 | 1.10 | 0.233 | 3.217 |
| **Mackerel, Atlantic** | **205** | **63.55** | 18.60 | **13.89** | 70 | 12 | 1.63 | 0.312 | **9.080** |
| **Salmon, Atlantic** | 142 | 68.50 | 19.84 | 6.34 | 55 | 12 | 0.80 | **0.380** | 7.860 |
| **Salmon, pink** | 116 | 76.35 | 19.94 | 3.45 | 52 | **0** | 0.77 | **0** | **0** |

| **Species** | **energy (kcal)** | **water (g)** | **protein (g)** | **fat (g)** | **cholesterol (mg)** | **calcium (mg)** | **iron (mg)** | **riboflavin (mg)** | **niacin (mg)** |
|---|---|---|---|---|---|---|---|---|---|
| **Trout, rainbow (wild)** | 119 | 71.87 | 20.48 | 3.46 | 59 | 67 | 0.70 | 0.105 | 5.384 |
| **Tuna, bluefin** | 144 | 68.09 | **23.33** | 4.90 | 38 | **0** | 1.02 | 0.251 | 8.654 |
| **Clam, mixed species** | 74 | 81.82 | 12.77 | 0.97 | 34 | 46 | **13.98** | 0.213 | 1.765 |
| **Crab, blue** | 87 | 79.02 | 18.06 | 1.08 | 78 | **89** | 0.74 | **0** | **0** |
| **Lobster, northern** | 90 | 76.76 | 18.80 | 0.90 | 95 | **0** | **0** | 0.048 | 1.455 |
| **Oyster, Pacific** | 81 | **82.06** | **9.45** | 2.30 | **0** | 8 | 5.11 | 0.233 | 2.010 |
| **Scallop, mixed species** | 88 | 78.57 | 16.78 | 0.76 | 33 | 24 | 0.29 | 0.065 | 1.150 |
| **Shrimp, mixed species** | 106 | 75.86 | 20.31 | 1.73 | **152** | 52 | 2.41 | 0.034 | 2.552 |

Source: U.S. Department of Agriculture, Composition of Foods, Agriculture Handbook no. 8–11.

## 3. THE FUTURE OF AQUATIC PRODUCT

According to assessments made by FAO, the annual production potential of seas and oceans is well above the estimated amount of meat needed to feed the population in 2050. This result is mostly due to the growth potential of aquaculture, highlighting the importance of establishing policies that promote the sustainable development of aquaculture and finding a low-cost alternative (OECD-FAO 2019).

It is stated by scientists that aquaculture production in the world cannot be increased through fishing and that the maximum amount of catchable stock has been reached, and a similar situation is also valid for our country.

Considering the increasing population and the improvement in the living standards of the societies, it is considered that the demand for aquatic products will increase and that the increasing demand can only be met through aquaculture; For this, it is necessary to expand the aquaculture investments by making maximum use of the existing water resources potential, to bring new species into aquaculture, and to operate and manage the fisheries resources within the framework of sustainability principles (Table 8).

**Table 8.** Aquatic Product Production Projection Results (OECD-FAO, 2021)

| | **Capture (Million tons/year)** | **Aquaculure (Million tons/year)** | **Consumption per capita (kg)** |
|---|---|---|---|
| **2020 (the last)** | 93 | 84 | 19.2 |
| **2050 (the better)** | 115 | 146 | 24.8 |
| **2050 (the same)** | 95 | 140 | 22.3 |
| **2050 (the worst)** | 81 | 127 | 19.8 |

Due to the increase in consumer incomes, the increase in demand for fish products is expected to continue in the future. There are predictions similar to the developments expected in the world in fishery production in Türkiye.

# 4. HOLISTIC APPROACH TO FISHERIES CONSUMPTION

In recent years, among the emphasis on the importance of fish consumption for human health, the importance of polyunsaturated omega-3 fatty acids in fish oil has been emphasized. For this reason, some consumers and parents are turning to fish oil. Omega 3 fatty acids are part, but not all, of fish's contribution to our health. Fish not only provides its consumer with omega-3 fatty acids, but a high-quality source of protein, essential amino acids, and most macro and microelements. Therefore, apart from the doctor's advice, instead of fish oil, fish consumption is recommended in terms of providing other essential nutrients that the individual needs.

Seafood is a food that should be included in an adequate and balanced diet as it is a source of high-quality protein, long-chain polyunsaturated omega-3 fatty acids, vitamins and minerals. To ensure the continuation of a healthy society, it is important to examine the contribution of aquatic products to public health and to provide adequate promotion and information to raise awareness in society about this issue, to introduce fish consumption habits by including fish frequently in places where mass feeding is carried out, and to break the prejudices against fish in the society and to raise healthy generations. In guidelines, models and randomized controlled studies, it is recommended to consume 300-400g of fish at least twice a week.

The aquaculture sector has an increasing share in providing rural development with the social and economic functions it undertakes. The seas that our country has, the experienced fishermen in the aquatic product sector, and the technological infrastructure, experience and knowledge of the industry make Turkey a country with important potential at the international level.

**1.** While the contribution of fisheries production to total aquaculture production, both in our country and in the world, is gradually decreasing, the contribution of aquaculture is constantly and steadily increasing.

**2.** Directing both fishing and production (aquaculture) in line with effective management plans in fisheries should be considered as it will positively affect the utilization coefficient of fishery products in biodiversity and socioeconomic contexts.

**3.** While the annual fish consumption per capita in Europe is approximately 25-26 kg, this rate is approximately 7.6 kg in our country. In addition, this figure decreases to approximately 1 kg in the Eastern and Southeastern Anatolia regions. It is necessary to raise awareness and emphasize the importance of fish in nutrition to get people into the habit of consuming fish.

**4.** It was emphasized that it is necessary to have a strong interaction with the market at the production stage of aquaculture and to be more aware of the trends and consumer needs of fish hunters breeders and market actors in the supply chain.

**5.** Development of the products obtained as a result of fisheries and aquaculture by using more modern production, techniques and technologies in the classification, processing, packaging and placing on the market, the necessity of carrying out effective publication and training activities for the domestic regions, the establishment of a market monitoring system for fishery products, other related activities. Strategies should be developed to create a regular production and consumption balance by monitoring the production, demand, stocks, imports and seasonal price changes of fishery products in harmony with all stakeholder markets.

**6.** In light of the collected sectoral data, there is a need for informative and sound guidance efforts aimed at the government, producers and processors as well as consumers.

**7.** Nutrition and Dietetics experts recommend consuming 300-400g of fish and seafood at least twice a week. Apart from the doctor's advice, instead of fish oil, fish consumption is recommended in terms of providing other essential nutrients that the individual needs. Recommended fish for this purpose are Anchovy, Sardines, Mackerel, Bluefish, Sea Bass, Sea Bream and Salmon, respectively.

**8.** Fishery products are healthy foods that are rich in nutrients such as essential amino acids, vitamins (vitamin D), iodine, selenium, magnesium, zinc, and especially protein sources. Fish is the most important source of

Omega-3 fatty acids, which are found in the structure of the cell membrane but are not synthesized in the body. Omega-3 fatty acids; In addition to being effective in preventing the development of colorectal, breast, prostate, and lymphoma cancers; There are also many heart protective mechanisms of action such as anti-inflammatory, antithrombotic, antiarrhythmic, cardiac remodeling effect, stabilization of atherosclerotic plaque, effect on lipid metabolism, protection of endothelial functions, exercise tolerance and cognitive functions. Similarly, people who consume fish have a lower risk of developing macular degeneration, one of the leading causes of vision impairment and blindness.

**9.** On the other hand, it was emphasized that the loss of the ratio of EPA/DHA unsaturated fatty acids due to the high heat generated during the cooking of fish meat will significantly reduce the benefit to be obtained from these products, and the importance of being cautious about the carcinogenic effect due to carbonization caused by sloppy exposure to fire.

**10.** Care should be taken in terms of the sources and contamination risks of fish and fish oil/fish oil capsules, to prevent poisoning and discomfort for fishery products, after purchasing the fish, it should be cooked and served in a short time without waiting too long in the kitchen and at room temperature. should be served and consumed.

**11.** Introducing fish consumption habits by including fish in society's diet at a sufficient frequency and breaking the prejudices against fish in society are also important in terms of raising healthy generations.

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# CHAPTER 4

## MICROSCOPIC, HISTOLOGIC AND ALLOMETRIC EVALUATION OF ALTRICIAL SERPAE TETRA (*Hyphessobrycon eques*, STEINDACHNER, 1882) LARVAE

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## INTRODUCTION

Aquarium has turned into an industry that attracts so many people on a global scale and has an increasing economic return. Thus, important large-scale producers have started to emerge in many countries of the world (Hekimoğlu, 2006). Due to the increase in the diversity of species involved in the hobby in the ornamental fisheries sector, the materials used in the aquarium setup and maintenance, equipment such as filtration, lighting and water regulating chemicals have created a new commercial field brought by the sector. This situation has further expanded the trade volume of the sector on a global scale (Alpbaz, 1993; Hunt and Koca, 2014).

Aquarium fishing in Turkey is mostly done in metropolitan cities. It is estimated that there are around 8.000 to 10.000 aquarists in total. Aquarium fish producers, defined as domestic producers, are mostly located in the west and south regions of Turkey (Çelik et al., 2014).

Many species belonging to different families are used in aquariums prepared with various aquarium concepts. Characidae (or characins) is one of the most important families that includes many economical and popular species used as ornamental fishes. The Characidae family is represented by 1152 species all over the world. Although there are primary freshwater species that also spread in brackish waters (FishBase, 2022). Several scholars have reported that the Hyphessobrycon is not a monophyletic community (Géry, 1977; Weitzman and Fink, 1983; Weitzman and Palmer, 1997; Mirande, 2010; Oliveira et al., 2011). *Hyphessobrycon eques* inhabits freshwater and tropical. It is a benthopelagic species. The properties of the water they live in should be in the pH range of 5.0 - 7.8: dH range of 10-25 and the temperature range of 22°C-26°C (Baensch and Riehl, 1995). It is oviparous. *H. eques* is a fish species in which male and female reproductive organs are present in separate individuals. In captivity, spawning is vigorously impelled by the male early in the day. The eggs are often set to the bottom (Mills and Vevers, 1989). It lives in shoal (Planquette et al., 1996). It feeds on worms, crustaceans, insects, and plants (Mills and Vevers, 1989). Humans use it in aquariums for commercial purposes. It is a species of high economic value. Synonyms of *H.eques* are *H. serpae, H. callistus* and *Tetragonopterus callistus* (Anonymous 2021a).

The highest mortality rates occur in the early larval stages in ornamental fish culture. Therefore, knowing the larval developmental stages is a key factor. Fish larvae are generally transparent up to the postlarval stage, this situation allowing the use of microscopes in ontogeny studies. Examination of various tissues and organs formed due to the development of larvae usually requires the use of histological methods. It is important to use microscopic and histological methods to determine the early feeding protocols of the larvae, to monitor the metamorphosis, and to determine the developmenta stages such as mouth opening, swim bladder formation, differentiation of the digestive tract, and functional stomach formation.

For the sustainable use of ornamental fish species that caught from natural waters, a thorough knowledge of the culture procedures is required. For this reason, the egg and embryological development of serpae tetra, which has an important place in the aquarium fish market, have been revealed. In order to shed light on those who will work on this subject in the future, the allometric growth curves and histological sections, which have not been included in recent studies, are presented in this article. In this trial, development and the allometric growth measurements of serpae tetra larvae were examined microscopically and histologically.

## 1. MATERIAL AND METHOD

The broodstock Serpae tetras have reached sexual maturity and have a total length of 2 cm and above (Figure 1). Fishes were separated into aquariums with 50 L volumes according to gender differences and each aquarium was stocked with 2 females and 1 male. They were fed with commercial flake and granule feeds of approximately 4% of their body weight twice a day. These feeds were used at certain rates (20% Tetra discus granules and 80% Tetra pro-energy flakes) (Ghosh et al., 2008). It was also fed with newly hatched *Artemia* sp. nauplii and bloodworms to adapt the fish to granular and flake baits. Water temperature in aquariums was 27.33±1.10 °C, pH 6.97±0.17, Electrical conductivity (EC) 151±24 µS/cm, salinity 0.02±0.01 mg/L and dissolved oxygen (DO) 6.97±0.15 mg/L during the acclimation process. Eggs and larvae which were reproduced after a certain adaptation period, were examined microscopically and histologically. And also, some allometric growth parameters were measured.


**Figure 1**. Images of broodstock fishes were used in the study.

### 1.1. Spawning of broodstock fishes

Breeding was carried out in 40 L glass aquariums with a water temperature of 27.30±0.41 °C, EC 21.1±1.2 µS $cm^{-1}$, and pH 6.10±0.21 and DO 7.17±0.11 mg $L^{-1}$ after 1 month of conditioning. In order to prevent predation of eggs, the broodstock fishes were taken into cages with 1 cm mesh size as 2 female and 1 male. After the eggs were observed on the aquarium bottom, the broodstocks were taken from the breeding tank and sampling was carried out here.

### 1.2. Egg and larva sampling and preparations

The first day of egg hatching was accepted as the first day of the larvae. Eggs were taken from the same broodstock fishes in a single batch. These eggs were sampled at 1, 2, 4, 8, and 10 hours and just before hatching. And also, larvae were sampled every day in the first 10 days (n:30). Randomly selected eggs and larvae were fixed as 10 samples in each sampling time. Eggs and larvae have used an overdose (250 mg $L^{-1}$) of Tricaine methane-sulfonate (MS-222) for euthanasia (AVMA, 2007; Topic-Popovic et al., 2012) firstly and then fixed in Bouin's solution (Ünal, 2010; Moore et al., 2002) for 24 hours at room temperature. Following the dehydration process in the alcohol series, the samples taken into base molds were embedded in paraffin. Sections (5 µm) were obtained using the rotary microtome (MICROM HM 315, Walldorf-Germany). Sections with Haematoxylin-Eosine staining were examined under a light microscope to determine embryonic and larval developmental stages according to Önal et al., (2008), Santos et al., (2016) and, Aminaghie and Esmaeili (2017).

External morphometric measurements were performed using Toup view and ImageJ 1.46 software. The first egg diameter, the number of oil droplets, the pigmentation pattern seen in the egg and larva, the egg hatching period and developmental stages, the first larva size, the absorption time of the yolk sac, the opening of the mouth and anus and the length of the first mouth opening were revealed. Mouth gap sizes were examined in fish larvae when the first exogenous feeding begins, sizes were measured at the point where the mouth was fully opened, as specified in Cunha and Planas, (1999), Ramezani-Fard et al., (2011) and Riar et al., (2018). And also, the allometric growth parameters were determined for the sampling days (Figure 2).

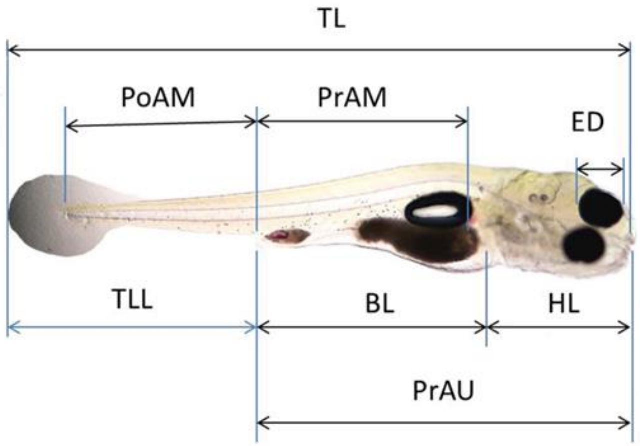


**Figure 2.** Abbreviations of Some Meristic Growth Characters on Fish Larvae (TL: total length, PoAM: Post-anal myomere length, PrAM: Pre-anal myomere length, PrAU: Pre-anal length, ED: eye diameter, TLL: tail length, HL: head length, BL: body length).

Allometric growth was defined by regression formulas which were determined by different meristic properties are compared with the total length (Fuiman, 1983; Gisbert et al., 2002; Çelik et al., 2011). Meristic growth characters were estimated using the $Y = aX^b$. The growth coefficients were calculated according to this equation model. Growth coefficient; If b=1 isometric, if b >1 positive, b<1 negative allometric growing was assumed.

## 2. RESULTS

### 2.1. Egg structure and reproductive characteristics

Totally 9 times spawning was carried out at different times for each of the groups during the trial. It has been observed that egg laying was completed in 6-7 hours (h.) and also it was determined that the eggs were round, transparent, and demersal (Figure 3). In the samples examined within the first 1-2 h. from the eggs, it was determined that the egg diameter was in the range of 1013-1057 µm and the average diameter was 1035.4±17.2 µm (n:60).

It has been detected that water temperature values directly affect the egg hatching time in the decapsulation of eggs. It was determined that the eggs hatched in 10-11 hours at 27.8 °C, and in 12-13 h. at 25.9 °C.

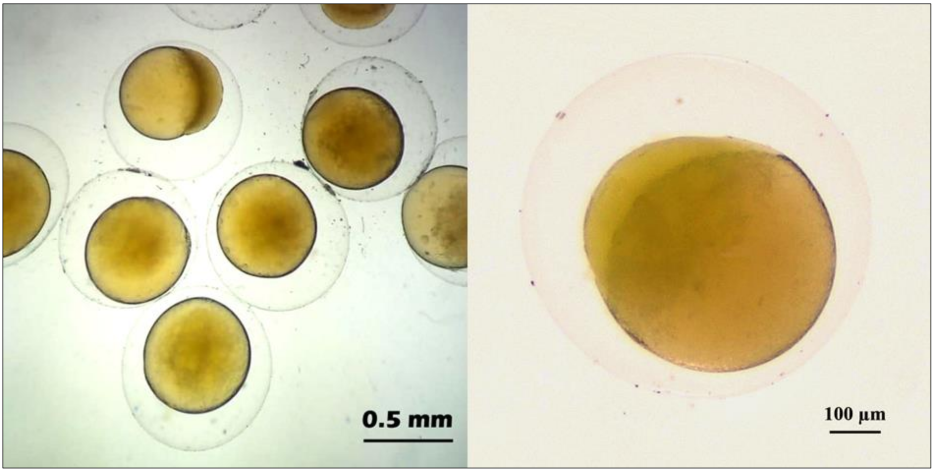


**Figure 3.** Image of egg shape and structure.

### 2.2. Microscopy of embryonic development

Microscopic images of the embryological development stages of eggs were given in Figure 4 and details about the stage of the eggs are in Table 1. In this study, all eggs were collected from a single batch of eggs. After laying eggs, pigmentation in the form of spots occurred on the chorion at the 8$^{th}$ h. (Figure 4d). From the first day onwards after hatching, black random pigmentation along the notochord to the tail became evident (Figure 4f).

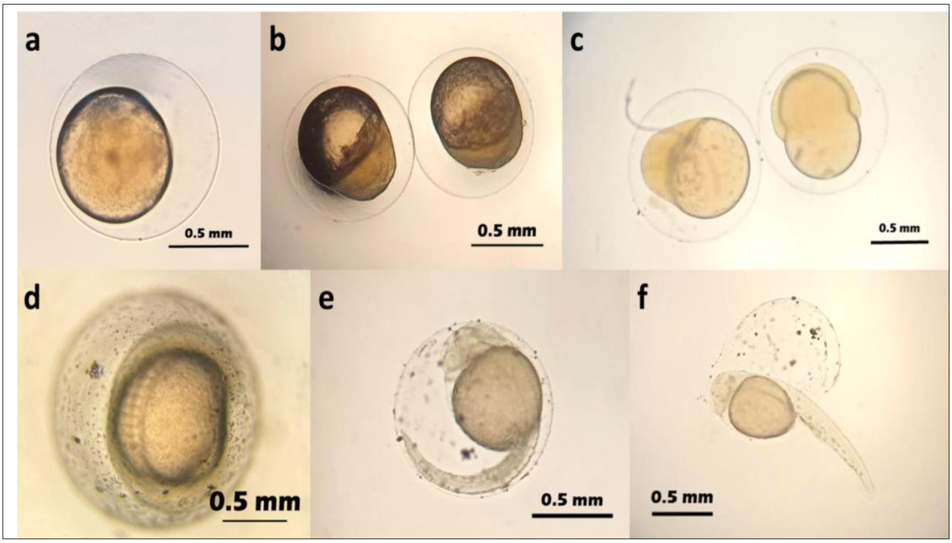


**Figure 4.** Developmental stages of eggs from newly laid eggs to 11-12$^{th}$ h. (hatching). (a: 1$^{st}$ h, b: 2$^{nd}$ h, c: 4$^{th}$ h, d: 8$^{th}$ h, e: 10$^{th}$ h, f: 11-12$^{th}$ h).

**Table 1.** Explanations of the embryonic development stages

| Figures | Descriptions/Measurements |
|---|---|
| **a** | 1$^{st}$ hour, VD 708 µm, CD 1055 µm, PS 244 µm |
| **b** | 2$^{nd}$ hour, blastula stage, blastodisc formation, $VD_1$ 732 µm, $VD_2$ 718 µm, $CD_1$ 1057 µm, $CD_2$ 1053 µm, $PS_1$ 138 µm, $PS_2$ 197 µm |
| **c** | 4$^{th}$ hour, multi-cell (>64) formation-early morula stage and two-somite embryo formation, $VD_1$ 935 µm, $VD_2$ 898 µm, $CD_1$ 1190 µm, $CD_2$ 1111 µm, $PS_1$ 107 µm, $PS_2$ 142 µm |
| **d** | 8$^{th}$ hour, embryo formation, ED 1603 µm |
| **e** | 10$^{th}$ hour, embryo formation ED 1596 µm |
| **f** | 11-12$^{th}$ hours, hatching period, TL 1588 µm |

*VD: Vitellus diameter, CD: Chorion diameter, PS: Perivitelline space, ED: Egg diameter, TL: Total length.
****** Eggs given embryonic development stages were incubated at 27.3 °C and hatched in 11-12 hours.

### 2.3. Microscopy and histology of larval development

Microscopic images of the sampled larvae are given in Figure 5. Morphometric values such as the first total length of the pre-larvae, the diameter of the yolk sac and the first mouth gap size were measured throughout the development from the first hatching to the post-larval stage (Table 2).

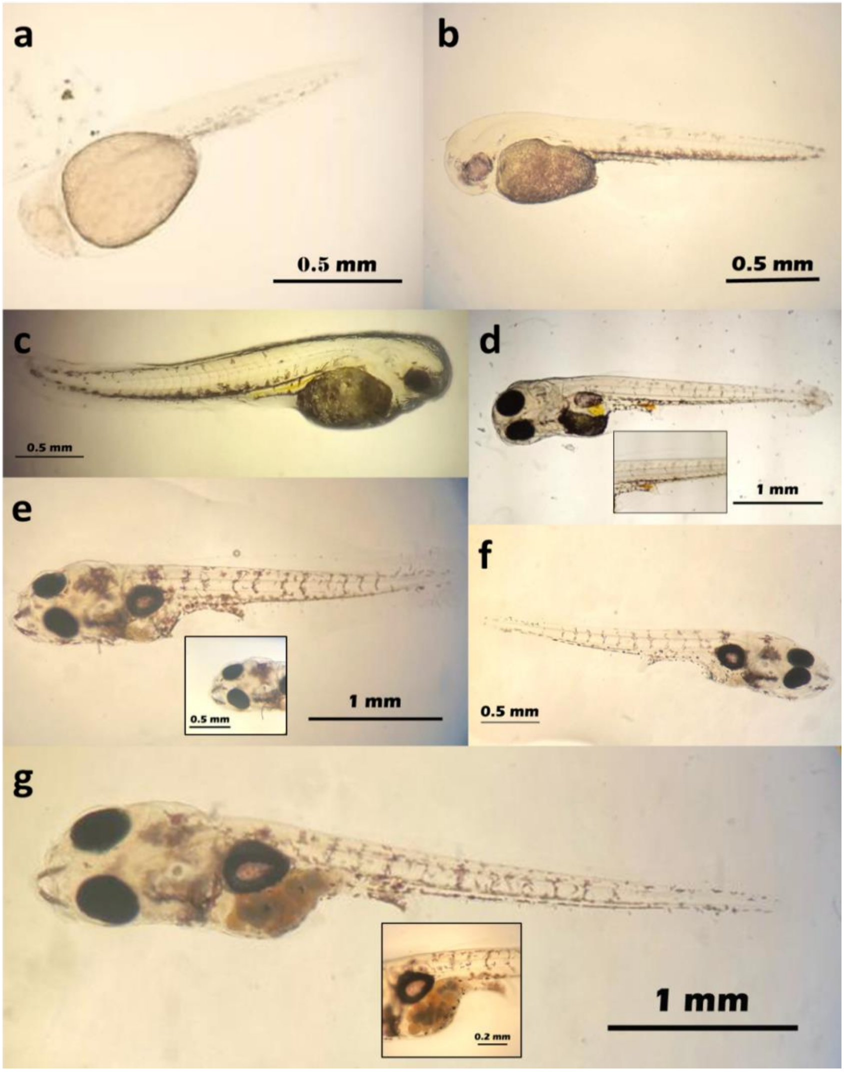


**Figure 5.** Developmental stages from newly hatched prelarvae to 7 DAH. (a: $14^{th}$ h, b: $24^{th}$ h, c: 2 DAH, d: 3 DAH, e: 5 DAH, f: 6 DAH, g: 8 DAH).

Water quality values during both egg hatching and larval development were measured as pH 6.21±0.7, EC 27.3±09 µS $cm^{-1}$, salinity 0.02±0.01 mg $L^{-1}$, DO 7.84±0.21 mg $L^{-1}$and 27.50±0.90 °C. It was determined that the larvae completely absorbed the yolk sac at a 72-75$^{th}$ h. and mouth opening occurred at 4 DAH (the day after hatching). At the opening of the mouth and anus, the yolk was completely or almost exhausted. A newly opened mouth gap size is not suitable for feeding with newly hatched *Artemia* at this stage, so egg yolk was given as the first food. Since egg yolk was seen in the stomach and digestive tract of all larvae, it was decided that the larvae started exogenous feeding at the end of the 4 DAH. In addition, it was observed that the larvae were generally motionless on the aquarium floor until 4 DAH, started to swim freely after exogenous feeding and used the entire water column from the 5$^{th}$ day. It was determined that the swim bladder did not show any differentiation until the post-larval stage. Larvae started to take newly hatched *Artemia* sp. at the end of the 7$^{th}$ day. The early-stage measurement and feeding procedures of the larvae are summarized in Table 3.

**Table 2**. Descriptions of images about morphometric measurements

| Figures | Descriptions |
|---|---|
| **a** | 14$^{th}$ hour, TL 1.96 mm, YD: SA 413 µm, LA 546 µm |
| **b** | 24$^{th}$ hour, TL 3.11 mm, YD: SA 334 µm, LA 556 µm |
| **c** | 2 DAH, TL 3,54 mm, YD: SA 302 µm, LA 687 µm |
| **d** | 3 DAH, TL 3.78 mm, MS 198 µm (mouth opening at the end of the day) |
| **e** | 5 DAH, TL 3.83 mm, MS 217 µm |
| **f** | 6 DAH, TL 3.91 mm, MS 265 µm |
| **g** | 8 DAH, TL 4.33 mm, MS 409 µm |

* TL: Total Length, YD: Yolk Sac Diameter, MS: Mouth Gap Size. SA: Short axis, LA: Long axis.

****** The larvae in the pictures were kept at 26.9-27.3 °C throughout the sampling.

When the larval development stages were evaluated histologically, it was observed that the digestive tract was not differentiated and segmented on the 2$^{nd}$ day and also the liver and the other organs became prominent on the 5$^{th}$ day.

**Table 3.** Early larval morphometric measurements and feeding protocol up to the post-larval stage

| DAH | Morphological Measurements/Descriptions (n:80) | Feeding Procedures |
|---|---|---|
| **1** | TL min 2990 µm, max 3114 µm, 3058±56 µm | *not exogenous feeding* |
| **2** | TL min 3299 µm, max 3546 µm, 3697±88 µm | *not exogenous feeding* |
| **3** | TL min 3603 µm, max 3784 µm, 3696±45µm. The yolk sac absorption and mouth opening at the end of the day (Almost all of the larvae), MS 198±13 µm | *not exogenous feeding* |
| **4** | MS 203±09 µm, TL min 3809 µm, max 3849 µm, 3826±14 µm | *Egg yolk* |
| **5** | MS 217 ±14 µm, TL min 3887 µm, max 3914 µm, 3902±10 µm | *Egg yolk* |
| **6** | MS 265±27µm, TL min 3986 µm, max 3997 µm, 3991±04 µm | *Egg yolk* |
| **7** | MS 373±11 µm, TL min 4101 µm, max 4161 µm, 4125±20 µm | *Egg yolk +AF* |
| **8** | MS 409 ±07 µm, TL min 4303 µm, max 4374 µm, 4343±21 µm | *AF* |

* TL: Total Length, MS: Mouth Gap Size, AF: Newly Hatched Artemia (AF type)
** The larvae were kept at 27.1 °C throughout the measurement.

The pharyngeal cavity and gill arches started to become evident on the 2$^{nd}$ day. The lens, ganglionic layer and pigment parts of the eye were observed on the 4-5$^{th}$ day. The oesophagus and pharynx became distinguishable at the end of the 5 DAH. The digestive tract is differentiated into 3 regions from the 7$^{th}$ day. It was observed that the metamorphosis of all nasal system structures, especially the olfactory organ was started at the end of the 5 DAH and also these system structures such as the olfactory organ and olfactory bulb became evident on the 8$^{th}$ day. The swim bladder did not show any constriction until the 8$^{th}$ day. The digestive tract, liver and heart have become distinct structures at the 8 DAH (Figure 6).

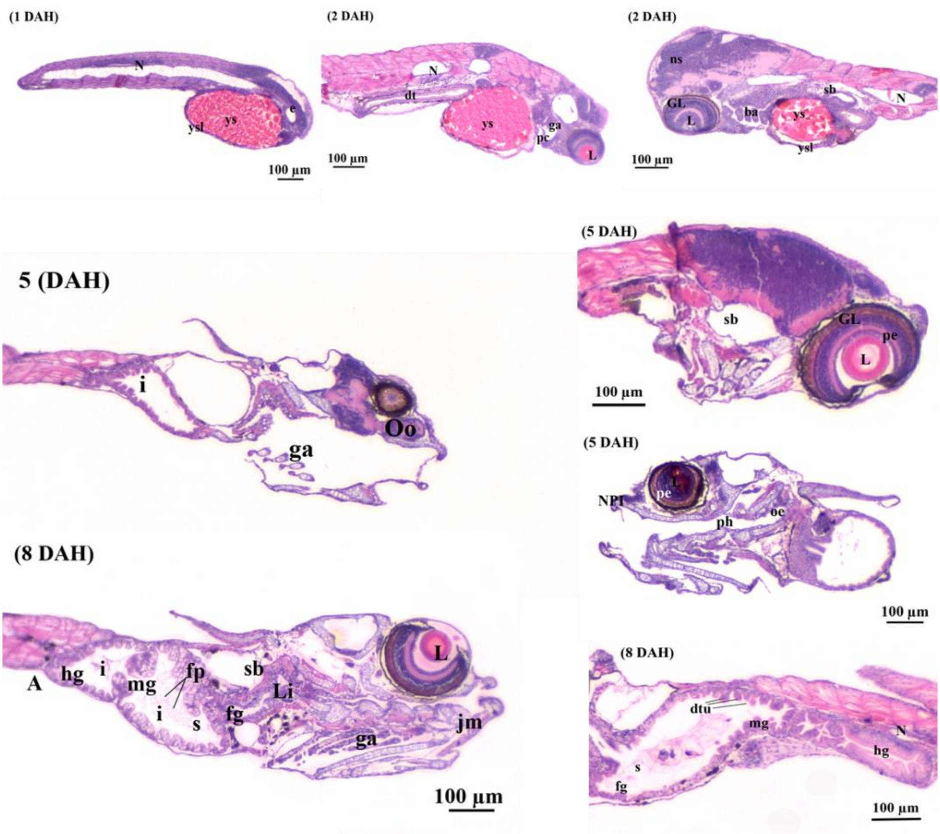


**Figure 6.** Histological images of larval development stages.

(*sb: swim bladder, N: notochord, fg: foregut, hg: hindgut, mg: midgut, pc: pharyngeal cavity, ga: gill arches, ns: nervous system, pe: pigment, ysl: yolk syncytial layer, L: lens, g: ganglionic layer, ba: branchial arches, NPI: nasal pit, fp: food particles, Li: liver, i: intestine, Oo: olfactory organ, jm: jaw muscle, s: stomach, GL: ganglionic layer, Oe: oesophagus, ph: pharynx).

Calculated using SPSS 21 software early growth formula of larvae is $y = 2.5735e^{0.0035x}$ ($R^2 = 0.6124$, n=70). 'y' in the formula; represents the total length (TL), and x represents the hours after hatching (HAH). Growth rates were estimated using to the $Y = aX^b$. The growth coefficients of the morphological characters are as follows, respectively: HL (a: 0.06, b:1.89), PoAM (a: 0.43, b: 0.90), PrAM (a: 0.55, b: 0.45), PrAU (a: 0.53, b: 0.97), ED (a: 0.04, b: 1,49), TLL (a: 0.53, b: 0.94) and BL (a: 0.58, b: 0.52). According to these data, HL and ED showed positive allometric growth, PoAM, PrAU and TLL showed isometric allometric growth, and PrAM and BL showed negative allometric growth from the prelarval stage (Figure 7).

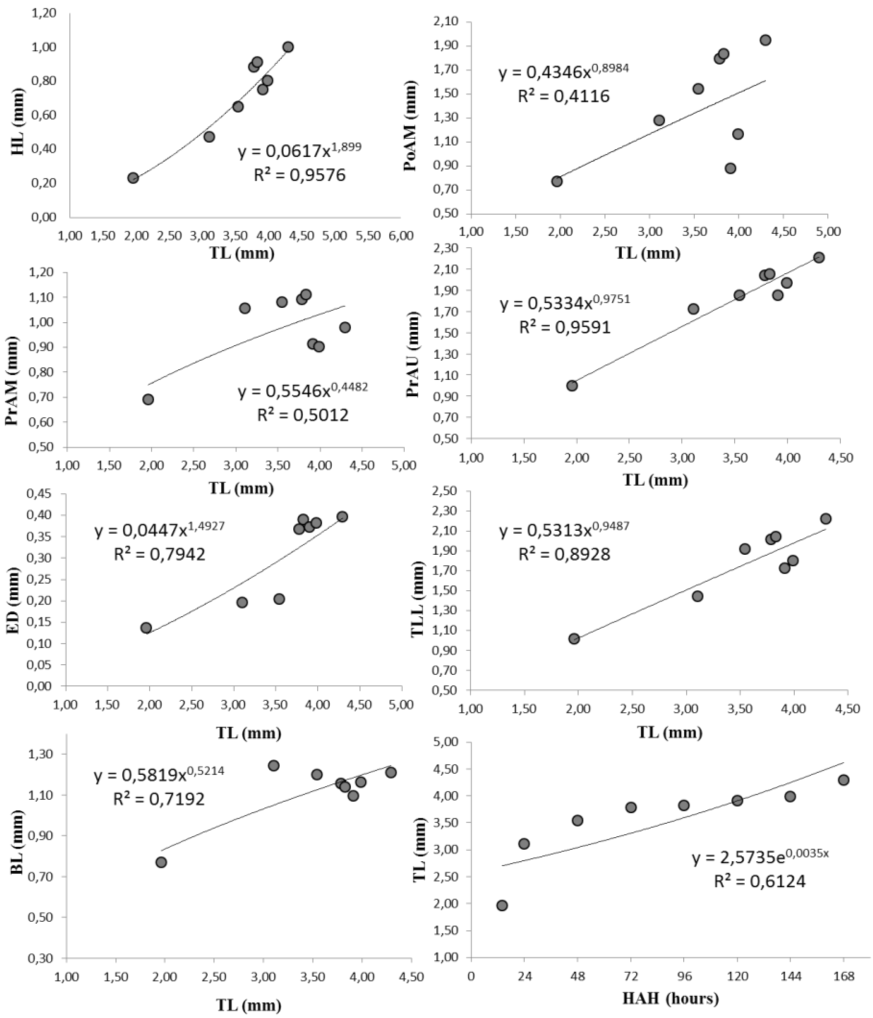


**Figure 8.** The allometric development curves of morphometric characters during the larval developmental stage (from Pre-larvae to 8 DAH) and their relationship graphs according to the total length.

## 3. DISCUSSION

Histological and microscopic methods are frequently used in larval ontogeny studies (Önal et al., 2008; Ramezani-Fard et al., 2011). These ontogeny methods are specially used for altricial fish larvae. Very limited or no enzyme activity and undeveloped digestive systems do not allow artificial feed intake. This situation necessitates the use of various zooplankton species in this stage. Especially, live baits are the highest cost in larval feeding in commercial fish culture (Person Le Ruyet et al., 1993; Önal, 2006). Therefore, determining how long zooplankton feeding will be followed by powder artificial baits depends on the mouth gap size and digestive system. So, these physiological and morphometric developments are closely related to the effective use of microscopic and histological methods in altricial fish larvae ontogeny studies.

The allometric growth pattern, which is a widely used method to analyze the early growth relationships of fish, was used in this study. (Peña and Dumas 2009, Çelik, 2011). Teleost larvae are usually described by high-level allometric growth patterns. (Osse et al., 1997; van Snik et al., 1997; Geerinck et al., 2008). These models predict growth patterns in aquaculture and fisheries management and can be used to define (Peña and Dumas, 2009). Allometric growth of fish groups in larval stages was investigated based on different families and species (Osse and van den Boogaart, 2004). However, there is almost no scientific literature on the allometric growth of commercially important tetra species. It is thought that this study will contribute to the knowledge of the determination of early-stage growth patterns of tetra species.

Tetras generally prefer to breed in soft and acidic waters. In ornamental fish production, some changes in water quality parameters (EC, temperature and pH) that will positively trigger some reproduction are required in line with the requirements of the fish species. There are lots of studies on breeding of ornamental fishes and larval development in order to determine the optimum water quality. In Amazonian discus species (*Sypmhysodon* spp.), EC 50-200 µS/cm, pH 3.9 - 5.7 and temperature 28.6 - 30.2 °C were found to have a reproductive- triggering effect (Çelik et al., 2008). The pH value is between 5.5 - 6.5, the EC value is in the range of 28 - 30 µS/cm and the using humic acid (0.04%) is important in breeding of Neon Tetra (*Paracheirodon innesi*) (Kucharczyk et al., 2010). 24±0.5ºC, 6-6.5 pH and 100-200 µS properties of water are used.for breeding Black neon tetra (*Hyphessobrycon herbertaxelrodi*) (Çelik et al., 2011). Optimum breeding values for Tiger Barb (*Puntius tetrazona*) water temperature, pH and DO have been reported as 28 ± 0.7 ºC, 7.2 ± 0.12 and 6.4 ± 0.34 mg/L (Abolhasani, et al., 2014). When Serpae tetra is evaluated (27.30±0.41 °C, EC 21.1±1.2 µS/cm, pH 6.10±0.21 and DO 7.17±0.11 mg/L), it is seen that serpae tetras prefer waters that are similar to the breeding conditions of other tetra species.

Optimum water quality values for breeding of some important ornamental species showed in Table 4 (Çelik et al., 2008; Kucharczyk et al., 2010; Çelik et al., 2011; Abolhasani, et al., 2014). When serpae tetra is evaluated (27.30±0.41 °C, EC 21.1±1.2 µS $cm^{-1}$, pH 6.10±0.21, and DO 7.17±0.11 mg $L^{-1}$), it is seen that serpae tetras prefer waters that are similar to the breeding conditions of other tetra species.

**Table 4**. Ideal water quality values of some important ornamental fish species in breeding period

| Species | Water temperature (°C) | pH | EC (µS/cm) | Hardness |
|---|---|---|---|---|
| Neon tetra | | 5.5 - 6.5 | 28 - 30 | |
| Black neon tetra | 24±0.5 | 6.0-6.5 | 100-200 | |
| Gold fish | 18-20 | 7.0-7.5 | | 90-200 |
| Koi carp | 20-22 | 7.0-7.5 | | 70-200 |
| Angel | 24-30 | 6.3-8.5 | | 70-200 |
| Gourami | 24-30 | 6.0-7.0 | | 60-100 |
| Tiger barb | 28 ± 0.7 | 7.2 ± 0.12 | | |
| Amazonian Discus | 28.6-30.2 | 3.9 - 5.7 | 50-200 | |
| Sword tail | 28-30 | 6.5-7.5 | | 80-250 |
| Platy | 28-30 | 6.5-7.5 | | 80-250 |
| Guppy | 28-30 | 6.5-7.5 | | 80-250 |
| Molly | 28-30 | 6.5-7.5 | | 80-250 |

The egg incubation time has been reported as 30-36 hours in striped gourami (Barman et al., 2013), 16 hours in serpae tetra (Park et al., 2014), 11 days in killifish (Sepil, 2020), and 3-4 days in bronze cory catfish (Huysentruyt et al., 2009). It is seen that the egg hatching time (10-11 hours at 27.8 °C, in 12-13 hours at 25.9 °C) determined in this study is similar to other tetra species. In addition, it is seen that the egg-hatching period in characins is quite short compared to other ornamental fish families. Park et al. (2014) reported that the relatively longer egg hatching time (16 hours) for serpae tetra is thought to be related to the lower water temperature used for the breeding trials.

Subsequent to the absorption of the yolk sac, in altricial fish larvae mouth opening is seen and exogenous feeding commences. When the larval metamorphosis of serpae tetra is investigated, it occures that the yolk sac is absorbed in a comparatively short time (72-75$^{th}$ hour) and commences to feed exogenously (4 DAH) in the newly hatched pre-larva compared to other ornamental fish species. In black neon tetra (*H. herbertaxelrodi*) the yolk sac is all consumed on the 5$^{th}$ day and the mean total length of the larva is round about 3.70 mm (Çelik et al., 2011). Park et al. (2014) reported the total length of the prelarva as 3.42 ± 0.38 mm on the 2$^{nd}$ day and 3.72 ± 0.24 mm on the 3$^{rd}$ day in a study about the larval development of the serpae tetra. These values are similar to the data obtained in the study (2$^{nd}$ day: 3.69±0.88 mm TL, and 3$^{rd}$ day: 3.69±0.45 mm TL).

When the mouths of Serpae tetra larvae are newly opened, the mouth gap size (198±13 µm, n:80) is quite small compared to other species. Due to the small mouth gap size in the early larval stage, they can be fed with egg yolk or rotifers. Wherefore these features, early larval losses of Serpae tetras are thought to be high.

## 4. CONCLUSION

In larval ontogeny studies, histological methods are preferred as well as microscopic examination of its development. Therefore, there are some larval histology studies similar to this study (Sarasquete et al., 1995; Monsefi et al., 2010; Santos et al., 2016). Various stages of tissue and organ differentiation were determined in histological sections that could not be visualized microscopically. The gill arch and pharyngeal cavity became prominent (at the end of $2^{th}$ day), the digestive tract differentiated into anterior and posterior intestines, and the digestive tubes and liver became prominent ($8^{th}$ day). The short duration of this period ensures the formation of a functional stomach in a short time. The fact that Serpae tetra larvae can be fed with powdered artificial feed in a short time compared to many other ornamental fish larvae is very important in terms of reducing the cost of aquaculture and larval losses.

In general, serpae tetras, which are thought to be among the species with high commercial returns, can be easily preferred by hobbyists, especially in mixed-type plant aquariums due to their attractive colors. In this respect, know well the larval development stages of tetras and other ornamental species culture.

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# CHAPTER 5

## A NEW APPROACH: AQUACULTURE APPLICATIONS OF GEOTHERMAL ENERGY

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## INTRODUCTION

Aquaculture is the activity of controlled and semi-controlled production of aquatic animals (fish, crustaceans, mollusks and arthropods) and aquatic plants (algae) under specific conditions, with the aim of increasing production quantity for human consumption, stock enhancement, ornamental, recreational and scientific purposes. It is currently the fastest-growing sector in the world's food industry and serves as a fundamental industry that meets a significant portion of global food demand. According to FAO (Food and Agriculture Organization) statistics, in 2020, global aquaculture production reached approximately 87.5 million tons, accounting for 57.28% of the total fish production (FAO, 2022). Aquaculture production in 2022 reached 514,805 tons in Türkiye (TUIK, 2023).

In aquaculture research is conducted to explore new practices to improve the production of existing species or cultivate alternative species. One of these practices is the utilization of geothermal energy sources. The temperature requirements for aquaculture applications are typically lower than those required for other geothermal applications. Therefore, many low-temperature geothermal sources remain underutilized.

The most crucial factor in the growth of cultured organisms is water temperature. When the water temperature falls below optimal levels, the organism's feeding capacity decreases, and its growth rate slows down. If the water temperature drops below the optimum level, the basic body metabolism of the fish is affected, making it unable to feed (Johnson, 1981). Geothermal energy provides a stable temperature, creating a natural and mild climate. External air temperature generally affects aquatic organisms more than terrestrial animals. Therefore, geothermal energy has more potential for use in aquaculture than in small-scale animal husbandry. The use of geothermal resources in aquaculture helps maintain water temperature at optimal levels, resulting in faster and more abundant product yields during the developmental period of the organisms (Barbier and Fanelli, 1977).

Temperature is a critical water quality parameter in aquaculture, along with dissolved oxygen, nitrogen compounds, pH, carbon dioxide, and salinity. Geothermal fluids contain high levels of chemicals, gases, dissolved solids and salts, which may not be directly suitable for use in the cultivation of all aquatic organisms. Particularly, depending on the system from which hot waters emerge and the geological rocks that control the hot waters, toxic metals may be present in significant amounts (Şimşek et al., 2005). These highly toxic elements can affect both the organisms and the people who consume them as a food source, which may hinder the direct use of hot waters. Instead, closed-loop systems can be used to transfer the energy of geothermal fluids to clean water in order to maintain the continuity of water temperature. In this case, the use of an exchanger system is necessary (Berdondini et al., 1995).

## 1. WHY GEOTHERMAL ENERGY SOURCES?

Geothermal energy is the heat energy that is continuously generated as a result of the large amount of energy accumulated within the Earth's crust, and it surfaces due to anomalies at various depths in the Earth's crust. Geothermal energy sources are defined as hot water and steam with temperatures consistently above regional atmospheric average temperatures, and they may contain more dissolved minerals, various salts, and gases than the surrounding underground and surface waters (Figure 1).

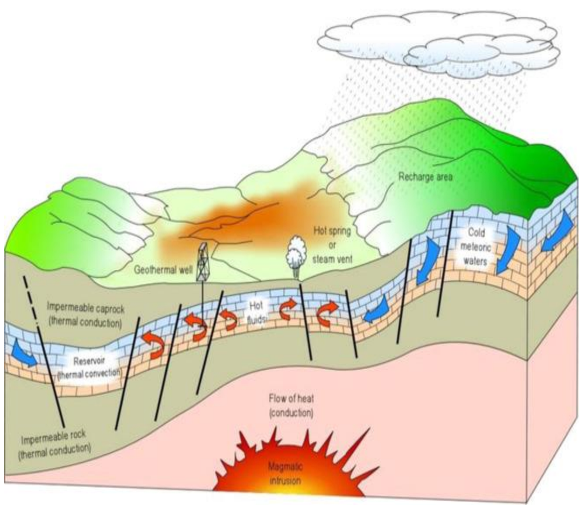


**Figure 1.** Schematic representation of an ideal geothermal system (Gonzalez and Garcia, 2018)

According to MTA's research, Türkiye ranks first in Europe and seventh globally in terms of geothermal heat potential, with a total potential of 40,000 MWt. Türkiye is among the top five countries in the world in terms of geothermal heat utilization and currently utilizes 3,488 MWt of the available resources for direct heat production (Taşkıran, 2023). Geothermal resources are classified into low, medium, and high enthalpy resources based on the temperature of the resource fluid. In Türkiye, resources with temperatures between 20°C and 70°C are considered low enthalpy, those with temperatures between 70°C and 150°C are classified as medium enthalpy, and resources with temperatures exceeding 150°C are regarded as high enthalpy resources (Kahraman, 2022).

Geothermal resources are used for various purposes, including electricity generation, space heating, air conditioning, greenhouse heating, and more industrial applications, such as process heat and drying production of chemicals and minerals, carbon dioxide, fertilizers, lithium, heavy water, hydrogen, etc., spa and thermal tourism, aquaculture(30°C) and drinking mineral water (Figure 2).

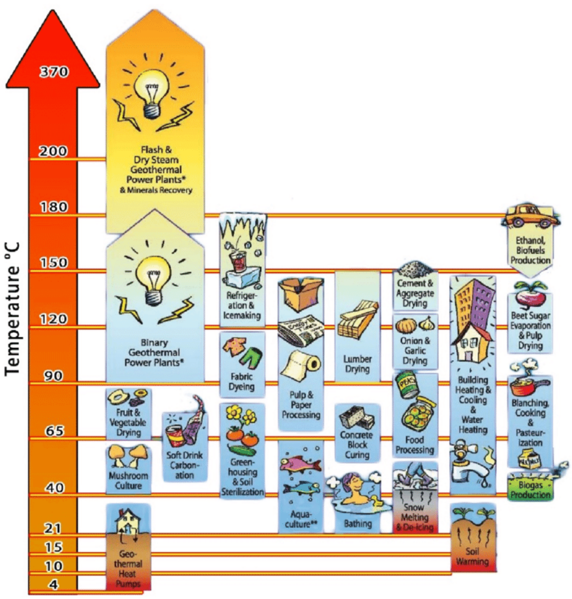


**Figure 2.** The Lindal Diagram (Climo et al., 2016)

Geothermal hot water is utilized in aquaculture to achieve the necessary temperatures by either blending it with cooler freshwater or employing heat exchangers. This application of geothermal energy extends aquaculture activities into regions with cold climates or where other heating sources are either unavailable or expensive. It not only shields the stock from adverse weather conditions but also enhances production rates. Additionally, geothermal heating is often a cost-effective alternative, ultimately leading to increased economic returns. Various aquaculture species are commonly raised, as shown in Figure 3. The use of geothermal heating in aquaculture is a well-established technology that is rapidly expanding in numerous countries, including the USA, France, Iceland, Greece, and New Zealand (Islam et al., 2022)

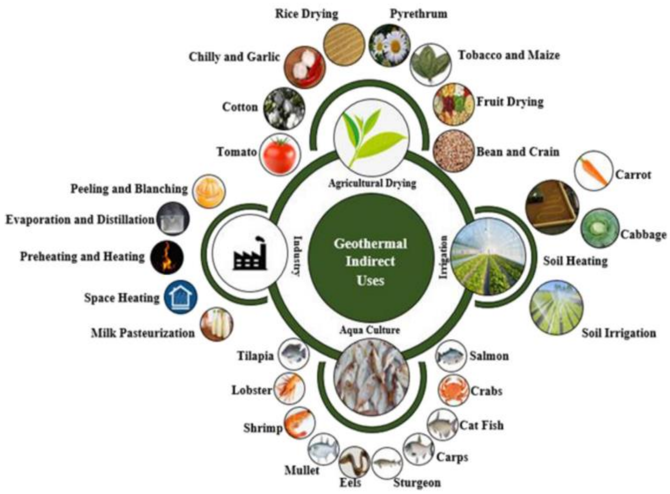


**Figure 3.** Direct means of geothermal energy utilization (Islam et al., 2022)

## 2. AQUACULTURE APPLICATIONS IN GEOTHERMAL ENERGY IN TÜRKİYE AND WORLDWIDE

Direct usage for utilizing geothermal fluids in aquaculture is one of the most used methods (Lienau et al., 1994; Borghetti et al., 2005). This method involves heating aquaculture ponds and channels, worldwide use of geothermal energy for aquaculture is 950 MWt in installed capacity and the energy use 13,573 TJ/yr. Geothermal-heated aquaculture facilities are used in twenty-one countries report this type of use, the main ones in terms of annual energy use being China, United States, Iceland, Italy and Israel – the same countries reported in WGC2015, accounting for 92 % of the annual use. Tilapia, salmon, bass and trout seem the most common species cultivated, but tropical fish, lobsters, shrimp and prawns, as well as alligators, are also being farmed. Based on work in the United States, it is estimated that 0.242 TJ/yr/tonne of fish (bass and tilapia) are required using geothermal water in uncovered ponds. Thus, the reported energy use of 13,573 TJ/yr. represents an estimated equivalent of 56,087 tonnes of annual production, representing a 13.5 % increase over 2015. It should be noted that if the fish are raised in covered ponds, say by a greenhouse, the energy requirements would be about half. The literature on the use of geothermal fluids in aquaculture applications mainly contains examples from the United States. The U.S. utilizes 35% of its current resources for aquaculture. The country has 58 farms using geothermal energy, with these facilities primarily located in states like Idaho, Utah, California and Oregon. The Geo-Heat center in Oregon is responsible for developing necessary projects and conducting feasibility studies for operators However, very few covered ponds are known to be in use (Lund and Toth, 2021).

Low-temperature geothermal fluids are used to prevent significant fish mortality in fishponds during extreme cold weather conditions due to freezing (Gelegenis et al., 2006). Low-temperature geothermal fluids are suitable for the direct use in aquaculture of tropical and subtropical fish species. Both low and high enthalpy geothermal fluids are suitable for the direct or indirect use in the cultivation of other species to maintain water temperature at optimum levels (Fridleifsson et al., 1995; Lund and Freestone, 2001; Lund et al., 2005; Ragnarsson, 2005).

In Imperial Valley, California, fish farming is carried out using geothermal energy in semi-closed earthen ponds. This allows for maintaining the required water temperature for optimal fish growth during the winter, resulting in the production of 4,500 tons of tilapia annually, meeting a significant portion of the region's demand (Rafferty, 1999).

In the U.S., Pacific Aquafarm conducts aquaculture practices, cultivating channel catfish. Water at 32°C is obtained from artesian geothermal wells at a rate of 380 liters per hour. This geothermal heat helps maintain a consistent water temperature, especially during the winter months, preserving thousands of dollars in annual operational earnings (Fridleifsson et al., 1995).

Geothermal fluids with temperatures ranging from 20-38°C are used to maintain a constant water temperature of 10°C in the cultivation ponds for salmon in Iceland (Fridleifsson, 2001).

At the Oregon Institute of Technology, a geothermal fluid with a temperature of 37°C, emerging from a greenhouse, was supplied to tropical fishponds. The ponds were 30 meters in length, 4 meters in width, and 1-1.4 meters in depth. They used 7 TJ/year of geothermal heat to maintain a constant temperature of 23°C. A total of 250,000 aquarium fish of 85 different species were transported to the U.S. West Coast for sale via air travel or trucks. This geothermal energy resulted in an annual profit of $100,000 (Lund, 1994).

In another study conducted in the U.S., geothermal fluid with a temperature of 36°C was used in January for the production of channel catfish. The temperature in closed pools, each with a volume of 400 $m^3$, was raised from 10°C to 27°C. For 40 days, a total of 61.6 x $10^3$ kJ (17.1 x $10^4$ kWh) of energy per pool was calculated to achieve and maintain this increase. The cost of diesel fuel for three and a half months was $6,388, while geothermal energy cost $3,432 (Lamoureux, 2003).

Low-enthalpy geothermal water was used to prevent freezing in fish traps during the winter months, providing a 5°C increase in water temperature in Greece. This system resulted in a three-fold increase in the production of sea bream (*Sparus aurata*) among other species (Gelegenis et al., 2006).

Geothermal sources are rich in micro and macro elements, providing a suitable nutrient environment for various algae species. In a study conducted in Greece and Bulgaria, spirulina (blue-green algae) cultures exhibited a 5-10% increase in growth and a reduction in the cost of mineral salts. Additionally, geothermal sources were utilized for algae drying, with better results achieved in Greece based on the local resources and seasonal conditions (Lund et al., 2005).

In Slovakia, geothermal fluid with a temperature of 42°C was passed through heat exchangers to provide geothermal heat input to tanks for the cultivation of eel. A constant temperature of 24-26°C was maintained, resulting in eel reaching market size in 18-20 months (Lund et al., 1998).

Geothermal sources were used to heat low-temperature waters for aquaculture in regions rich in geothermal resources in Hungary. In particular, channel catfish were cultivated in a facility with a total volume of 1,200 $m^3$. Geothermal fluid with an average temperature of 32°C, obtained from four underground wells, was used to maintain a constant water temperature of 24°C throughout the year (FAO, 1996).

At the Middle East Aquarium ornamental fish production farm in Bergama, Türkiye, geothermal fluid with a temperature of 61°C and a flow rate of 30-35 liters per second was used to modify the temperature regime to 58/48°C. This allowed the maintenance of a required temperature of 26°C for 6,000 aquariums and 800 fiberglass pools, reducing the consumption of fuel oil by four tankers used for monthly heating (Geothermal Journal, 2007).

In China, low-temperature geothermal sources are used to heat fishponds in 300 facilities with a total area of 5.5 million $m^2$. The most cultivated species include carp, grass carp, eel, frogs, turtles and shrimp. Studies have identified 6 $m^3$ of geothermal water per kg of fish. (Tian et al., 2020)

Fish farming is conducted using geothermal energy in semi-closed earthen ponds in California. In this region, 15 fish farms use geothermal energy to maintain the necessary water temperature for optimal fish growth during the winter, resulting in the annual production of 4,500 tons of tilapia and meeting a significant portion of the region's demand (Rafferty, 1999).

In Italy, a closed-loop cultivation system with an average temperature of 110°C was created using geothermal fluid obtained from a well. This energy was transported to the system via pipes and transferred to the water using heat exchangers. The facility, with a total capacity of 350 $m^3$, produces 20 tons annually and cultivates species such as *Acipenser sp., Tilapia sp., Cyprinus carpio* and *Anguilla anguilla* (Berdondini et al., 1995).

In India, there is currently an aquaculture facility in Lampung that uses a traditional freshwater fishery that mixes natural geothermal hot water (outlet) with freshwater from a river to grow catfish. The farmer reported that fish grow better in a mixture of geothermal fluid and fresh water (Darma et al., 2020).

A study in Türkiye was conducted to examine the suitability of geothermal resources for aquaculture, focusing on the growth and survival rates of European eels (*Anguilla anguilla*, L. 1758). The study also investigated the accumulation of heavy metals in certain tissues of the fish taken from the geothermal water environment. In a closed-loop system using geothermal resources, it was found that the growth and development rates of the cultured European eels were parallel to those in similar studies conducted in the natural environment. As a result, there is no apparent problem in consuming the European eels cultured in the study area (Ferhatoglu, 2010).

## 3. RESULT AND DISCUSSION

One of the main reasons for the relatively slow development of geothermal energy is the lack of investment in this sector over the years. This is due to the absence of comprehensive geothermal energy laws, the high initial investment costs, and the absence of necessary incentive systems. Furthermore, projects and applications that involve low-enthalpy geothermal fields, such as using low-temperature waters in residential heating, greenhouse farming, and certain industries, are typically initiated by local governments or individuals rather than driven by a clear national geothermal energy policy.

In addition to environmental concerns, there is a growing global recognition of the importance of diversifying the aquaculture sector. Instead of increasing the production capacity of saturated species like salmon, key organizations such as the European Union Commission prioritize modernizing existing facilities and conducting research to enhance the variety of species produced. Diversification in the aquaculture sector is considered one of the core principles of sustainability, and countries should prioritize it in their future planning.

Fishponds and cages, especially those for warm water fish, cannot protect against the halt in fish growth due to cold winters in Türkiye. While the direct use of low-temperature geothermal fluids is suitable for the cultivation of tropical and subtropical fish species, both low and high enthalpy geothermal fluids are suitable for the cultivation of other species. Using geothermal energy in this way allows aquaculture to be conducted in colder climates or in locations where conventional heating methods are not economically feasible.

Geothermal fluids have the potential to be used for various applications in aquaculture, such as direct heating, indirect heating through heat exchangers, and even for the cultivation of algae. The rich micro and macro elements present in geothermal fluids provide a favorable nutrient environment for different species, reducing the costs associated with mineral supplements. Additionally, the use of geothermal fluids in drying algae has been demonstrated in Greece and Bulgaria.

The issue of heavy metal content in geothermal fluids can pose challenges, as these metals may not be suitable for direct use in aquaculture. However, in many cases, especially when considering the continuous threat of heavy metals in natural waters and the increased risk during the summer months, geothermal fluids can provide a more controlled and manageable source for aquaculture.

The discussion underscores the potential of geothermal energy in aquaculture, highlighting its benefits in enhancing growth, maintaining stable water temperatures, reducing operating costs, and contributing to sustainability. It also points out the need for further research, investment, and policy development to unlock the full potential of geothermal energy in this sector.

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# CHAPTER 6

## CALCULATING CARRIYING CAPACITY ON INLAND AQUACULTURE

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## INTRODUCTION

The significant increase in fish farming in recent years has led to an awareness of the effects of fish farming on the environment, as well as an increase in the importance given to sustainable aquaculture (Kaya & Pülatsu, 2017). Various methods are needed to ensure that aquaculture continues sustainably and at the same time to detect its effects on the environment. One of these methods that can be used effectively is the calculation of aquaculture carrying capacity. By calculating the aquaculture carrying capacity, it is ensured that the aquaculture continues sustainably and it becomes easier to predict the environmental impacts occurring in the aquaculture environment.

## 1. EFFECTS OF AQUACULTURE ON INLAND AQUATIC ENVIRONMENT

In areas where aquaculture farms are located, soluble and insoluble nutrients enter the receiving environment from net cages through feeds not consumed by the fish and feces and excretory products that emerge after the fish use the feed (Topçu et al. 2017). As a result of intensive fish farming, unconsumed feed, feces and excretory waste cause large amounts of organic matter to accumulate in the sediment (Ackefors and Enell, 1990). It is also known that this accumulation resulting from aquaculture can cause significant changes in the macrofauna and the chemical structure of the sediment (Hawkes, 1992).

Its negative effects on the sediment in the receiving environment where the aquaculture facilities are located can subsequently affect the water quality of the receiving environment. Changing the negative conditions occurring on the sediment takes more time than the renewal in the water column (Kaya and Pülatsu, 2017).

The impact of waste resulting from aquaculture in net cages in inland water areas can be much greater than the environmental impacts of marine aquaculture, and changes in the organic nutrient level may occur due to intensive aquaculture in dam lakes and reservoirs where aquaculture is carried out (Pulatsü and Kaya, 2016). Since the renewal period of the water mass in freshwater environments takes longer than in marine receiving environments, it is thought that the effects of nutrients derived from aquaculture may be greater in freshwater environments than in marine environments (Beveridge et al. 1997).

The environmental effects of intensive fish farming in net cages on the receiving environment vary depending on the farming method, applications, hydrographic structure of the region, aquaculture stock density, aquaculture system management, feed type used, and biological, chemical and physical properties of the receiving environment (Davies, 2000; Beveridge, 2004; Tokgöz Yayan, 2015).

Insufficient quality of the feed given and mistakes made in feeding strategies can be seen as the main pollutant effects in net cage farming systems. In order for the nutrients in the receiving environment to be considered as pollutants, it is important that the carrying capacity of the ecosystem and the water quality concentration limits determined by the standards are not exceeded. In order for aquaculture to continue sustainably, it is recommended to calculate the aquaculture carrying capacity and make aquaculture planning accordingly.

## 2. CARRYING CAPACITY AND IMPORTANCE FOR THE AQUACULTURE

The values of nutrients in the feeding environment may vary with changes in the location of the cages, the duration of culture and changes in feeding regimes. Determination of aquaculture carrying capacity is very important for both sustainable aquaculture, selection of cultivation area, and compliance with environmental standards (Ross et al. 2013). Knowing the carrying capacity, feed conversion rates and determining the aquaculture biomass to be obtained are also helpful (Collings et al. 2007). In intensive aquaculture, it can be estimated what level of phosphorus can accumulate from the feed into the environment, how much phosphorus in the system can contain and what level of aquaculture can be done (Beveridge, 1984).

In general, carrying capacity can be defined as the maximum usage levels that ensure that the environments used by both humans and living beings are renewed with the natural regenerative power of the environment and used sustainably in the long term. In terms of aquaculture, carrying capacity can be seen as the whole of ecosystem-based methods or methods that help determine the upper limits of aquaculture and prevent unavoidable changes in both natural ecosystems and social structures (Inglis et al. 2000; McKindsey et al. 2006; Ross et al. 2013).

While determining the carrying capacity, certain standards should be used for the receiving environment and its environment where the cultivation takes place. Objective methods and models should be used when determining carrying capacity. During the use of these models, samples should be taken from the receiving environment and evaluated in comparison with established national and international water quality standards. The models used must be compatible with the hydrodynamic and ecological processes of the environment (Stigebrandt, 2011).

The production level calculated in the carrying capacity can be limited to smaller areas within a water basin so that the total biomass of the water basin does not exceed the ecological carrying capacity. An example of this is net cage culture in dam lakes (Ross et al. 2010). In lakes and reservoirs, models that estimate the phosphorus load entering the aquatic environment from the intensive net cage culture environment by adapting the phosphorus mass balance model and using a small number of variables have been tested and validated (Beveridge, 1984).

The development of the aquaculture sector in inland waters by guaranteeing the sustainable use of the water body and without restricting other areas of use is the responsibility of researchers working in this field, public institutions, non-governmental organizations and aquaculture companies. One of the most important elements in determining the environmental effects of net cage aquaculture in dam lakes is the determination of the carrying capacity of the lake. Using models to estimate the environmental impact of cage culture in inland waters, or in other words, determining the carrying capacity of inland waters, is considered an important stage in the development of sustainable aquaculture (Beveridge, 1984).

It has been stated in previous studies that the phosphorus budget model can be used to determine the carrying capacity of receiving environments for intensive aquaculture in deep and shallow lakes (Dillon and Rigler, 1974).

## 3. STUDIES ON CARRYING CAPACITY IN TURKEY AND THE WORLD

Wang et al (2012) determined that 404 000 tons of carbon, 50 600 tons of nitrogen and 9400 tons of phosphorus were released annually as a result of 1 million tons of salmon production. It has been estimated that 48% of the carbon in the feed is exhaled as $CO_2$, 45% of the feed nitrogen is excreted as dissolved inorganic nitrogen, and 18% of the phosphorus in the feed is excreted as dissolved inorganic phosphorus. It has been determined that approximately 44% of the phosphorus in feed is released as particles with solid waste predominating.

Husa et al. (2014) examined the effects of aquaculture in Hardangerfjord (Norway) between 2008 and 2010, and found that chlorophyll-a values varied between 3–6 μg/L (Spring period) and 0.97- 1.15 μg/L (Summer period), while legal water quality limits were exceeded. It was found to be below. They concluded that the effect of fish farming on the water quality of the fyord region was low in periods when there was no intensive farming.

David et al. (2015) in their study, they determined the total phosphorus release as 14.8 kg per 1 ton of production, based on the results of average feed compositions, fish meat biochemicals and feed evaluation ratio in tilapia breeding farms in Southeastern Brazil.

Demir et al. (2015) determined in their study that Çat Dam Lake (Malatya) has mesotrophic properties, stated that Çat Dam Lake is suitable for trout farming according to the water quality data they obtained, and estimated that around 500 tons of trout can be grown in the lake according to its phosphorus-based carrying capacity.

In Oakley (2015) study calculated the carrying capacity of the lake in Lake Toba (North Sumatra, Indonesia) according to the Beveridge (2008) model, found the total annual allowable production amount to be 55,232 tons with the method calculated according to phosphorus, and achieved this with a better feed management model. suggested that capacity could be improved.

Kaya (2016) aimed to measure and evaluate the sediment-related environmental quality parameters of rainbow trout net cage enterprises with a total aquaculture capacity of 4000 tons in Almus Dam Lake (Tokat). It was concluded that in both months of sampling, organic matter and total nitrogen levels in the sediment of the cage stations increased by 1.23 and 1.0 times compared to the control stations.

Pulatsü and Kaya (2016) examined the environmental situation of the rainbow trout farm with a capacity of 274 tons located in Karaova Dam Lake (Kırşehir) and determined the carrying capacity values for the months of October (2015) and April (2016) according to the Beveridge (1984) Model. respectively; As 0.64 kg/m$^3$ and 3.71 kg/m$^3$, Tookwinas et al. According to the (2004) model, they determined them to be 10.91 kg/m$^3$ and 72.47 kg/m$^3$, respectively.

Ondoğan (2017) estimated the carrying capacity of Ataköy Dam Lake (Tokat) for rainbow trout cultivation in net cages according to the phosphorus loading model. When the acceptable phosphorus load is taken as 45 mg/m$^3$, the feed evaluation rate is accepted as between 1.0-1.5, and phosphorus input from other sources is not taken into account, the amount of rainbow trout farming in net cages in Ataköy Dam Lake (average depth of 5.2 m and surface area of 0.50 km$^2$) is determined as follows: It was determined as approximately 1346-2336 tons/year.

Topcu et al. (2017) in their study, the lowest phosphorus release value in Almus Dam Lake was in April at the control station selected 500 m away from the cage stations (14.58 µg/m$^2$/day), and the highest value was 31.73 µg/day at the cage station No. I in April. They determined it as m$^2$/day.

In their study, Kaya and Pülatsu (2017), determinated that Almus Reservoir (Tokat); When organic matter and total nitrogen levels were examined in the samples made in April and October 2015, in terms of total phosphorus in cage stations compared to control stations, respectively; They concluded that there was an increase of 1.23 and 1.70 times.

Ayekin et al. (2018) estimated the carrying capacity for intensive rainbow trout cultivation in net cages in Karakaya Dam Lake (Malatya). Vollenweider (1968) phosphorus concentration model was used, and Dillon and Rigler's model (1974) was used as a reference to evaluate the carrying capacity. It has been calculated that the aquaculture carrying capacity of rainbow trout in net cages in Karakaya Dam Lake may vary between 55 000-80 000 tons per year. According to the Dillon-Rigler model, it was determined that it should be at the level of 43 000 tons in July.

In the study of Yüzer (2019), in Kılıçkaya Dam Lake (Sivas), under conditions where the acceptable phosphorus load is taken as 24 $mg/m^3$, the feed conversion rate is taken as a basis between 1.5–2.0, and the phosphorus loads of other polluting sources in the environment are not taken into account, the surface area is 64.4 $km^2$ and the average depth is 64.4 $km^2$. The amount of rainbow trout aquaculture in Kılıçkaya Dam Lake, which is estimated to be approximately 16 m, was determined as 2500 thousand tons per year.

Türk (2020) in his study, in Hasanuğurlu Dam Lake (Samsun, average depth is approximately 47 m and surface area is 22.70 $km^2$), in mesh cages based on the acceptable phosphorus load between 30 and 60 $mg/m^3$ and the feed conversion rate between 1.0-2.0. The maximum carrying capacity for rainbow trout farming has been determined as 34,000 tons/year.

Keskin (2020) found the average depth of Süreyyabey Dam Lake (Yozgat) as 31.6 and the area of the lake as 41.34 $m^2$, and the acceptable phosphorus load was 30-60 mg/m3 when the feed evaluation ratio was used between 1-2. determined the maximum aquaculture carrying capacity of the lake as 13,600 tons/year.

In their study, Law and Law (2020) determined the total nitrogen and total phosphorus levels in Bengoh Dam Lake (Malaysia) as 0.119 tons/day and 0.114 tons/day, respectively, and determined that it is a 1st class lake according to Malaysian national water quality standards, with a maximum They determined that it allowed a production of 218 cages.

In their study, Aslantürk and Çetinkaya (2021) determined the aquaculture carrying capacity of Sücüllü Dam Lake (Isparta) as 0.4 tons/year at the minimum water level (26.28 ha area and 0.57 $hm^3$ volume) according to the Dillon-Rigler method and the current at water level (82.32 ha area and 6.4 $hm^3$ volume); They calculated it as 18.94 tons/year. The average carrying capacity was found to be 9.67 tons/year.

## 4. CALCULATION OF THE CARRYING CAPACITY

Dillon and Rigler (1974), who developed Vollenweider's (1968) model regarding the total phosphorus concentration in a certain water mass, created a phosphorus loading model using the dimensions of the lake (volume and area), the renewal time of the water, the entering phosphorus load and the remaining phosphorus load in the sediment. According to this model;

$[P]= L(1-R) / z \rho$

[P] = Total phosphorus ($g/m^3$)

L = Total phosphorus accumulation ($g/m^2$/yıl)

z = avarege depth (m)

ρ = lake water renewal period (year or production period)

R = It refers to the portion of phosphorus retained by the sediment (0.45-0.55).

The phosphorus loading model developed by Dillon and Rigler (1974) was revised by Beveridge (1984) to calculate the amount of fish that can be cultured in the aquatic environment (carrying capacity), and this model is listed in steps below.

Step 1: In order to determine the carrying capacity of a lake or reservoir for intensive net cage culture, it is necessary to determine the phosphorus concentration present in the receiving environment. Dillon and Rigler (1974); Vollenweider (1976) and OECD (1982) showed that phosphorus values obtained during the spring and autumn mixing would represent the phosphorus concentration in lakes in temperate waters.

Step 2: The phosphorus input concentration [P] resulting from net cage culture in a water body is determined by the difference between the maximum acceptable phosphorus concentration for the receiving aquatic environment [Pf] and the phosphorus concentration [Pi] of the water body in its current state. Dillon and Rigler (1974) reported the acceptable phosphorus concentration [Pf] for temperate region lakes as 60 $mg/m^3$; According to Turkish legislation for eutrophic lakes, it is taken as 50 $mg/m^3$.

$\Delta[P] = [P]f – [P]i$

If the phosphorus loading model developed by Dillon and Rigler (1974) is applied to phosphorus loading resulting from aquaculture, the following model emerges:

$\Delta[P] = LB – (1\text{-}RB) / z \text{ X } \rho$

$C.C = [\Delta[P] \text{ X } z \text{ X } \rho] / [1\text{-}RB]$

Here;

C.C = Carrying capacity of lakes or reservoirs for intensive fish culture ($mg/m^2$/year)

Δ[P] = Difference between maximum acceptable phosphorus concentration [Pf] and phosphorus concentration [Pi] before the aquaculture period ($mg/m^3$)

Z = avarege depth (m) (Z = V/A; Average depth is the lake's volume (V) divided by the lake's surface area (A))

ρ = lake water renewal period (year or production period) (the waiting time of the water in the lake or the time required for the entering water to leave the lake. It is equal to the ratio of the amount of water leaving the lake in a year (Q) and the lake volume (V): ρ = Q/V)

RB = The portion of phosphorus from aquaculture retained by sediment

$RB = x+ [(1\text{-}x) R]$

$R= 1/ (1+0,515 \rho 0,551)$

The portion of the phosphorus entering the lake retained in the sediment (retention coefficient; R) is determined by determining the average phosphorus amount of the inlet and outlet waters and the average phosphorus concentration in the lake and sediment.

X= Ratio of phosphorus precipitated in sediment (0,45-0,55)

Step 3: By using the feed evaluation ratio, the amount of fish that can be grown in the receiving environment can be calculated. It is calculated based on how much of the phosphorus contained in the feed will be released into the environment, using the feed evaluation rate data obtained during the production period.

Amount of Phosphorus Released to the Environment: ($P_{env}$): ($P_{feed}$)- ($P_{fish}$)

## 5. ENVIRONMENTAL CARRYING CAPACITY

Nitrogen and phosphorus loads are critical to aquaculture carrying capacity. Knowing the amount of total nitrogen and total phosphorus in annual production is critical for sustainable cultivation. The environmental carrying capacity of the dam lake, which depends on total nitrogen and total phosphorus, is based on the model proposed by the Organization for International Economic Co-operation and Development (OECD, 1982) by Zhou et al. (2011) and is explained step by step below.

$L = qs X Cs X [1 + 2.27 (V/Q_{quit})0,586]$

$q_s = Q_{entering} / A$

$W = 0,001 X L X A$

L: Allowable load of nitrogen or phosphorus per square meter per year in receiving environments (g/m$^2$)

$Q_s$: Discharge per square meter per year in reservoirs (m)

$C_s$: TN and TP standard of surface water environmental quality (mg/L),

V: Volume of lake (m$^3$),

$Q_{quiting}$: Amount of water coming out of the lake (yearly)(m$^3$)

$Q_{entering}$: Amount of water entering the lake (yearly)(m$^3$)

A: Area of lake ($m^2$)

W: It is the environmental carrying capacity of annual or seasonal Total nitrogen or Total phosphorus.

## 6. CONCLUSION AND RECOMMENDATIONS

Calculation of carrying capacity in areas operating in the field of aquaculture or in places such as dam lakes or basins with production potential is of great importance in terms of being an indicator of both regional development and environmental impacts. In terms of sustainable aquaculture, it is recommended to use environmental carrying capacity models based on phosphorus and nitrogen in order to see to what extent the places currently in production have an impact on the environment and to use it if there is an opportunity to exceed production without harming the environment.

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# CHAPTER 7

## A VIEW ON CULTURING OF ALGAE: A GREENER STRATEGY TOWARDS SUSTAINABLE PRODUCTION ON MICROALGAE

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## INTRODUCTION

The food and nutrition! It is the most important factor in providing the energy necessary for the survival of all living species. In order to meet this need, there are various types of food that will provide nutrition from the most primitive organism to the most advanced living thing. In this life cycle, microalgae have an important nutritional value. It is estimated that the human population in the 21$^{st}$ century world will reach approximately 9.22 billion in 2075. Therefore, nutrition and healthy food varieties gain importance. Increasing knowledge of the relationship between food biochemistry and positive health shows the urgency of using food sources that are not only sustainable but also affect human health beyond basic nutrition.

Nowaday, the functional role of foods has changed from providing energy and essential nutrients alone to providing non-nutritive bioactive compounds that may protect against the development of certain diseases. Plant food groups with these dual characteristics (cereals, legumes, nuts, fruits and vegetables, algae etc.) are defined as functional foods that can be consumed as fresh foods and consumed as ingredients in minimally processed or formulated foods (Khan et al., 2018; Ampofo and Abbey, 2022). New trends in the food industry are microalgae, an underutilized group of organisms with a potential source of functional food. This situation is a contributing factor in the development of the vegan diet today. Today, a typical example of such new food is algae, specifically microalgae, a phytoplanktonic organism that contains a wide variety of bioactive compounds, including protein, phenolics, carotenoids, vitamins, and peptides (Fig. 1,3).

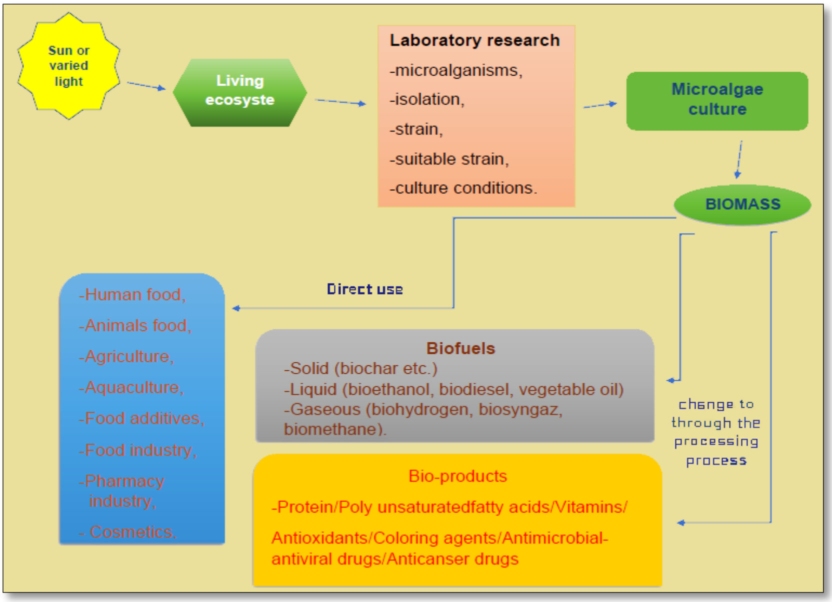


**Figure 1:** Microalgae convert atmospheric $CO_2$ to carbohydrates, lipids, and other valuable bioproducts by using light. Microalgae biomass is a rich source for food, biofuels and bioactive compounds.

Algal organisms are the world's first photosynthetic organisms to grow in a variety of aquatic environments, including terrestrial areas, ponds, lakes, rivers, sea, oceans, as well as wastewater. They can tolerate a wide range of temperatures, salinities and pH values; they can grow in different light intensities; in reservoirs or deserts and in extreme conditions and other organisms that can grow alone or in symbiosis. Research results have shown that microalgal bioactive compounds have positive health effects such as antihypertensive, anti-obesity, antioxidative, anticancer, cardiovascular protection and beneficial nutrition. Microalgae have shown potential to meet the population's need for a more sustainable food supply, particularly with regard to nutritional demand. These promising food sources offer several environmental advantages over other raw materials currently used. On the other hand, the use of microalgal biomass by the functional food industry has faced many challenges due to species diversity and differences in biomass and cultivation factors. The main difficulties encountered in the use of microalgae as functional food are attributed to inefficient bioprocess techniques, insufficient literature knowledge regarding the bioavailability and safety of microalgal bioactive compounds, and changes in functional structures due to extraction, purification, practicality and cost due to odor and taste when applied in food formulations. Despite these challenges, there are great opportunities to explore their use in the development of functional foods. Therefore, detailed research is needed to overcome these challenges to pave the way for the large-scale commercialization of microalgae-based healthy foods, which are new renewable food sources and have a rapid growth rate in nature. Microalgae show potential in meeting the population's need for a more sustainable food supply, especially with regard to nutritional demand. These promising food sources also offer several environmental advantages over other currently used raw materials. In recent times, microalgae have attracted great interest in livestock, agriculture, chemical, pharmaceutical, food, textile and many other industries due to their nutritional, therapeutic and commercial applications, important sources of food components and bioactive products with health benefits (Egardt et.al., 2013). For this reason, it is not difficult to guess that food companies, which are constantly in search of innovative products, make a significant investment. This global trend clearly demonstrates the need to monitor the quality of microalgae products often promoted as "superfoods", although this term, used as a marketing tool, has no official definition by the regulatory authority. Cost and potential for growing microalgae using nutrient-rich wastewater and harvesting performance with biocoagulants/bioflocculants: Physiology of the mechanism, multiple conversion of biomass to valuable products and future challenges are some of the issues still being investigated. The use of microalgae in new food products continues to increase every year (Fig. 2,3).

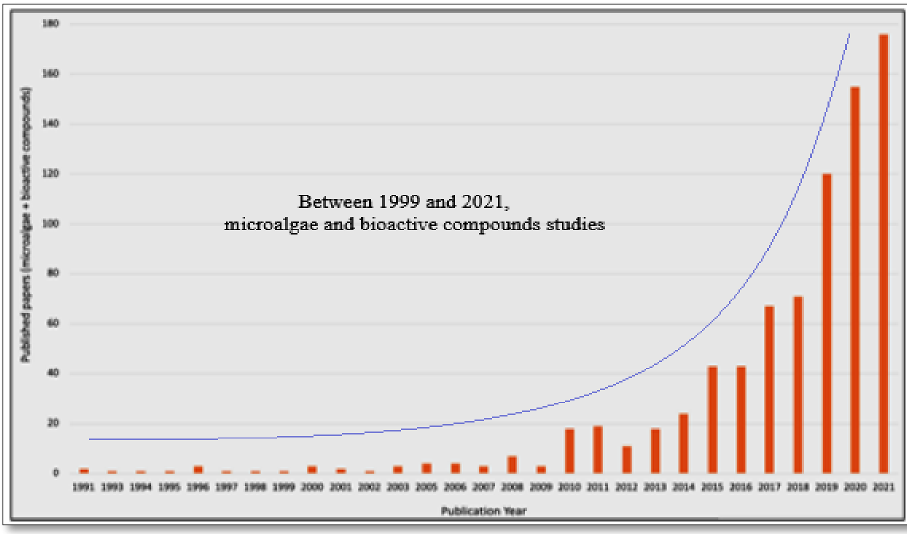


**Figure 2:** Bibliometric analysis on "microalgae and bioactive compounds" from the Web of Science database. The chart displays published research papers from January 1991 to December 2021 (Ampofo and Abbey, 2022).

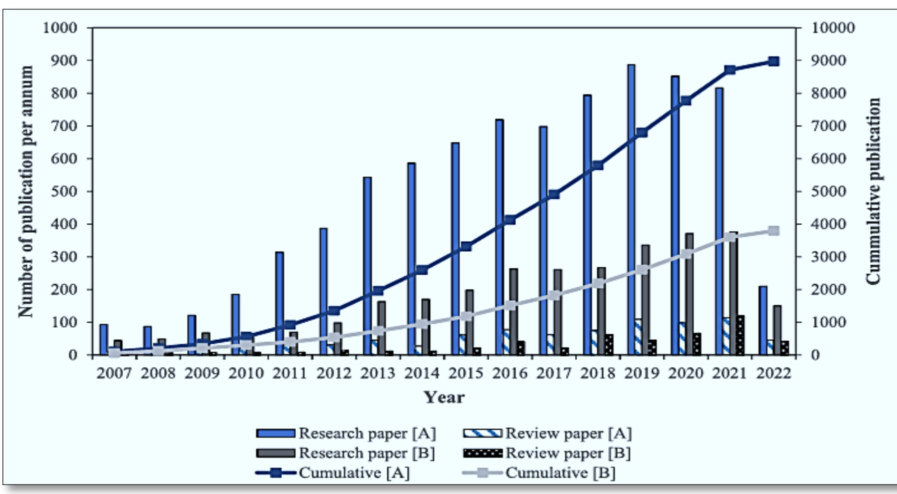


**Figure 3:** Number and type of literatüre on algae species [A] and biocoagulants/bioflocculants [B] indexed by SCOPUS in the last 15 years (Kurniawan et.al., 2022).

This review describes the use case of microalgae as an ingredient in innovative food products with potential health benefits. The focus of this section is to discuss the potential of microalgae as natural components for functional food development, factors limiting their acceptance and use in the food industry, and consumption trends for incorporating microalgae into innovative food products with potential health benefits.

## 1. WHAT ARE MICROALGAE

Microalgae, microphytes or phytoplancton are microscopic algae invisible to the naked eye. They are unicellular species which live individually, or in chains or groups. Relate to the species, their sizes can range from a few micrometers (μm) to a few hundred micrometers (Fig. 4).

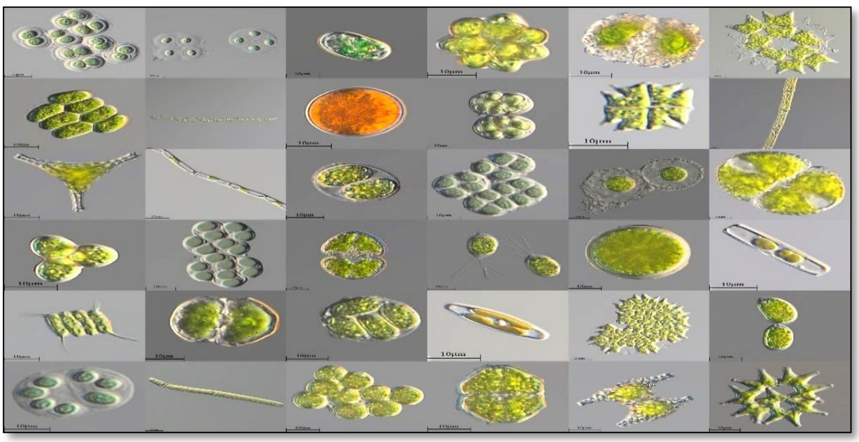

**Figure 4:** Some various species of unicellular and colonial microalgae in nature (Alexander Klepnev, 2017: Wikipedia, the free encyclopedia, 2023. https://en.wikipedia.org/wiki/Microalgae)

Algae do not represent a formal taxonomic group of organisms, but rather form a heterogeneous collection of phyla or divisions that have representatives in various kingdoms and possess many of the described characteristics. Divisions are distinguished from each other based on a combination of features, including photosynthetic pigments, starch-like (or other) reserve products, cell coat, and other aspects of cellular organization (Sheath & Wehr 2015). There is little consensus among phytologists on the exact number of algal divisions, and modern methods are likely to replace higher classification categories within the next few years. Although the systematic naming of algae changes every year as a result of the discovery of new species and increasing research, the known general systematic classification shows their classification according to their basic characteristics. In this chapter, the basic characteristics of algae in 13 main groups recognized in research are compared and given as shown in Table 1. Unlike higher plants, microalgae do not have roots, stems, or leaves system. They are specially adapted to an environment possess by viscous forces. Microalgae are photosynthetic organisms that can grow in water and extreme environments and often to use light and carbon dioxide to build biomass. Microalgae play an important role in the nutrient cycle and fixing inorganic carbon to organic molecules and denoting oxygen in the world biosphere. They are the primary producers in the food pyramid in nature (Figure 5).

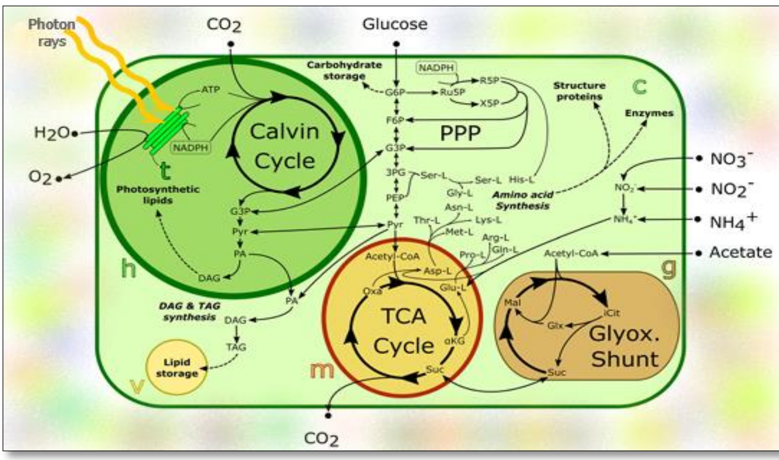


**Figure 5:** Inorganic carbon assimilation and metabolic biosynthesis through the Calvin-Benson cycle in algae in relation to biological reactions in the nature ecosystem (Vuppaladadiyam et.al., 2017).

In addition, microalgae can live in marine or brackish water and wastewater. Microalgae are photosynthetic organisms that play a key role in aquatic ecosystems. Approximately 40% of global photosynthesis is due to these microorganisms. They usually have a fast-growing rate and can reach high biomass per unit area due to simple structures. Today, as a result of the development of technology, a number of microalgae species have been identified that contain sufficient material for industrial application (Table 1) (Sheath&Wehr 2015; Lopez et.al., 2018)

**Table 1:** Major characteristics of algae in the 13 major groups.

| Main Identifying Features of Common Algal Groups | | | | | | |
|---|---|---|---|---|---|---|
| **Algal group** | **Photosynthetic Pigments** | **Chloroplast Outer Membranes** | **Thylakoid Associations** | **Starch-Like Reserve** | **External Covering** | **Flagella** |
| **Cyanobacteria** | Chl *a*, PE, PC, APC | 0 | 0 | Cyanophycean | Pepitoglycan matrices or walls | 0 |
| **Red algae** | Chl *a*, PE, PC, APC | 2 | 0 | Floridean | Walls with galactose polymer matrix | 0 |
| **Green algae** | Chl *a,b* | 2 | 2-6 | True | Cellulosic walls, scales | 0-many |
| **Euglenoid algae** | *Chl a,b* | 3 | 3 | Paramylon | Pellicle, lorica | 1-2 emergent |
| **Xanthophytes and Raphidophytes** | Chl *a,c*, diadinoxanthin, heteroxanthin, vaucheriaxanthin | 4 | 3 | Chrysolaminarin | Mostly cellulosic walls, some naked | 2 unequal |
| **Eustigmatophytes** | Chl *a*, violaxanthin, vaucheriaxanthin | 4 | 3 | Chrysolaminarin | Mostly cellulosic walls | 1-2 (unequal) |
| **Chrysophyte algae** | Chl *a,c*, fucoxanthin | 4 | 3 | Chrysolaminarin | None, scales, lorica | 2 unequel |
| **Haptophyte algae** | Chl *a, c*, fucoxanthin, diadinoxanthin | - 4 | - 3 | - Chrysolaminarin | Non-siliceous scales | 2 equal+haptonema |
| **Synurophyte algae** | Chl *a,c*, fucoxanthin | 4 | 3 | Chrysolaminarin | Siliceous scales | 2 unequal |
| **Diatoms** | Chl *a,c*, fucoxanthin, diatoxanthin, diadinoxanthin | 4 | 3 | Chrysolaminarin | Siliceous frustule | 1 (only rarely) |
| **Dinoflagellates** | Chl *a,c*, peridinin | 3 | 3 | True | Cellulosic theca | 2 unequal |
| **Cryptomonads** | Chl *a,c*, PC or PE, alloxnathin | 4 | 2 | True | Periplast | 2 equal |
| **Brown algae** | Chl *a,c*, fucoxanthin | 4 | 3 | Laminarin | Walls with alginate matrices | 2 unequal: transverse+ longitudina |

According to information obtained from various physiology textbooks: Chl=chlorophyll (green), PE=phycoerythrin (red), PC=phycocyanin (blue), APC=alophycocyanin (blue), fucoxanthin and peridinin (gold to brown).

## 1.1. Importance of Microalgae

Why are microalgae important? Historically, they were used as food in ancient civilizations such as the Aztecs (Mexico) in the American continent. Aztec society used cultures of *Arthrospira* (Spirulina) *maxima*, a Cyanophyceae, to prepare a type of cake called "Tecuitlatl". Likewise, natives to Chad used the same microalga species to prepare a foodstuff named “dihe” in Africa continent (Ahsan et al., 2008). *Nostoc flagelliforme* has been used for food for more than 2000 years in Asia continent (China). They have been named such nourishment “fa cai,” which means “vegetal hair” (Sánchez et.al., 2019). Microalgae can be a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics. They also have applications in wastewater treatment and atmospheric $CO_2$ mitigation. Microalgae produce a wide range of bioproducts, including proteins, polysaccharides, lipids, pigments, vitamins, bioactive compounds, and antioxidants. The interest in microalgae as a renewable and sustainable feedstock for biofuels production has inspired a new focus in biorefnery. Growth enhancement techniques and genetic engineering may be used to improve their potential as a future source of renewable bioproducts (Khan et.al., 2018). To summarize; As it is tried to be expressed visually in the diagram in (Fig. 6), microalgae metabolism behaves to changes in the external environment together with the changes in the intracellular environment. therefore, manipulation of culture conditions or the presence or absence of certain nutrients stimulates the biosynthesis of certain compounds (Gohara-Beirigo et.al., 2022; Tibocha-Bonilla, 2018).

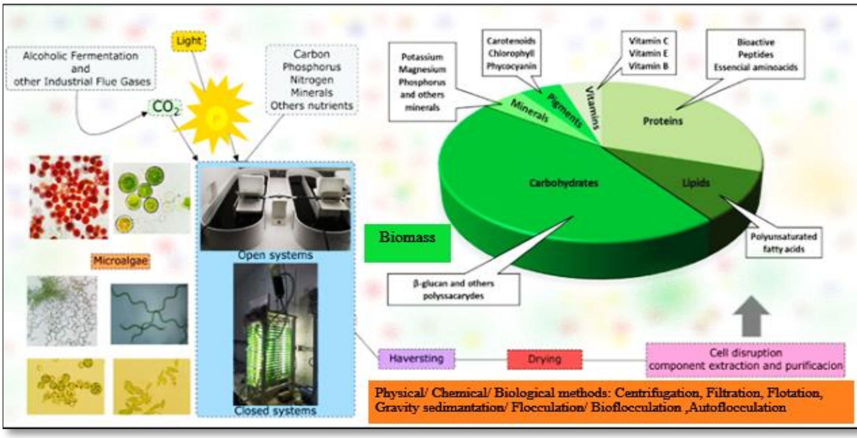


**Figure 6:** Microalgae biotechnology as sustainable source of bioactive compounds. Biomass briefly exhibits the main steps of a microalgae biotechnological course, starting from the absorption of carbonic gas, nutrients and light by microalgae cultivated in open and closed systems. The cell suspension is treated and form the microalgal biomass, rich in several biomolecules, as shown in the pie chart (Gohara-Beirigo et.al., 2022).

Different studies have been carried out not only to understand the nature of microalgae metabolism products, but also to investigate substances with possible applications to humans in different fields of use. Separation of extracts from different microalgae or isolation of metabolites is a widely used method to determine the biological activities of these components. Microalgae have been identified as rich sources of various biological compounds of commercial importance and secondary metabolism. Such compounds may exert antifungal, antiviral, antialgal, antienzymatic or antibiotic effects. Many of these compounds (cyanovirin, oleic acid, compounds like linolenic acid, palmitoleic acid, vitamin E, $B_{12}$, $\beta$-carotene, lutein, zeaxanthin, and phycocyanin) have antimicrobial antioxidant and anti-inflammatory capacities and have the potential to reduce and prevent diseases (G. de Morais et.al., 2015). Bioactive compounds in microalgae accumulate in the biomass; however, in some cases these metabolites are excreted; these are known as exometabolites. Bioactive metabolites from microalgae are of particular interest in the development of new products for the food, feed, pharmaceutical, medical, cosmetic and chemical industries. Further research should be conducted with these bioactive compounds to confirm their beneficial effects for humans, their degradability when released into the environment, and their effects when used in animals. In this context, the aim of this review is to discuss the possible applications of bioactive metabolites produced by microalgae in life sciences.

## 2. CULTURE OF MICROALGAE

Microalgae or phytoplanktonic organisms are one of the most widely known living organisms with multiple benefits, serving as feedstock in varios industrial sectors, pollutant remediation tool, phenolic compounds and a rich source of pigments. Therefore, it has led to an increased demand for algal biomass in recent years. However, the natural environment (e.g., ponds and lakes) cannot produce sufficient algal biomass. Therefore, man-made algae cultivation systems are being developed to increase microalgal biomass yield. Microalgae cultivation systems commonly used for this aim can be classified as open and closed systems. The biomass yield of microalgae species also depends on the cultivation mode of the microalgae species. This method varies depending on the genetic characteristics of the species, the characteristics of the geographical region and the physico-chemical characteristics of production. Phytoplankton can be cultured under four different conditions; autotrophic, heterotrophic, mixotrophic or photoheterotrophic microalgae, including autotrophic microalgae relatively higher biomass yield (like 60-70 t biomass ha/year) and higher oil productivity (20%–40%) when grown in open ponds (Fig. 7) (Ray et al., 2022). The autotrophic cultivation mode includes atmospheric $CO_2$ and/or industrial flue gases as carbon source under the condition of light source, while in heterotrophic conditions, an external of organic carbon source is supplied to the medium and grown without any light source. Mixotrophic production mode involves the use of an additional source of organic carbon, with the condition that the light source is used as the energy source.

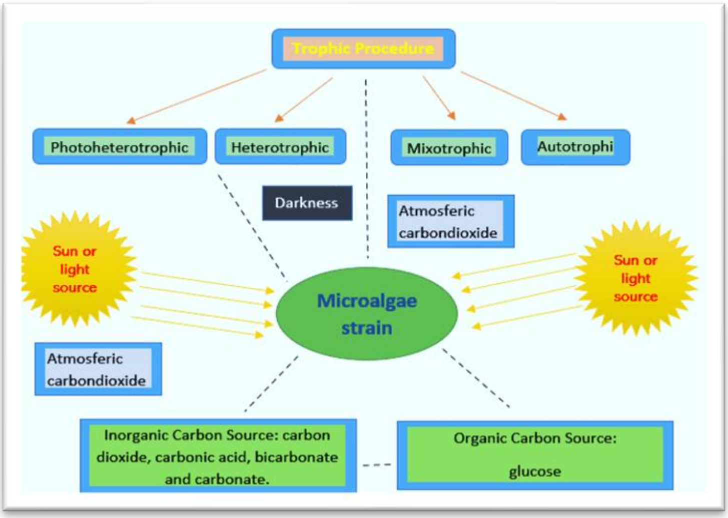


**Figure 7:** A schematic example of different trophic modes in microalgae cultivation systems in general.

## 2.1 Different cultivation techniques of microalgae.

**2.1.1. Photoautotrophic cultivation:** Autotrophic or Photoautotrophic process of cultivation is one of the earliest ways of cultivating microalgae, where the natural sunlight serves one's time as the light source and microalgae depends on atmospheric $CO_2$ for carbon-source. However, external industrial exhaust gas or flue gas can also be supplied additionally in microalgae production (Fig. 7). Autotrophic or Photoautotrophic cultivation process is one of the oldest ways of growing microalgae where natural sunlight serves as the light source and microalgae depends on atmospheric $CO_2$ as the carbon source. However, in microalgae production, industrial exhaust gas or flue gas can also be supplied from outside. In industrial production, this is an economic cost. Despite this, the most common technique for growing microalgae today is autotrophic growth. Since all microalgae are photosynthetic organisms and many microalgae are particularly efficient converters of solar energy, microalgae are grown naturally or artificially in illuminated environments. Under autotrophic cultivation, cells harvest light energy and use $CO_2$ as a carbon source. Providing sufficient natural or artificial light to allow growth in biomass and dense populations is the main goal and limiting factor of cultivation: the more appropriate light intensity and regime available to the species, the better. Therefore, open ponds that mimic the natural environments of microalgae are the most common option for mass production when implemented with other microbial communities producing economic products (Tredici, 2004). Large open pond cultivation for intensive algal production of single-cell protein, healthy food and b-carotene (Borowitzka and Borowitzka, 1989; Chisti, 2007) is one of the oldest industrial systems, used since the 1950s (Perez-Garcia, 2011). Large outdoor pools can be made of glass, plastic or similar materials. concrete, brick or rammed earth in various shapes and sizes. The most common is the oval-shaped "raceway pond" that resembles a car racing track (Pulz, 2001; Chisti, 2007). These cultivation systems offer relatively low construction and operating costs, and large ones can be built on degraded and non-agricultural land, avoiding the use of high-value land and crop production areas (Perez-Garcia, 2011; Tredici, 2004). Considering all these advantages, outdoor pools also have some disadvantages: (1) Poor light propagation indoors.

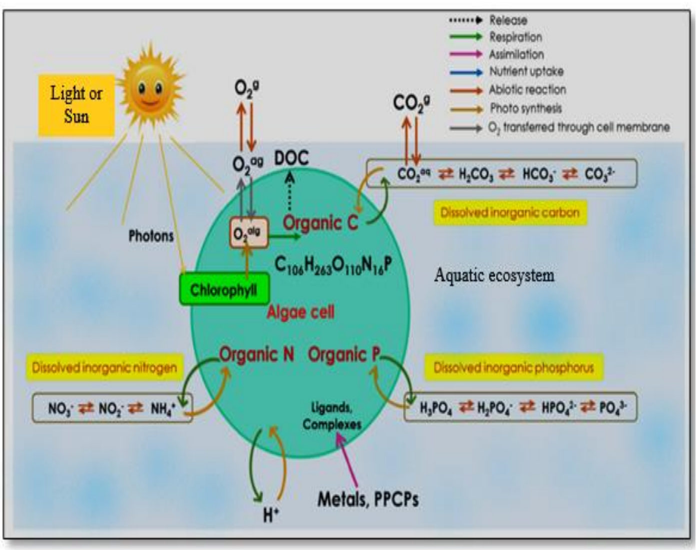


**Figure 8:** General characteristics of metabolic process in open pond (outdoor) system (Sleger et.al., 2013: Marsullo et.al., 2015)

This mode of cultivation can be general categorized into **(a)** open pond system (Fig. 8), **(b)** closed photobioreactor (PBR) system (Fig. 9), the two major cultivating methods (Andrade et.al., 2016; Dormido et.al., 2014; Marsulo et.al., 2015). In this production method, raceway pond is one of the most commonly used outdoor systems for microalgae cultivation. It consists of a series of closed-loop channels and impellers, approximately 30 cm deep, that provide recirculation of microalgae biomass to promote even distribution of nutrients and prevent sedimentation of microalgae biomass. Outdoor system may provide the ease of operation, also accessibilityof the microalgae to atmospheric $CO_2$, and introducing submerged aerators to improve $CO_2$ absorption represents a potential option for microalgae cultivation (Borowitzka, 1999; Ray et. al. 2022).

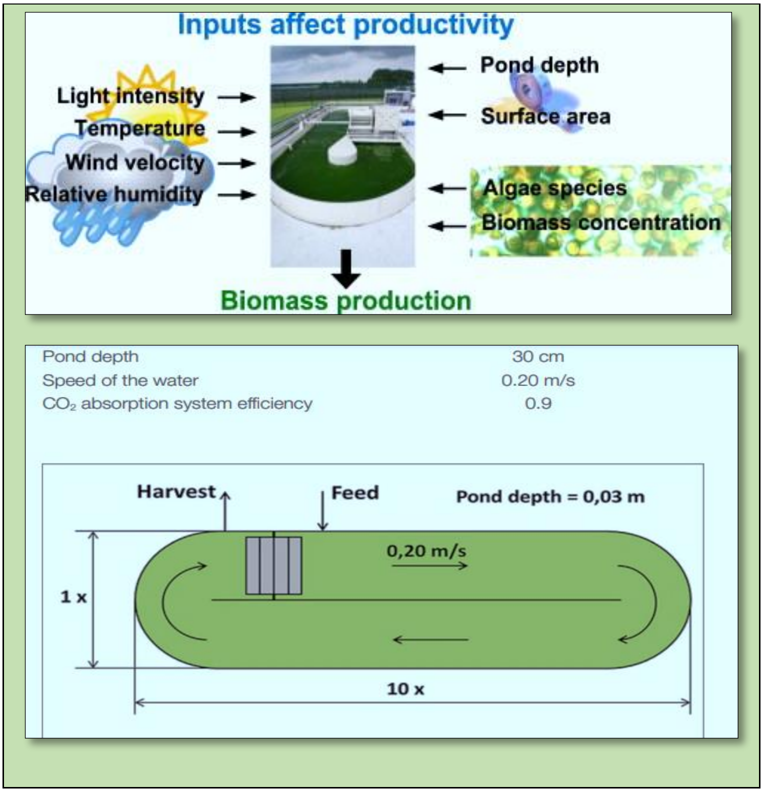


**Figure 9:** The details of biochemical processes in microalgae-based PBR (Vo et.al, 2019).

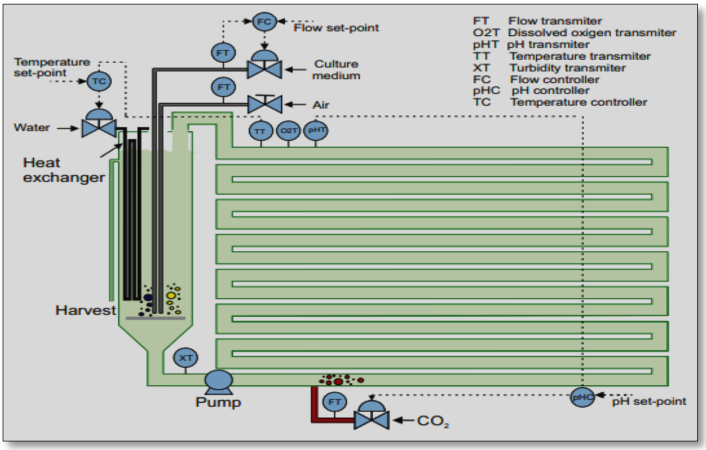


**Figure 10:** General characteristics of closed photobioreactor (PBR) system (Andrade et.al., 2016)

The advantage behind using open pond system for cultivation could be explained by the fact that it facilitates a larger contact surface area for the accessibility to light and atmospheric $CO_2$ to the microalgae, additionally submerged aerators enhance the dissolved inorganic carbon concentration in the medium and supply optimum carbon for microalgal growth. Moreover, the ease of operation is also because it does not include a complex working design and is not labor-intensive which leads to an overall lower cost of operation than that of a closed photobioreactor. Also, outdoor systems provide a larger volume for cultivation, thus high amount of biomass can be harvested at once. However, there are few drawbacks, such as tending to get contaminated frequently, suffering a huge temperature difference between day and night hours, different physicochemical environmental conditions and etc. This situation making it tedious to maintain the culture conditions (Ray et. al. 2022; Chisti, 2007). A closed photobioreactors (PBR) system was developed to cope with the limitations of the open pond system. Photobioreactors are available in different forms, where the three major classifications are plate photobioreactor, tubular photobioreactor, and vertical column photobioreactor even so, it was suggested that tubular PBR was comparatively more efficient for algal cultivation (Sánchez Mirón et al., 1999) due to higher surface to volume ratio, even though, it was established that operation and production costs of closed cultivation systems are evidently higher than open pond cultivation systems (Table 2,3).

**Table 2:** Algal biomass productivities in open pond cultivation systems (Vuppaladadiyam et.al., 2017).

| Genus/species | Production system | Cell density ($g\ L^{-1}$) | Areal productivity ($g\ m^{-2}\ day^{-1}$) | Volumetric productivity ($g\ L^{-1}\ day^{-1}$) | Solar energy conversion (%) |
|---|---|---|---|---|---|
| *Spirulina platensis* | Outdoor raceways pond | 0.9 | 12 | 0.15 | - |
| *Spirulina* sp. | Outdoor raceways pond | 1.6 | 19 | 0.32 | - |
| *Spirulina maxima* | Outdoor raceways pond | 0.5 | 14 | 0.05 | - |
| *Anabaena* sp. | Outdoor raceways pond | 0.2 | 24 | - | >2 |
| *Chlorella* sp. | Outdoor raceways pond | 40 | 32 | - | 5.4 |
| *Chlorella* sp. | Flat plate | 400 | - | 22.8 | 5.6 |
| *Chlorella sorokiniana* | Inclined tubular | - | 1.5 | 6 | - |
| *Phaeodactylum tricornutum* | Outdoor helical tubular | 75 | - | 1.4 | 15 |
| *Phaeodactylum* | Flat plate | 5 | 7.3 | 31.8 | 4.8 |
| *Haematococcus pluvialis* | Airlift tubular | 55 | - | 7 | - |
| *Haematococcus pluvialis* | Bubble column | 55 | 1.4 | 0.1 | - |
| *Porphyridium cruentum* | Airlift tubular | 200 | 3 | 1.5 | - |
| *Tetraselmis* sp. | Column | 1000 | 1.7 | 38. | 9.6 |
| *Dunaliella* sp. | Flate plate | 3 | - | 1.5 | 6.8 |

**Table 3:** General characteristics of the culture of some cultivated microalgae species (Apandi et.al., 2017; Zakaria et.al. 2017; Lafarga, 2020).

| Genus | Species | Growth phase | Protein (g/100 g) | Lipid (g/100 g) | Carbonhydrate (g/100 g) | Ash (g/100 g) |
|---|---|---|---|---|---|---|
| **Phylum: Bacillariophyta** | | | | | | |
| *Nitzschia* | *pafeacea* | Exponential | 30.2 | 23.4 | 17.1 | 24.0 |
| *Nitzschia* | *pafeacea* | Early/late | 30.3 | 27.1 | 16.1 | 23.6 |
| *Phaeodactylum* | *tricornutum* | Exponential | 38.3 | 17.1 | 19.9 | 13.9 |
| *Phaeodactylum* | *tricornutum* | Early/late | 44.5 | 17.3 | 25.7 | 11.0 |
| *Minidiscus* | *trioculatus* | Exponential | 21.9 | 19.6 | 19.3 | 34.0 |
| *Minidiscus* | *trioculatus* | Early/late | 40.9 | 27.3 | 22.3 | 22.6 |
| **Phyllum: Chlorophyta** | | | | | | |
| *Chlorella* | *vulgaris* | Exponential | 39.6 | 15.9 | 8.1 | 6.3 |
| *Chlorella* | *vulgaris* | Early/late | 20.3 | 41.7 | 51.0 | N.A |
| *Chlorella* | *pyrenoidosa* | Exponential | 50.0 | 20.0 | 15.0 | 4.45 |
| *Dunaliella* | *salina* | Exponential | 38.0 | 18.8 | 16.8 | 11.7 |
| *Dunaliella* | *salina* | Early/late | 9.9 | 9.3 | 42.8 | 11.2 |
| *Dunaliella* | *tertiolecta* | Exponential | 40.0 | 2.9 | 30.3 | 12.0 |
| *Dunaliella* | *tertiolecta* | Early/late | 45.6 | 7.9 | 9.2 | N.A |
| *Tetraselmis* | *suecica* | Exponential | 45.6 | 7.9 | 30.3 | 18.5 |
| *Tetraselmis* | *suecica* | Early/late | 25.2 | 9.8 | 9.2 | 15.2 |
| *Scenedesmus* | *sp.* | Exponential | 35.0 | 15.1 | 11.2 | 10.2 |
| **Phyllum: Cryptophyta** | | | | | | |
| *Rhodomonas* | *salina* | Exponential | 47.5 | 18.3 | 16.0 | 10.0 |
| *Cryptomonas* | *sp.* | Exponential | 47.6 | 21.1 | 4.1 | 6.0 |
| Phyllum: Cyanobacteria | | | | | | |
| *Arthrospira* | *platensis* | Exponential | 63.0 | 6.6 | 15.3 | 8.4 |
| *Arthrospira* | *platensis* | Early/late | 50.3 | 18.1 | 7.2 | N.A |
| *Arthrospira* | *maxima* | Exponential | 63.0 | 7.0 | 13.6 | 6.8 |
| *Nostoc* | *paludosum* | Exponential | 50.3 | 18.1 | 15.3 | 5.5 |
| *Nostoc* | *paludosum* | Early/late | 43.1 | 6.6 | 7.2 | N.A |
| **Phyllum: Haptophyta** | | | | | | |
| *Diacronema* | *lutheri* | Exponential | 32.6 | 11.3 | 16.4 | 6.4 |
| *Diacronema* | *lutheri* | Early/late | 26.5 | 23.4 | 21.8 | N.A |
| *Isochrysis* | *galbana* | Exponential | 34.5 | 20.8 | 14.0 | 14.8 |
| *Isochrysis* | *galbana* | Early/late | 32.6 | 25.2 | 12.8 | 12.2 |
| *Pseudoisochrysis* | *paradoxa* | Exponential | 42.4 | 23.6 | 10.5 | 10.1 |
| *Pseudoisochrysis* | *paradoxa* | Early/late | 21.2 | 33.7 | 13.7 | N.A |

Abbreviations: N.A., data not available. Data of biomass expressed on a dry weight basis.

Nevertheless, autotrophic mode requires the dependence on natural weather conditions for various culture conditions (such as temperature, light), which is challenging to maintain at a steady state; moreover, high production expenses it not the most appropriate approach for industrial-scale microalgal cultivation (Zhan et al., 2017).

## 3. INNOVATIONAL, BIOACTIVE, AND TECHNO-FUNCTIONAL INGREDIENTS DERIVED FROM MICROALGAE BIOMASS

Microalgae have been used as food, feed and fertilizer since ancient times, and today nearly 200 species are used in different sectors around the world. Today, algae are used in the production of ethanol or biodiesel, and research continues, especially on genetic engineering of microalgae, to produce them more efficiently and cheaply specially in the field of pharmaceuticals and cosmetics. However, there are currently no genetically modified microalgae on the market. Today, the global market for microalgae-based food and feed additives/nutraceuticals is highly developed and has great growth potential.

Microalgae are currently used both as dried whole algae and for the extraction of high-value food/feed supplements and colorants. on the contrary. The total production capacity and market size of food and feed supplements/nutraceuticals derived from microalgae, which are still relatively small compared to alternative sources, have increased five-fold since the turn of the century, and some microalgae applications now already have a long history. tradition. Microalgae have been used as food, feed and fertilizer since ancient times, and today nearly 200 species are used in different sectors worldwide. Today, algae are used in the production of ethanol or biodiesel, and research continues, especially on genetic engineering, to produce microalgae more efficiently and cheaply, especially in the field of medicine and cosmetics. However, there are currently no genetically modified microalgae on the market. Today, the global market for microalgae-based food and feed additives/nutraceuticals is highly developed and has great growth potential. Microalgae are currently used both as dried whole algae and for the extraction of high-value food/feed supplements and colorants. on the contrary. The total production capacity and market size of food and feed supplements/nutraceuticals derived from microalgae, which are still relatively small compared to alternative sources, have increased fivefold since the turn of the century, and some microalgae applications now have a long history (Enzing et.al., 2014). As a reality, bioactive and techno-functional components of biomass obtained from microalgae have a wide range of uses as food and raw material agents. Microalgae biomass or valuable compounds derived from it can be used as ingredients with both techno-functional and bioactive properties that can be used in the development of new foods. There are dozens of studies on this subject. The most important compounds for food applications obtained from microalgae biomass include proteins, pigments, oils and other compounds such as polyphenols with high antioxidant activity. More than 100 companies, mostly located in Australia, China, Japan, Israel, Spain, Portugal, France, Germany, the Netherlands and the United States, are currently commercializing high-value compounds from microalgae (Figure 2,3). On the other hand, algae organisms contain suitable protein/carbohydrate/lipid/vitamin/pigment etc. as large scale commercial production (Fig. 11). A resource that has an impact on food-feed safety can still be considered an industry in its infancy.

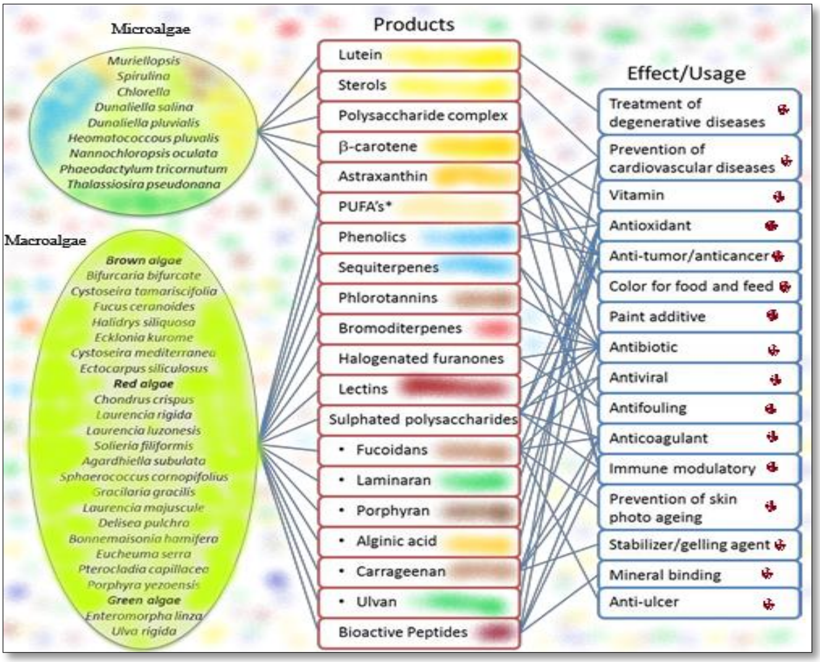


**Figure 11:** Components of secondary metabolites of some algae species and their possible application (Alassali et.al., 2013)

## 4. PROBLEMS, RECOMMENDATIONS AND FUTURE PERSPECTIVES

### 4.1. Complexity of mixing algae strategy

Bacterial-microalgae dual culture, in addition to the benefit that the co-culturing strategy provides us with, also provides us with the protection of various algae toxic metabolites, cell wall degrading enzymes, etc. ensures extracellular secretion. It brings with it many disadvantages, such as causing algal cell lysis and inhibiting algal cells. their growth. (Cole,1982; Fukami et al., 1997; Zhang et al., 2007b; Janpum et. al., 2022). Furthermore, in an industrial-scale production, both open (surface open ponds) and closed (conventional photobioreactors) systems are frequently prone to contamination. (Dias et al., 2019). For this reason, there is a need for a laboratory where stocks are stored in fully controlled, sterile environments. Therefore, industrialization of such co-culture methods involves sterilizing the grown culture system and using low-cost substrates; where such a large-scale sterilization step increases the global production cost (Dias et al., 2019).

Selection of suitable strains for binary culture is one of the biggest challenges because in the absence of symbiosis, the growth of microalgae can be negatively inhibited, leading to undesirable consequences. Moreover, to commercialize the co-culture method, the grown system will be subjected to various physico-chemical changes such as changing nutrient availability, temperature and pH fluctuations due to gradients resulting from unequal and insufficient mixing of the culture medium. It leads to a more heterogeneous environment, making these factors important limitations for the proper implementation of this hybridization strategy on an industrial scale. Therefore, developing an optimal culture medium that will support the growth of both organisms in common dual culture is another important task. Another critical point when operating large-scale bioreactors for such co-cultivation processes is that during the cultivation of obligate aerobic microorganisms (mostly yeasts) or autotrophic microorganisms such as microalgae, some oxygen or carbon dioxide depleted areas are formed (Ray et.al., 2022) and very dead. zones will trigger poor mass transfer, precipitation of biomass and thus cellular stress. Fortunately, It is possible to alleviate these depleted areas of space. Synergistic oxygen and carbon dioxide exchange between mixotrophs/autotrophs/heterotrophs in non-axenic cultures (da Silva and Reis, 2016). It is therefore important to optimize aeration and agitation rates for binary cultures at both laboratory and industrial scales; Otherwise, high aeration and mixing speed may also negatively affect the cells, causing hydrodynamic stress and cell damage (Dias et al., 2019). Moreover, designing a reactor with optimized culture parameters is a prerequisite for a successful co-cultivation process (Ray et.al., 2022). Another important aspect of this co-culture system to consider is the accessibility of the light source during co-cultivation of symbiotic (microalgae/bacteria) organisms with high cell density. Such a dense population will block the light path, inhibiting microalgal growth and lipid productivity (da Silva and Reis, 2016).

### 4.2. Recommendations of the co-cultivation strategy

Since microbial co-culture involves growing two or more microorganisms in the same closed environment, it is considered a promising strategy to induce cryptic pathways. Then, it is necessary to find the most appropriate, healthiest and least costly production process in this common culture. However, some problems arise. Different researchers in the scientific community have made different suggestions regarding this problem. Olguín (2012) stated, extrapolating data from laboratory-scale studies should be avoided in terms of biomass and lipid or any valueadded product yield and biofuel productivity. Another recommendation is to reduce the high operating cost of sterilization steps. However, at this stage, sudden contamination problems that may arise in the future will always be a problem. If the system collapses as a result of contamination, the production costs will be wasted and the cost of re-production will not be economical. In studies conducted on an industrial scale, detergent use and phenol-based disinfection/sanitization to prevent contamination culture broth. Apart from these approaches, many other cheaper methods can be applied to control contamination. Due to the photosynthetic activity of microalgae, the culture broth is subjected to an increase in pH and saturated oxygen values, which can be affected by other fungal and bacterial invasions, especially during the growth period (Ray, et. al., 2022). A different approach has also been proposed by Mata-Gómez et al. (2014) and Chagas et al. (2015) suggest that the evaluation of different microbial biomasses may be a preferable option, as various oleaginous microbial strains provide efficient yields for triacylglycerol production as well as value addition. Compounds of commercial importance, such as carotenoids and polyunsaturated fatty acids, can lead to increased revenue from the overall process and increase the economic viability of the bioprocess (Mata-Gómez et al., 2014; Chagas et al., 2015).

## 5. CONCLUSION

Microalgae are widely recognized as one of the most potential renewable energy feedstocks. Despite their many advantages, such as exponential growth per unit volume, microalgae have low biomass productivity and a costly harvesting process. Applying co-culturing techniques on microalgae with other microbes increases overall biomass production and facilitates biomass harvest through bioflocculation. Nowadays, many studies have focused on co-cultivation of microalgae in this way and found that the productivity of lipids as well as biomass and other value-added products increases, hence the potential for biofuel production, healthcare, cosmetic industries, etc. He observed that it became suitable for culture. Microalgal-based fuels and other byproducts largely depend on optimization of growth conditions, pretreatment costs and methods, efficient solvent separation, harvesting, and conversion to biofuel. In this section, common breeding strategies are discussed in detail to critically evaluate the pros and cons of their implementation in technology advancement. An attempt has also been made to give brief information about various trophic processes and their effects on various co-cultivation strategies. Systematic studies and species isolation can make great progress in economically and environmentally friendly co-cultivation of microalgae to scale up microalgae-based fuels. Moreover, it is hoped that the use of biodegradation of waste materials as the main nutrient source for co-cultivation of microalgae with bacteria and parameter optimization to achieve versatile values can make positive contributions in developing an effective algal biorefinery leading to greener and carbon-neutral energy.

Finally, two technological areas may have a dominant influence on the marketability and competition of microalgae-based metabolites, molecules, extracts and biomass in the algae industry: the possibility of transforming species to obtain modified (improved or genetic strains) microalgae with high metabolic potential and innovations in the fields of biomass: microalgae growth, harvesting and extraction new technologies on an industrial scale. In this context, Europe and especially the Euro-Atlantic Region regions have many assets that will significantly improve the microalgae bioeconomy and orientation of the current production and market model.

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# CHAPTER 8

## PHYTOREMEDIATION ECO-FRIENDLY USE OF AQUATIC PLANTS IN WASTEWATER TREATMENT

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## INTRODUCTION

Protection of the ecosystem in various water resources and wetlands is carried out in many different ways. With polluted water, our usable water is decreasing even more, and with global warming, living conditions around the world are changing. Due to rapid population growth in recent years, sustainable use of our water resources has become even more important. The need for fresh water increases every year due to water pollution and decrease in water resources in parallel with the increasing world population. Pollutants; are dissolved or suspended organic and inorganic substances from domestic or industrial sources (Lalevic et al., 2012; Gandhi et al., 2013; Gavrilescu et al., 2015; Lal, 2015). For this reason, wastewater treatment has an important place in environmental protection plans. Furthermore, wastewater treatment plays an important role in protecting environmental health (Barceló et al., 2011).

Since the 1980s, the rate of increase in water use worldwide has been around 1% per year (Aquastat, 2018). According to UNESCO (2019), global water needs for industrial and domestic use are expected to increase globally by approximately 1% annually over the next 30 years in usage levels until 2050 (Global Water Report, 2022). It estimates that this would correspond to an increase of 20% to 30% above current water demand (UNESCO, 2019; Burek et al., 2016).

The total amount of water in the world is estimated to be 1,338,000,000 $km^3$. 96.54% of it is in the oceans, 0.007% in lakes, and 0.93% in salty waters on land. 1.74% of fresh water is in glaciers and 1.69% is in underground water. Salt water constitutes approximately 97.4% of the world's total water reserves. Fresh water that can be used by plants and animals on Earth; ~2.5% (or $3.19\times10^{19}$L) of total water. It is estimated that 70% of the available water on Earth is for agriculture, 19% for industrial use and 11% for urban use (Gleick, 2000; FAO, 2015; Lal, 2015).

In countries that are not rich in water resources, the treatment and reuse of domestic and industrial wastewater provides opportunities for developing technologies, improvement, reduction of pollution and increase of clean water. People's awareness of environmental issues; It has led them to seek alternative solutions for the protection and sustainability of water resources. A clean environment and the development of new ecological technologies; It is possible to reduce the load of wastewater treatment facilities, increase treatment efficiency and recycle wastewater (Jones, et al. 2021; UNESCO, 2019).

## 1. WASTEWATER TREATMENT

Wastewater treatment; is a critical aspect of preserving environmental sustainability. In this in-depth analysis, the effectiveness of aquatic plants; It shows solutions as a natural and cost-effective solution method. These plants are unlocking the potential to revolutionize wastewater treatment processes by discovering their ability to metabolize pollutants and remove harmful substances.

The oldest system used to treat wastewater is conventional wastewater treatment plants. The construction of conventional wastewater treatment plants is quite costly. Energy prices, manpower costs, maintenance and usage costs are quite high. Green remediation (phytoremediation) technology, which is environmentally friendly, is purified by natural methods; It provides great advantages due to reasons such as low energy requirements and low-cost installation, no use of chemicals, and environmental benefits such as obtaining quality products. (USEPA 1998; Tekoğul, 2023a; Tekoğul2023b; Stefanakis et al., 2014). First, they increase the removal of pollutants such as nitrogen and phosphorus thanks to their natural absorption and uptake capabilities. Secondly, they improve water quality by facilitating the breakdown of organic matter. They also support biodiversity by providing a natural habitat for beneficial microorganisms. Finally, the use of aquatic plants is economically viable and sustainable in the long run.

**Figure 1.** Modern industrial treatment plant

1. Conventional waste water treatment systems:

There are commonly used methods to clean wastewater and purify it from harmful substances, and a few of them are the traditional wastewater treatment system as follows.

2. Physical Purification:

Physical treatment; It is a method in which wastewater is cleaned through various physical processes. These processes may include pre-treatment, sedimentation ponds, sand filters and micro screening. In this method, solids, suspensions and other large particles in the wastewater are removed.

3. Biological Treatment

Biological purification; It is a process that purifies wastewater with the help of microorganisms. The biological purification system may consist of different systems such as activated sludge, biological filters or stone beds. Microorganisms' clean wastewater by using organic materials and other harmful substances as nutrients.

4. Chemical Treatment:

Chemical purification It is a method in which chemical reagents are used to remove harmful substances in wastewater. In this method, processes such as pH adjustment, flocculation, precipitation and adsorption are performed using various chemicals. Chemical treatment is effective in removing certain contaminants, especially the removal of heavy metals.

5. Disinfection

At the end of the waste water treatment process, a process called disinfection is performed. This process is used to destroy microorganisms in wastewater. Methods such as chlorination, ultraviolet (UV) irradiation and ozonation can be used for disinfection.

These traditional wastewater treatment systems combine different processes to clean the wastewater and ensure it is discharged safely without harming the environment. However, each system has advantages and disadvantages, and the method to be applied may vary depending on wastewater characteristics, treatment requirements and local regulations.

According to the Wastewater Treatment Facilities Technical Procedures Communiqué and Water Pollution Control Regulation; The most ideal treatment systems for villages are natural treatment systems and natural treatment systems are systems created by creating artificial wetlands. Purification; It is much more convenient than traditional conventional purification systems. Reinforced concrete pools built in traditional systems create environmental problems not only with their bad appearance but also with the bad smell they emit (Figure 2). The construction and maintenance of these facilities is very costly.

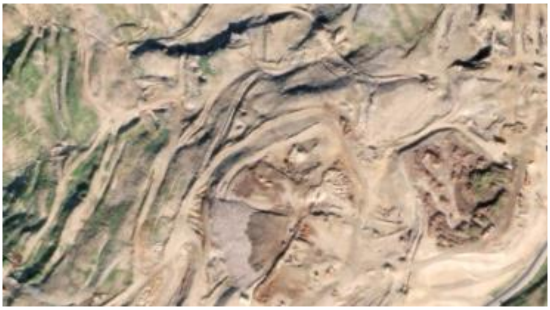

**Figure 2.** Solid waste accumulation area

Even though industrial wastes meet the standards required for facility discharge, they have negative effects on the lives of aquatic creatures.

Conventional wastewater treatment systems are insufficient in purification because they cause high energy consumption and greenhouse gas emissions. It is not a suitable method both ecologically and economically. In order not to harm the environment in waste management, the collected waste is stored in rural areas. Organic or inorganic contaminants (pesticides, solid wastes and persistent organic pollutants) can be removed from soil, sediments and water. Some of the substances stored in the field liquefy and form leachate (Figure 2). Leachate; It contains heavy metals (mercury, copper, manganese and iron) that pose a great danger to groundwater (Topal et al., 2011; Trebouet et al., 2001; Şişman-Aydın, 2022; Bhatia and Goyal 2014; Bauddh et. al., 2015). Wastewater must be completely purified before being released into the ecosystem (Paredes, 2003).

## 2. ARTIFICIAL WETLANDS AND PHYTOREMEDIATION

Various applications, large and small, have been used to purify domestic wastewater since ancient times. The most natural technology of wastewater treatment; It is carried out using self-purification processes with naturally occurring vegetation in the soil and water environment. The use of wetlands in wastewater treatment offers an alternative solution to traditional wastewater treatment facilities. While it is more economical in terms of cost, it also provides a natural cleaning process. In addition, the formation and protection of wetlands also plays an important role for biodiversity. Disposal of waste by natural methods; It has become the center of attention in recent years. There is a need to transform waste materials, especially to protect our waters. Surface and underground flow artificial wetlands, natural lagoons and aerated lagoons are used as natural treatment systems for rural areas. Among the increasing eco-living solutions in recent years, natural purification studies with aquatic plants have become even more important as they eliminate all these negativities.

**Figure 3.** Black Sea Region Network Project for Promotion of Integrated Natural Wastewater Treatment Systems WASTEnet project

Using wetlands in wastewater treatment is an important method in terms of environmental sustainability. Wetlands can be effective in cleaning wastewater since they have natural filtering and cleaning properties.

The term "wetlands" is generally used to refer to areas where water resources are located and host various plant and animal species. These areas; It contains various ecosystems such as wetland meadows, marshes, lakes, rivers and deltas. Wetlands; swamps, marshes, peatlands or areas of water, whether natural or artificial, permanent or temporary, with static or flowing water, fresh, brackish or salt; Areas, including areas of sea water, whose depth at low tide does not exceed six meters. They are extremely important for biodiversity and the water cycle. They also provide ecosystem services such as water purification, flood control, and percolation of water underground. Therefore, conservation and sustainable management of wetlands is of great importance. Wetlands are valuable for their natural beauty and ecosystem health (MacDicken, 2015).

Phytoremediation; It is the use of plants to clean polluted water and soil. Phytoremediation, which is a method where plants are used to reduce or eliminate environmental pollution, plays a very important role in aquatic environments. In this process, plants absorb and break down harmful substances and produce clean water and soil without harming the environment. They take advantage of the ability of aquatic plants to absorb harmful substances through soil, water or air. These plants absorb and metabolize or store toxic substances through their roots. Phytoremediation can be an effective solution against different types of pollution such as oil spills, heavy metal pollution, nuclear waste and other environmental problems. This method is used as a natural and sustainable environmental remediation strategy. For this purpose, the use of wetlands for wastewater treatment requires comprehensive planning and management. It is important that they are designed in accordance with the composition and treatment goals of the wastewater. Therefore, it is of great importance to get support from local experts, fisheries engineers and environmental engineers. However, there are some limitations and challenges associated with using wetlands for wastewater treatment. These include the need for specific environmental conditions, potential invasive species concerns, limited knowledge of plant requirements, and the need for appropriate maintenance and monitoring. For example; Factors such as the size of wetlands, their hydrological characteristics, that is, the flow rate and volume of water, can affect the effectiveness of wetlands. Factors such as plant selection and care requirements are taken into account. In addition, it is necessary to follow the relevant local and national legal regulations. Arrangements need to be made regarding the diversion and management of wastewater to the wetland (Koyuncu, et al., 2012).

Artificial wetlands; These are wetlands designed and created by humans to fulfill the functions of natural wetlands, improve habitats, protect water resources and mimic the natural cycle of water. Wetlands use natural processes to purify wastewater. When wastewater is directed to wetlands, it is cleaned by vegetation, microorganisms and the water layer. In this process, vegetation and microorganisms absorb and break down harmful substances found in wastewater. Additionally, thanks to the slow flow of water in wetlands, particles settle and are cleared. The use of aquatic plants to purify wastewater is the application area of phytoremediation.

## 3. ADVANTAGES OF WETLANDS IN WASTEWATER TREATMENT:

1. Natural purification processes

Wetlands allow the treatment of wastewater using natural processes, which reduces the use of chemicals and offers a more sustainable treatment method.

2. Biodiversity:

Wetlands; It is home to a rich biodiversity of various aquatic plants, birds, fish and other creatures. This is important for restoring ecosystems and protecting habitats.

3. Water retention capacity:

Wetlands absorb water from precipitation and retain water, playing an important role in flood control and protection of water resources.

4. Aesthetic and recreational value:

Wetlands are attractive places for people with their natural beauty and recreational opportunities. These are places where activities such as walking, bird watching and fishing can be done.

Planning wetlands according to wastewater treatment is very important and there are criteria to be considered:

1. Wastewater analysis:

The first step is to determine the composition and contamination levels of the wastewater. Wastewater analysis enables the detection of organic substances, nutrients, heavy metals, toxic substances and other pollutants contained in wastewater. This analysis provides data to be used in wetland design and plant selection.

2. Wetland design:

When designing a wetland for wastewater treatment; It is necessary to take into account factors such as the size, shape and water distribution of the wetland. The wetland being sufficiently large allows the processes required to completely clean the wastewater. Additionally, a suitable water distribution system should be considered to ensure water circulation.

3. Plant selection:

Selection of plants to be used in wastewater treatment is an important part of wetland design. Phytoremediation abilities and adaptability of plants should be taken into consideration. Plants function to clean wastewater by absorbing nutrients and harmful substances. The plants' growth rate, care requirements, and toxicity potential should also be evaluated.

4. Hydrological planning:

The water cycle of the wetland should be optimized through hydrological planning. Factors such as management of water resources, reaching of wastewater to the wetland, circulation of water and discharge to outside when necessary should be taken into account. This ensures the healthy functioning of the wetland and the efficiency of wastewater treatment.

5. Monitoring and maintenance:

Regular monitoring and maintenance must be carried out to ensure the proper functioning of the wetland. This includes monitoring plant health, controlling the accumulation of harmful substances and intervening when necessary. Regular cleaning and maintenance of the wetland increases the effectiveness of the treatment process.

Vegetation in artificial wetlands is of critical importance for the healthy functioning of ecosystems. Plants not only provide a visually pleasing appearance but also offer many ecological benefits. Plant selection in artificial wetlands should be made depending on the climatic conditions of the region, water quality and the purpose of the wetland. In addition, the care requirements, growth rate and adaptability of the plants should also be taken into account.

Aquatic plants; macrophytes; aquatic plants; They are plants that live in water or under the influence of water and in aquatic environments. They are aquatic plants that grow completely submerged in the water (floating) on the water surface (floating) and the stem is above water (emerging) in the varying depths of wetlands, in streams, lakes and stream banks (Tekoğul, 2022; Gettys, et.al., 2014).

Aquatic plants have been found to have the ability to detoxify organic pollutants and take up inorganic nutrients (nitrogen and phosphorus) as compounds. They function to clean wastewater by absorbing nutrients, organic matter and some harmful substances. The extraordinary abilities of macrophytes and their suitability for use in phytoremediation technologies are emphasized (Dhir, 2013; Dhir et al., 2009; Plante, 2007).

Phytoremediation abilities of aquatic plants; It may vary depending on the plant species, growing conditions and type of pollutant. More research is needed to fully understand the phytoremediation capacity of plants. In addition, appropriate plant selection should be made by taking into account the characteristics of the wetland where the plants will be used and the type of pollutants. In terms of habitat, macrophytes are classified as plants in areas under the influence of water (emers), submerged or free-floating. Some aquatic plants used in wastewater treatment: Water hyacinth, water mint, duckweed, hydrilla, reeds, sparganium, zizania, potamogeton, sagittaria, carex, chara, eichornia, typha, cyperus, pragmites, eichornia, pistia, ipomoea, salvinia, lemna, myriophyllum, scirpus, cabomba, utricularia, elodea, compsopogon, lagarosiphon, enteromorpha, vallisneria, alfalfa etc. submers plants, hornwort, nuphar lutea, emers plants such as water lily and above-water plants such as lemna are some of the aquatic macrophytes frequently used for the treatment of polluted waters. At the same time, water mint can add a pleasant smell and taste to water. Many of these plants are used in phytoremediation. The most commonly used are lemna (duckweed) and eichornia (water hyacinth) species (Patel and Kanungo, 2012; Vymazal and Kröpfelova, 2008; Haslam, 1998; Singh et al., 2012; Tekoğul, 2023a, Sharma et. al. 2015; Singh et al., 2012; Hua et al., 2012).

These aquatic plants represent just a few of the common plants used for wastewater treatment. The plants used in wastewater treatment may vary depending on the composition of the wastewater, the type of pollutants and other factors.

There are approximately 500 species of aquatic plants belonging to 50 families in Turkey's wetlands (Environment Foundation of Turkey, 1993). These plants can be used to purify different types of wastewaters, especially irrigation water, industrial wastewater and domestic wastewater. Plants absorb nutrients, nitrates, phosphates and organic substances in wastewater through roots, stems or leaves. Accordingly, they help clean wastewater while promoting the growth of aquatic plants. Additionally, the roots of aquatic plants can help precipitate and retain harmful substances. In addition, the microbial interaction of plants and the photosynthesis process can also have a positive effect on wastewater treatment.

The ability of plants to survive and grow in polluted environments is an indicator of phytoremediation efficiency. Especially in underdeveloped countries, aquatic plants have been used for many years in the removal of nitrogen and phosphate in wetlands (Tunçsiper Akça, 2011; Iamchaturapatr et al., 2007). Wastewater treatment with aquatic plants can be a natural and sustainable method. However, before application, plant selection, water quality, wastewater composition and other factors must be considered, and this method may need to be integrated with other treatment technologies (USEPA, 1998).

Artificial wetlands, one of the treatment types, can effectively remove pollutants from wastewater by utilizing the power of aquatic plants. In this way, the quality of our water resources can be guaranteed. More than 75% of facilities monitor the quality of wastewater discharges, according to Key Performance Indicators (KPIs) for water safety, and companies are moving away from polluting and water-intensive products, according to the 2022 Global Water Report.

**Figure 4.** Screened Artificial Wetland Pilot Project to Improve the Quality of Water Returning from Irrigation" by the General Directorate of Water Management of the Ministry of Agriculture and Forestry

Plant selection in wastewater treatment with aquatic plants is an important step for the success of the project. In the phytoremediation technique, aquatic plants, which are hyperaccumulator plants, are used in the treatment of wastewater. Hyperaccumulator plants; They accumulate 50 to 500 times more metal in their leaves, branches and trunks than the metal content in the soil (Clemens, 2006). Plant selection; It should be done depending on the wastewater composition, water quality, climatic conditions and the objectives of the project.

Some factors to consider when choosing plants:

1. Phytoremediation abilities:

The ability of plants to absorb and metabolize harmful substances is important. For example, some aquatic plants can remove heavy metals, nitrates or phosphates more effectively. Therefore, it is important to choose plants that are effective against specific pollutants in your wastewater.

2. Growth rate and adaptability:

The ability of plants to grow quickly can increase efficiency in wastewater treatment. At the same time, the ability of plants to adapt to various water conditions is important for the success of the project. There are plants that grow on the edges of artificial wetlands, in areas where the water is shallow. These plants must be compatible with the rise and fall of water levels. Plants that are tolerant to climatic conditions, water temperature, pH levels and water movement should be preferred. Examples include reeds, marsh roses and water hyacinths.

3. Maintenance requirements:

The care requirements of the plants should be taken into consideration. Some plants may require more maintenance and regular pruning, while others may require less maintenance. Perennial plants are preferred. These plants increase the stability of the wetland and provide permanent habitat. It is important to choose plants that are appropriate for the scale of the project and your resources. Perennials, such as trees and shrubs, provide homes and food for waterfowl and other animals.

4. Ecosystem compatibility:

It is important that the plants used in wastewater treatment are compatible with the local ecosystem. Native aquatic plants in particular may be advantageous in terms of adaptation and interaction with the local ecosystem. Choosing local plant species can contribute to the protection of the natural environment. Examples include water ivy, water daffodil, and water hyacinth. At the same time, plants that help clean the water should be chosen.

5. Toxicity and intended uses:

Some aquatic plants can be toxic to humans and other organisms. Therefore, it is important to evaluate the toxicity potential of plants and their intended use. You should make sure that it is safe for plants, especially if it will be used as irrigation water.

Artificial wetlands are aquatic ecosystems designed and created by humans and are versatile systems that provide environmental and economic benefits. However, it is a process that requires expertise to ensure design, management and sustainability. These wetlands are used for various purposes such as wastewater treatment, water management, habitat restoration and biodiversity conservation. Some features of artificial wetlands:

1. Wastewater Treatment:

Artificial wetlands are used as an effective method for wastewater treatment. Wastewater is directed to the wetland and goes through a natural purification process through plants and microorganisms. While plants absorb harmful substances and nutrients, microorganisms clean wastewater by breaking down organic substances.

2. Water Management:

Artificial wetlands are used for water management and water retention. They can prevent floods by holding water during periods of high rainfall and allow rainwater to seep into the ground. They can also be used for water resource management and water recycling purposes.

3. Habitat Restoration

Artificial wetlands play an important role in the restoration of natural habitats and conservation of biodiversity. Wetlands serve as habitat for aquatic plants, birds, fish, and other aquatic ecosystem creatures. These areas help protect threatened species and revitalize ecosystems.

4. Aesthetic and Recreational Value:

Artificial wetlands provide aesthetic value for humans and can be used for recreational activities. They can also be used as areas where activities such as water bird watching, fishing, hiking and nature excursions are carried out.

Plants get all the nutrients they need from the surrounding water, and during this process, they take in pollutants as nutrients. They synthesize it in their bodies, using it as a nutrient source for their development. Thanks to the oxygen they add to the environment, the purification process is accelerated. As the water purification technology of the century, they work as purification machines with mechanisms to remove substances from water. It has many applications in wastewater treatment due to its lower costs and higher efficiency.

They grow at least twice as much as terrestrial plants. For example, in research on aquatic plants, the use of Lemna minor is very common due to its fast growth rate (0.10-0.35 g/day) (Özkoç, 2011). The small structure and fast growth characteristics of duckweed provide advantages in toxicity tests. This also gives us information about the characteristics of other aquatic plants.

Aquatic plants act as natural purifiers in wastewater treatment. They reduce the possibility of eutrophication in rivers and lakes by absorbing excess nutrients such as nitrogen and phosphorus from the water. Aquatic plants also provide habitat, shelter, and food for aquatic organisms while acting as a physical barrier, preventing the re-entry of pollutants into the water. Its versatility and efficiency; making them a valuable component in sustainable wastewater treatment systems. It is possible to use it in purification, toxicity tests, as a fertilizer, to reduce mosquito larvae, in medical and cosmetic compositions, as an alternative feed material in aquaculture, as food, in genetic studies, in various landscaping studies, as ornaments in aquariums and small ponds. It is used to purify agricultural fertilizer waste and provides nitrate conversion.

Methane gas obtained during fermentation can be used as a renewable energy source in the energy sector. In addition, the plant itself can be used as organic fertilizer (Matache, 2022; Kop et al., 2016, Ramírez, et al., 2006;

Surrency, 1993; Hutchinson, 1975). They are used as raw material sources in pharmacy and medicine due to the valuable essential amino acids, phosphate, xanthophyll, potassium, chlorophyll-a carotene, vitamins A and B and mineral substances they contain. Aquatic plants; It is used in the treatment of antipruritic, depurative, antiscorbutic, astringent and diuretic, soporific, febrifuge, measles, colds, edema and problems encountered in urine excretion. It is used as a wash in the treatment of skin diseases, wound healing and ophthalmia (conjunctivitis) cleaning (Cantó-Pastor et al., 2015; İpek and Gültekin, 1995; Leng et al., 1995; Gülçin et al., 2010; Bolotova, 2015) .

Aquatic plants with high nutritional values have metabolically active tissues. High growth rate, high protein content, ability to purify dirty water, some species show superior endurance in brackish waters, ability to absorb dissolved nutrients and solids in water, low cellulose amounts. It is a plant rich in β-carotene and xanthophyll, containing high amounts of pigment and mineral substances. It has been determined that it has 10 times more carotenoids than land plants (Skillocorn et al., 1993). It contains dry matter between 6-8% of its average fresh weight. 92-94% of it is water. They grow successfully in wastewater and convert degradable pollutants into biomass rich in proteins and amino acids(Verma and Suthar, 2014). They can be used as an application area for education. Moreover, it creates beautiful areas with flowers.

Purified effluent can be used for irrigation purposes. Since the plants produced contain high levels of protein (6-45%), lysine, methionine and carotenoids, they are used as animal feed and high-quality meat yield is obtained. (Men, 1997; Ahn and Preston, 1997, Leng et al., 1995; İpek and Gültekin, 1995).

Today, they also play a role in reducing carbon dioxide emissions. If we reduce the fact that 70% of the oxygen source on Earth is made by aquatic plant creatures, we can include them in the game at this stage.

These plants are of great importance for bioremediation studies due to their ability to absorb nutrients. Phytoremediation; In wastewater treatment, environmentally appropriate and low-cost treatment using macrophytes and/or microalgae in water has attracted great attention. (Brix and Schierup, 1989; Dipu et al., 2011)

Phytoremediation refers to the use of plants to reduce or remove water pollution. Plants used in the phytoremediation process carry out the cleaning process by absorbing or breaking down various pollutants, which have the ability to absorb harmful substances in water.

Here are some common aquatic phytoremediation plants: Duckweed (*Lemna minor*), Calamus (*Phragmites australis*), Water Mint (Mentha), Water Hyacinth (Typha), Rice (*Oryza sativa*), ceratophylum demersum. The phytoremediation abilities of different aquatic plants are given in Table 1. (Information was also taken from the table 'Mohebi and Nazari, 2014).

It cleans water by absorbing high amounts of nutrients such as nitrogen and phosphorus. It cleans water by absorbing pollutants and prevents soil erosion.

These plants are just a few examples of common plants used in the phytoremediation process. The most effective plant species for each pollutant may vary and selection should be made depending on the needs of the project.

These plants can help reduce water pollution, but the application of phytoremediation methods may vary depending on the specific conditions and type of pollutants.

Waste treatment management; Developing systems for purifying and recycling water. It is easier and simpler to maintain than conventional systems.

The biotechnological solution to wastewater involves the creation of artificial wetlands that will act as natural filters. When a suitable area for artificial wetlands is found, the biological process suitable for wastewater treatment begins. These systems are marvels of engineering.

Artificial wetlands are the most economical. These systems have been used for the treatment of industrial wastewater for the last twenty years. Early applications in purification were in the meat, dairy, petrochemical, paper, textile, wine, vinegar and aquaculture industries (Temel, 2017). Plants use it as filter material. And in this case, the use of aquatic plants appears as a natural and cost-effective solution.

The use of artificial wetlands in our country has started experimentally. Applications were supported through projects. The General Directorate of Rural Services initiated practices for rural settlements in 43 villages in 2003. Studies continue in 174 villages (Eremektar, et al., 2005). Some of the artificial wetlands currently in use in our country are;

Ankara- Haymana-Dikilitaş Köyü Artificial Wetland Facility

Ankara- Kazan- Orhaniye Artificial Wetland Facility

Şanlıurfa–Viranşehir Artificial Wetland Facility

Mersin–Toroslar- Musalı Köyü Artificial Wetland Facility

İstanbul- Paşaköy Artificial Wetland Facility Built Within the Treatment Plant

İzmir-Torbalı-Korucuk and Çakırbeyli Villages Artificial Wetland Facilities

Manisa-Akhisar-Sakarkaya Village Artificial Wetland Facility

Manisa-Saruhanlı-Yeni Osmaniye Village Artificial Wetland Facility

Konya- Meram- Karaağaç and Kızılören Artificial Wetland Facilities

Aksaray-Yenikent Artificial Wetland Facility

Isparta–Eğridir–Ağılköy Artificial Wetland Facility

Kazımkarabekir (Karaman) Artificial Wetland Treatment Plant (Yiğit and Kayranlı, 2022).

**Table 1**. Different aquatic plants' phytoremediation capabilities

| Aquatic Plant | | Pollutant | Wastewater type | References |
|---|---|---|---|---|
| Common Name | Scientific Name | | | |
| Mosquito Fern | *Azolla pinnata* R.BR | Cr, Hg and Cd | Sewage waterfor | Rai 2008, Rai,2010 |
| | | | Purification of coal mine waters | Juwarkar and Jambhulkar, 2008 |
| Water hyacinth | *Eichhornia crassipes* (Mart.) Solms | BOD, COD, Fe, Zn, Ni, Oil, grease. DO, NH3-N, TSS, EC, Salinity, TDS and NH4 | Industrial wastewater | Odjegba and Fasidi 2007; Rezania, S., et, al., 2016 |
| | | | Domestic wastewater | Patel and Kanungo. 2012; Priyanka saha et al. 2017 |
| Water lettuce | *Pistia stratiotes* | Cd, Zn, Ni, Pb, Cu, NO2, Ag, Cr and Hg., BOD, COD | Industrial wastewater | Odjegba and Fasidi 2004; Reddy et al. 2015; Lu et al. 2018 |
| Narrow-leaf cat-tail | *Typha angustifolia* L. | BOD, COD, color, TDS Textile wastewater | Textile wastewater | Chandanshiet al. 2020; Al-Baldawi. 2018 |
| Common duckweed | *Lemna minor* | BOT, Cl-, $SO_4^{2-}$, BOD, COD, TDS, NH3-N, NO3-N, PO4-P, Cu, Ti, Pb, As, Hg, Cr, Co and Zn | Industrial wastewater | Sood et al. 2012; |
| | | | Textile wastewater | Saha et al. 2017; Balarak et al., 2015, Khan, et al., 2022 |
| | | | Domestic and Municipal wastewater, | Tekoğul 2023a |
| Duckweed | *Spirodela polyrrhiza* | Pb, Cr,Zn, Cd, NH3-N, NO3-N, | Heavy Metals | Sharma and Gaur, 1995; Topal et al., 2013 |
| Swollen Duckweed | *Lemna gibba* L. | Pb, Cr, Cd, Co ,Zn, NH3-N, NO3-N, PO4-P, Cu, Pb and Zn | Heavy Metals from Aqueous Solutions, Purification of coal mine waters | Abdallah 2012; Megateli et al. 2009, Juwarkar and Jambhulkar, 2008, Topal et. al., 2013 |
| Water spinach | *Ipomeo aquatica* | COD, TDS, Nitrate, NH3-N, Phosphorous, Ni, Pb, Cd | Palm oil mill effluent, | Kumar et al. 2013; Sa'at and Zaman. 2017 |
| | | | textile wastewater | Chandanshive et al.2016 |
| Manyflower Marshpennywort | *Hydrocotyle umbellata* L. | Cr, Zn, Na and Cu | Rhizofilteration of heavy metals | Bareen and Khilji 2008 |
| Parrot-feather | *Myriophyllum aquaticum* | BOT, Cl- | River water | Coetzee et al., 2011; Mustafa and Hayder. 2020 |
| Manyflower Marshpennywort | *Hydrocotyle umbellata* L. | Cr, Zn, Na and Cu | Rhizofilteration of heavy metals | Bareen and Khilji 2008 |
| Coon's Tail | *Ceratophyllum demersum* | N, P, Pb and Cr | Fish pond wastewater, | Abdallah. 2012; Matache et al. 2013 |

| | | | | |
|---|---|---|---|---|
| | | | Municipal wastewater | Tekoğul 2023b |
| Floating fern | *Salvinia natans* | BOD, COD, NH4-N | Raw wastewater | Dhir et al. 2008; Ayache and Boudehane.2019 |
| Vetiver | *Vertiveria zizaniodes* | NH3, NO2, NH4, PO4 | Fish pond wastewater | Yeboah et al. 2015; Effendi et al. 2020 |
| Spiked water-milfoil | *Myriophyllum spicatum* | COD, TN, TP, NH4-N | Polluted rural river water | Milojković et al. 2016; Saleh et al. 2019 |
| Bulrush | *Typha orientalis* | BOD, Na, TOC, turbidity, NO2 | Municipal wastewater | Oladejo et al. 2015; Di Cesare et al. 2020 |
| Water-thymes | *Hydrilla verticillata* | BOD, COD, TSS, TP | Secondary domestic wastewater | Patel and Kanungo. 2012; Abu Bakar et al. 2013 |
| Broadleaf Cattail | *Typha latifolia L.* | Cu, Ni, Fe, Mn Ca, Cr, Pb and Zn | toxic elements in the largest coastal wetland | Haghnazar et al., 2023 |
| | | | | |

1. Water hyacinth (*Nymphaea* spp.): Water hyacinth is a plant commonly found in aquatic environments. Thanks to its high growth rate and large leaf surface, it can be effective in absorbing harmful substances. It can adsorb heavy metals and organic pollutants to the leaf surface on water.

2. Reed (*Phragmites* spp.): Reed is a type of plant commonly found in wetlands. It can absorb harmful substances thanks to its hairy structure on its roots and stems. The phytoremediation abilities of reed include the sorption of heavy metals and the degradation of organic pollutants.

3. Water ivy (*Hydrilla verticillata*): Water ivy is a plant that spreads rapidly in aquatic environments. Thanks to its high growth rate and dense root system, it can be effective in the absorption of harmful substances. It can reduce the nutrient load of water by absorbing nutrients, especially nitrogen and phosphorus.

4. Salvinia (*Salvinia molesta*): Salvinia is a type of plant that floats on water. Thanks to its dense feather structure, it can absorb harmful substances. It may be effective in the absorption of heavy metals and the breakdown of organic pollutants.

5. Watercress (*Lemna* spp.): Watercress are small plants that float on the surface of water. Thanks to its high growth rate, it can quickly absorb harmful substances. It may be effective in the absorption of heavy metals and the uptake of nutrients.

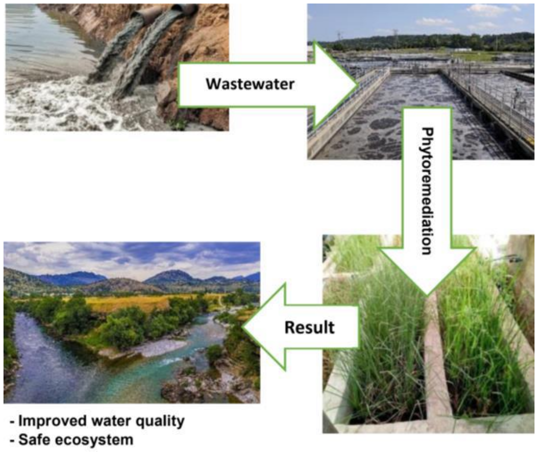


**Figure 5**. Phytoremediation by plants for refining and absorbing metals in wastewater (Mohebi, and Nazari, 2014).

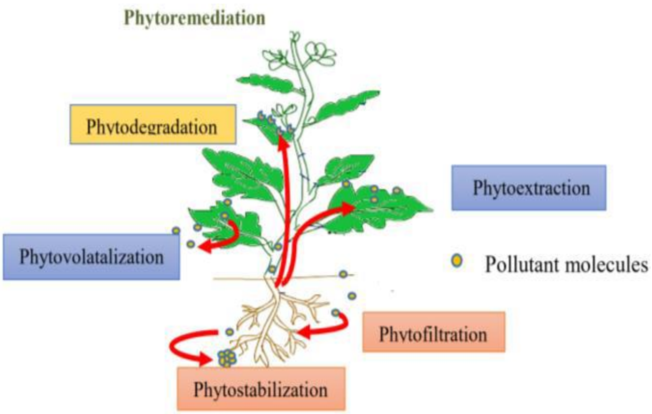


**Figure 6.** Aquatic plant treatment mechanisms of treat wastewater (Mohebi and Nazari, 2014)

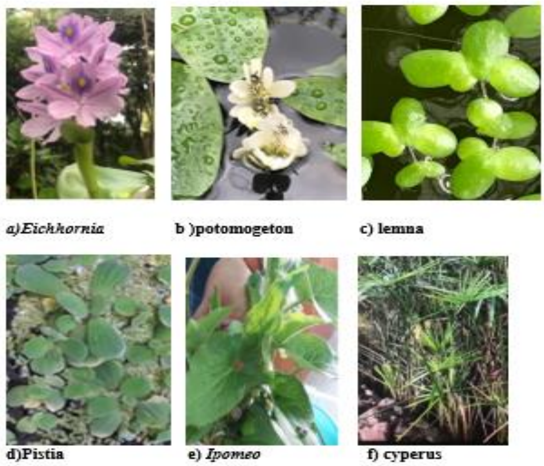


**Figure 7**. Some of the aquatic plants utilized for phytoremediation of water and wastewater (original)

By taking advantage of these natural purification processes, we can effectively treat wastewater and reduce its environmental impact. The maintenance of the plants we use is incomparably lower in cost than the maintenance of the facility itself. Since ancient times, farmers in low-income countries have been collecting their own waste in small ponds and using aquatic plants. This process not only protects public health, but also preserves aquatic ecosystems and promotes sustainable water management practices.

Studies on the successful application of aquatic plants in wastewater treatment demonstrate their effectiveness in improving water quality and reducing pollution. Will be able to perform environmentally friendly treatment by making use of nature's cleaners. By taking advantage of these plants' natural abilities to filter and clean water, we can effectively remove pollutants and improve our environment. These studies have demonstrated the ability of certain plant species to effectively remove contaminants, improve water quality, and increase the overall efficiency of treatment systems. This sustainable and environmentally friendly solution is a testament to the power of bioengineering in solving our water pollution problems. By implementing innovative solutions, we can gain valuable information about the potential applications and benefits of using aquatic plants in wastewater treatment.

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# CHAPTER 9

## CELL CULTURE AND APPLICATION IN AQUATIC VIRAL DISEASES (RTG CELL LINE EXAMPLE)

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## INTRODUCTION

Aquaculture diseases can cause the spread of many viral, bacterial and parasitic diseases affecting marine and freshwater organisms. These diseases can affect aquaculture industries, damage natural fish populations and affect food safety.

If we look at the situation of aquaculture diseases worldwide, Viral Diseases: Many viral diseases pose a serious threat to aquaculture industries. For example, Infectious Salmon Anemia Virus (ISAV), which is the causative agent of the chicken cockroach, is a serious problem affecting the salmon farming industry. In addition, viruses such as Koi herpesvirus (KHV) and Viral Hemorrhagic Septicemia Virus (VHSV) can also adversely affect commercial aquaculture. Bacterial Diseases: Bacteria such as Vibrio, Aeromonas and Francisella can cause aquaculture diseases. These diseases are common in marine and freshwater organisms. Parasitic Diseases: Aquaculture can be the target of a number of parasitic infections. These parasites can seriously affect the health of fish and reduce populations. Examples include parasites such as *Ichthyophthirius multifiliis* (Second Name: Ich) and Gyrodactylus spp. Epidemiological Variations: The rate and pattern of spread of aquaculture diseases can vary depending on a number of factors such as climate change, seawater temperature, water pollution and aquaculture practices. Therefore, the epidemiology of diseases can evolve dynamically. Control and Treatment Efforts: A number of strategies and treatment methods have been developed for the control of aquaculture diseases. This aims to ensure the sustainability of aquaculture and reduce commercial losses. International Co-operation: Aquaculture diseases are a global problem that requires cooperation between international organisations (e.g. the World Organization for Animal Health - OIE) and countries. Standards and protocols are being developed worldwide to limit and control the spread of these diseases (Aslan and Yıldız, 2021).

## 1. VIRAL DISEASES OF AQUACULTURE

Academic studies on viral diseases in aquaculture are very rich. The fisheries and aquaculture industries have long recognized that viral diseases can cause serious damage to fish populations and commercial aquaculture operations. Disease control and management in the aquaculture industry is constantly being improved through research and innovation. Therefore, it is important to regularly follow academic journals and resources to update the relevant literature. A brief description of the major diseases seen worldwide in aquaculture is given below. Viral diseases in aquaculture are diagnosed by isolation and identification of the virus, by morphological examination under electron microscope or by the presence of antibodies in serum. Diagnosis of viral infections in fish can be performed in complex virology laboratories. Only sending samples for viral diagnosis will be discussed here. For virus examination, it is best to send live samples. However, when viral infections are characterized by high mortality, it may not be possible to send live samples. For this reason, samples can be sent in viral transport medium (VTM) by sending the organs to be cultured from dying fish with typical symptoms. For fish under 4 cm in length, after cutting and discarding the part from the anus to the tail, the part with the head is placed in VTM at 10 times its weight at 4°C. In large fish, a total of 0.5 grams of brain, liver, spleen and kidney tissue taken from 5 fish are placed in 4.5 ml of cold VTM and sent to the laboratory so that they can be cultured in 24 hours at 4°C by following aseptic conditions as much as possible (Çağırgan, 2007).

### 1.1. Oncorhynuchus masou Virus (OMV)

Infection The virus in the Herpesviridae family was temporarily named *Oncorhynuchus masou* virus (OMV). The optimum replication temperature of OMV is around 15°C. The agent is sensitive to heat, ether and acid (pH 3) (Kimura et al., 1986).

### 1.2. Viral Nervous Necrosis (VNN)

Infection The causative agent of viral nervous necrosis infection, also known as viral encephalopathy and reticulopathy, is Betanodavirus in the family Nodaviridae. The agent has a double stranded genome and carries positive stranded RNA (Castric, 1997). VNN is known to cause serious diseases in juveniles and larvae worldwide, except for the American and African continents. Today, this disease is found in 22 fish species from 11 families (Noga, 2010).

## 1.3. Smallpox (Fish Pox)

The causative agent of the Poxviridae family, which has a DNA genome, is easily produced in cell cultures obtained from carp. Sporadic disease is commonly seen in cyprinid species and mortality is very low. The virus causes infection by entering through ports on the body surface (Erer, 1995).

## 1.4. Infectious Pancreatic Necrosis (IPN)

The causative agent of infectious pancreatic necrosis (IPN), first seen in the USA, is Infectious Pancreatic Necrosis Virus (IPNV) belonging to the Aquabirnavirus genus of the Birnaviridae family. Although the agent is resistant to UV, it is inactivated by chlorine and iodine, sensitive to alkaline pH and slightly resistant to acidic pH (Johnson et al., 2000).

## 1.5. Infectious Haematopoietic Necrosis (IHN)

It was first found in North America and then spread to Europe and Asia. According to WAHIDOIE data, it was reported that the disease was found in farmed fish in 9 countries (Austria, China, Czech Republic, Germany, Italy, Japan, Netherlands, Poland, Slovenia), in both wild and farmed fish in 2 countries (France, USA), and only in wild fish in 1 country (Canada). In previous studies conducted in Turkey, it was found that the virus was observed in farmed fish (Dixon et al., 2016).

## 1.6. Viral Haemorrhagic Septicaemia (VHS)

The causative agent of VHS, one of the most important viral diseases of rainbow trout, is Novirhabdovirus in the Rhabdoviridae family. It has 4 different genotypes and inactivates in ether, glycerol, chloroform, formalin, sodium hypochlorite, UV rays and at 56°C-60°C. VHS is an aquatic pathogen that infects freshwater and marine fishes in many regions of the Northern Hemisphere (Işıldan, 2006).

## 1.7. Spring Viremia of Carp (SBV)

The causative agent, which has a very wide distribution area in Europe, is located in the Vesiculovirus genus of the Rhabdoviridae family. The SBV virus, which has a high contagious character and causes serious infection in carps worldwide, was reported as a notifiable pathogen in the list published by OIE in 2011. The virus is sensitive to ether, heat and acid. Optimal replication pH is 7.0-10.0. While the mortality rate is much higher in the spring months when water temperatures are 10°C-17°C, humoral antibodies produced by infected fish at high temperatures can stop the spread and they can be protected from re-infection thanks to this immunity (Ahne et al., 2002).

### 1.8. Stomatopapilloma

The causative agent of the infection is Stomatopapilloma virus of the Papoviridae family containing DNA genome. Stomatopapilloma, also known as cauliflower disease, was first reported in Europe in the 20th century. Eels of all ages and sizes can be infected (Erer, 1995).

## 2. ISOLATION AND IDENTIFICATION IN VIRAL DISEASES

Fish virology is a fairly new branch of science. The first studies on fish viruses started after 1960. In other words, studies on fish virology were developed about 20 years after the isolation of viruses of warm-blooded animals in practical terms and the beginning of studies on them. Fish viruses develop and reproduce in a very wide temperature variation. This temperature variation is wider than that of the viruses of warm-blooded animals. This wide temperature tolerance depends on the ecology of the host and this situation, which is defined as ecological dependence, is related to the temperature and oxygen status of the water. For example, viruses of fish (Salmonidae) living in cool waters reproduce between 4°C-25°C. Known fish viruses easily reproduce in continuous cell cultures. One of these cells, the RTG (*Rainbow trout* gonade) cell, was obtained from the gonads of *Rainbow trout*. They have fibroblast structure, i.e. they are long cells. Another cell type, FHM (Fathead minnow) cell, was obtained from skin epithelial cells taken from the skin epithelial cells taken from near the tail of Dikence fish. Therefore, they are epithelial characterized cells. Apart from these, the continuous cell culture used today is BB (Brown bullhead) cell culture. These are also fibroblast characterized cells. BB cells are easily used in the production and isolation of CCV agent which is an epidemic disease of catfish (Burgu, 1979).

## 2.1. Purification of Viruses

Isolation and identification of a virus forms the basis of many areas such as solving problems caused by a viral disease, conducting epidemiological studies and developing vaccines or drugs against the virus. The basic steps for the isolation and identification of a virus can be listed as follows, respectively, Patient Sample Collection: The first step is to collect samples from the person or organism with the disease. These samples can be blood, saliva, urine, faeces, body fluids or infected tissues. Processing of Samples: The collected samples must be processed in a sterile manner under laboratory conditions. For example, samples can be centrifuged to remove cell debris and large particles. Virus Isolation: The virus to be isolated is inoculated into a suitable cell culture. This cell culture is used for the multiplication and replication of the virus. Replication of the Virus: The virus is propagated in cell culture. This allows the virus to increase in number and be used for further studies. Clearing the Virus: The virus separated from the cells must be cleaned of cell debris and other contaminants. This allows a pure characterization of the virus. Identification: The isolated virus is identified using molecular biology and virological methods. This helps to determine the genus, species and genetic structure of the virus. Serological Tests: Serological tests are used to assess the reactivity of the virus to various antibodies. This allows for more detailed characterization of the virus. Genetic Analysis: The genetic structure of the virus is analyzed using molecular biology techniques. PCR (Polymerase Chain Reaction), sequence analysis and other genetic methods help to determine the genetic structure of the virus. Electron Microscopy: Electron microscopy can be used to study and visualize the morphology of the virus. This allows you to visually assess the physical properties of the virus. Epidemiological Studies: Isolation and identification of the virus helps to understand the spread and epidemiology of viral diseases. This is important for disease control and prevention (Değirmenci and Çağırgan, 2017).

## 3. CELL CULTURE APPLICATION IN AQUACULTURE

Cell culture studies in aquaculture are the process of growing and reproducing cells of aquatic organisms under laboratory conditions. These studies are used in various research areas of fish, shellfish, mussels and other aquatic organisms. The basic steps in cell culture applications in aquaculture are, respectively, Cell Line Selection: The first step is to select a suitable aquaculture cell line for your study. These cell lines contain previously isolated and characterized aquaculture cells. You have to decide which organism's cell you will use and which cell line you will use. Cell Isolation: To isolate the cells of the aquatic product of interest, you need to take samples. These samples will usually be tissues or tissue samples. It is important to handle these tissues in a sterile manner. Cell Culture Media Preparation: You need to grow the isolated cells in a suitable cell culture medium or media. These media should contain the nutrients necessary for the growth and proliferation of the cells. Inoculation of Cells into Culture Dishes: Isolated cells are seeded into appropriate culture dishes using a sterile technique. In this step, cell density and seeding conditions should be carefully controlled. Ensuring Culture Conditions: Cell cultures should be maintained at appropriate temperature, humidity and gas conditions. These conditions may differ for each cell line and organism. Maintenance and Replication: Cell cultures should be maintained at regular intervals by feeding and passaging. This ensures that the cells grow and multiply in a healthy manner. Experiments and Research: Cell culture can be used for various experiments and research. For example, it can be used in areas such as the study of aquaculture viral diseases, genetic manipulation, toxicology studies and drug testing. Analysis and Data Collection: At the end of cell culture studies, the results of the experiments performed on the cells are analyzed and data are collected. This data will help you evaluate your research goals. Storage and Sharing of Cell Culture: The cell cultures obtained can be frozen and preserved for long-term storage. They can also be catalogued in accordance with appropriate protocols for sharing with other researchers (Şahin, 2003; Dikmen et al., 2020).

## 4. PRIMARY CULTURE OF RTG-2 CELLS

The worldwide increase in aquaculture has led to the development of technology in this field, and the realization of the importance of regulating populations of valuable aquatic organisms and disease control in this field has led to an increase in research on aquatic diseases, including viral diseases (Babin and Tarazona, 2005).

Fish constitute 48% of all known vertebrate species, making them a unique resource for researchers (Pandey, 2013). However, access to some species, difficulties in aquaculture, time and most importantly, the decline in fish populations have led researchers to in vitro studies. Thus, past research has been more easily consolidated and future research has been broadly and substantially guided (Pichardo et al., 2006).

In vitro biology studies generally refer to specific studies at the cellular and subcellular level at the laboratory level. Cell culture is basically the process of growing cells under controlled conditions. Cell culture studies in fish, initially to support the growth of fish viruses, have subsequently increased to cover a wide range of species or origin tissues and their applications. Fish immunology, physiology, genetics and development, toxicology, ecotoxicology, endocrinology, biomedical research, disease control, biotechnology and aquaculture are some of the areas where these studies have contributed (Wagg and Lee, 2005).

Cell culture systems, like other laboratory animals, are biological entities with specific physiological needs. They require constant care, adequate nutrition, suitable environment and regular controls. The fish health biologist must provide the cultures with an optimal environment for their survival. If this environment is not provided, the cells are not acceptable for viral testing of free-ranging fish populations (Ott, 2004).

There are two main approaches to cell culture. Primary culture and secondary culture, which refers to cell lines. Primary culture begins by placing tissue and organ cells of an animal directly into culture and ends when the primary culture is used in an experiment or taken into a subculture (secondary culture), at which point it becomes a cell line (Pichardo et al., 2006; Bols et al., 2017).

### 4.1. Primary Cell Culture

The preparation and preservation protocol of fish cell cultures is generally not different from that of homeotherm vertebrate cell cultures. The main advantages are that tissues from organs such as kidney, retina and liver can be rearranged into a monolayer cell configuration in contrast to their complex structure, facilitating the identification of the differentiated properties of the cells. In addition, the isolation afforded by the culture process allows precise control of hormonal, substrate and physical conditions that can alter cell and tissue function. Thus, the effects of prolonged exposure of tissues to low levels of humoral or xenobiotic substances can be assessed (Hightower and Renfro, 1988). The choice of fish species and appropriate tissues for the initiation of primary cell cultures usually depends on the type, function or utilization of the cell to be studied. For general purposes, various donor tissues such as heart, kidney, liver and spleen provide an adequate source of cells, but gonadal and embryonic tissues usually produce the most actively dividing cells (Nicholson, 1989).

## 4.2. RTG-2 Cell Line

The rainbow trout *Salmo gairdneri* gonadal cell line, RTG-2, developed by Wolf and Quimby (1962) is the first permanent cell line of fish origin (Bols et al., 2017). It was obtained from the gonad tissue of rainbow trout (Oncorhynchus mykiss) and shows a fibroblast-like cell structure (Çiçek, 2023).

This chapter summarizes the isolation of rainbow trout (*Oncorhynchus mykiss*) gonadal cell line (RTG-2), the conditions required for optimal growth of the cell line, laboratory standardization and quality standard procedures for tissue culture.

## 4.3. RTG-2 Cell Isolation and Cultivation

### 4.3.1. Precautions to be taken at the beginning of culture

a) Donor fish should come from fish farms, hatcheries, aquaria or other facilities where their health history is well documented and known to be free of systemic pathogenic parasites, bacteria and viruses.

b) Only one cell line should be worked with at a time and surfaces should be decontaminated with 70% isopropanol if different cell lines are or may be touching surfaces.

c) Ensure that the culture medium in which the cells will be grown is clean and free of microbial or fungal contamination.

d) Wipe all internal surfaces of the laminar flow cabinet with 70% isopropanol.

e) All items that will enter the laminar chamber, such as media containers, micropipettes, etc., should be disinfected with 70% isopropanol.

f) Wash hands and wrists thoroughly with an antibacterial soap before working with cell lines and perform additional disinfection of hands and wrists with 70% isopropanol before and after each procedure.

g) Dissolve trypsin-EDTA and keep this solution cool during use (Ott, 2004; Rubio et al., 2019; Wolf and Ahne, 1982).

### 4.3.2. Enzymatic Isolation of RTG-2 Cells

a) Donor fish are euthanized with 1:5000 tricaine methane sulfonate (MS 222), 1:2500 benzocaine or any other fish anesthetic.

b) The fish is cleaned with 70% alcohol (ethanol or isopropanol) without opening the body cavity.

c) The tissue is removed and cleaned with phosphate buffered saline (PBS) and cut into 1 to 2 mm pieces.

d) The pieces are suspended in PBS containing 25% trypsin, 5% fetal bovine serum (FBS) and 1% penicillin streptomycin at 4-5°C overnight (12-16 hours).

e) During this process, there is a preliminary period (approximately half an hour) during which cellular and tissue debris is released. As the first materials released have little growth potential, they are discarded. Subsequent cells and small pieces of tissue are collected for culture.

f) The cells are passed through filter paper to remove unwanted fibrous material and larger pieces of tissue.

g) To separate these cells from trypsin, they are centrifuged in a refrigerated centrifuge at +4°C 200-600 g for 3 minutes. The upper liquid is discarded and the cells are washed again by adding 3ml PBS. This process is repeated once more.

h) Cells should be of sufficient density for the initiation of primary cultures.

i) Cells should be cultured in 25cm flasks at 200.000 cells per ml in culture medium at 15-20°C in an oven containing CO2 (Cell medium contains Minimum Essential Medium (Eagle), 10% Fetal bovine serum (FBS) and L-glutamine (200 mM)) (Wolf and Ahne, 1982).

### 4.3.3. Subculturing of Cells

a) After the cells have been obtained and placed in the incubator, the medium should be changed at regular intervals. Subculturing should be done before the cells become too dense.

b) Firstly, the medium should be removed and the cells should be washed with PBS.

c) Then, approximately 2-2.5ml trypsin-EDTA should be added to the cells in 25cm flasks and the cells should be observed to rise from the surface. This may take approximately 10 minutes.

d) Centrifuge in a refrigerated centrifuge at 200-600 g for 3 minutes and remove excess trypsin.

e) After washing the cells with PBS, centrifuge again and add the medium.

f) It is more convenient to divide the cells into two or three in the first stage.

g) The separation of cells into subcultures is called passaging (Işık, 2022).

### 4.3.4. Freezing and Reuse of Cells

a) When the cells reach the culturing stage, they can be frozen and stored without re-passaging.

b) When subculturing the cells, after washing with PBS, they are taken into a 3ml cryo tube in media containing at least 15% FBS or Origen Freezing Medium with 1x106 cells per ml.

c) 10% DMSO is added into the tube.

d) It can be preserved by keeping in -80°C or liquid nitrogen tank.

e) It is recommended to write the name of the cell line, passage number and the date of freezing on the tube when the cells are left to freeze.

f) When the cells are thawed; the tube is defrosted and 2ml of cell medium is added into it and centrifuged at 200-600 g for 3 minutes and the supernatant is discarded.

g) Cells are washed with PBS and reseeded in culture medium.

h) One more than the frozen passage number is used as the passage number (Ott, 2004; Rubio et al., 2019; Wolf and Ahne, 1982).

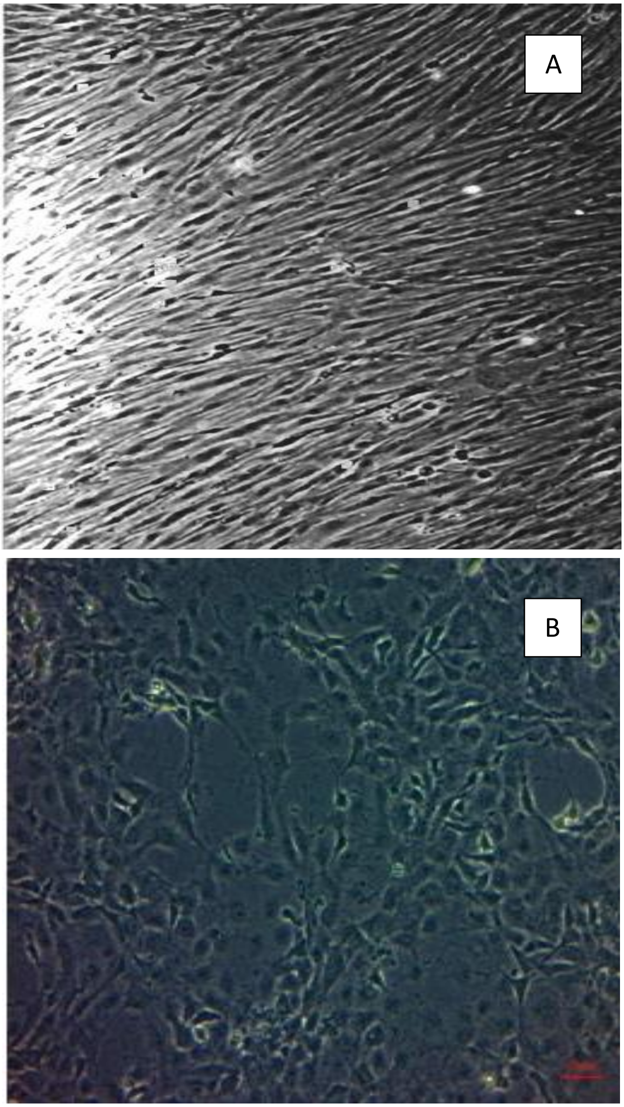


**Figure 1.** RTG cells (A) (Novoa et al., 1993) and invert microscobe screen (B) (Işık, 2022).

In conclusion; Diseases have a great share in the field of aquaculture. Because such diseases can negatively affect both fish populations and aquaculture industry. Here are some main points that explain the importance of aquaculture diseases; Population Decline: Aquaculture diseases can seriously damage wild fish populations. The spread of these diseases can lead to the decline of natural fish populations in aquatic ecosystems. This can lead to an imbalance of marine life and affect ecosystems. Economic Impacts: Aquaculture diseases can also negatively affect commercial fisheries and the aquaculture industry. Fish losses caused by these diseases can result in economic losses for fishing and aquaculture businesses. In addition, losses due to aquaculture diseases may lead to price increases. Food Safety: Aquaculture diseases can also harm human health. If diseased fish are consumed, people can be exposed to these diseases. Therefore, controlling aquaculture diseases is important for food safety. Environmental Impacts: Aquaculture diseases can damage aquatic ecosystems and water resources. The spread of these diseases can lead to the degradation of ecosystems and adversely affect water quality. Trade and Export: Aquaculture diseases can affect trade in aquaculture products. A country's ability to deal with aquaculture diseases can affect aquaculture trade and exports. Restrictions or embargoes due to disease can reduce the competitiveness of the country's aquaculture industry in the international markets.

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# CHAPTER 10

## THE REPLACEMENT OF WHEAT FLOUR CONTAINING GLUTEN BY DUCKWEED (*Lemna minor*) FLOUR IN BAKERY PRODUCTS AGAINST CELIAC DISEASE

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## INTRODUCTION

Microalgal aquatic organisms (*Tetrasselmis, Arthrospira (Spirulina), Dunalliela, Chlorella,* etc.), and duckweed (*Lemna spp*., and *Wolffia spp.* etc.) are a good source for producing protein-rich biomass and many other high-value compounds (vitamins, fatty acids, pigments etc.) due to very necessary for human health (Markou et al., 2018). Duckweed are free-floating, too small, aquatic green plants that are usually found in slow-moving bodies of water. Members of the *Lemnaceae* family not only contain the world's smallest angiosperms, but also, they are commonly recognized as duckweed. This family consists of five aquatic genera (i.e. *Lemna, Spirodela, Landoltia, Wolffiella, Wolffia,*) and 38 member species (Ali et al., 2016). Edible duckweed that is the smallest plant in the world and includes polyphenols, dietary fiber, vitamin B12, essential amino acids, iron, and zinc (Kaplan et al., 2019). Duckweed species are nutritionally very important plants that grow wildly in different areas. Therefore, understanding the metabolite content in *Lemna minor* is essential for designing a future food product because of a good potential of α-linolenic acid, glutamic acid, 5-Hydroxyl-L-tryptophan, Tocopheryl acetate, and Naringenin (Tekoğul, 2021; Yahaya et al., 2022). In addition to this, it is a potentially ideal succinic acid (SA) raw material because of its low lignin content and high starch content (Shen et al., 2016). Various usages and advantages of duckweed have been found as follows. Potential usage of *L. minor* as an ingredient in fish meal showed that *the* findings reduced the usage of fish oil and fish meal in tilapia feeds, whereas increased the values of EPA, DHA, omega-3 LC-PUFA, which were the content of farmed tilapia feeds (Opiyo et al., 2022). The total carotenoid amount of duckweed was found to be 10 times higher than land plants (Skillocorn et al., 1993). The level of crude protein ranged from 26.3% to 45.5% of the dry mass of the duckweed species, whereas the amino acid content of the plant compared favorably with that of soybean, and cottonseed meals and also significantly exceeded that of the peanut meal. The levels of essential amino acids except for methionine, which met 61.4% of the recommended value. Additionally, mineral levels were high, but should not pose any toxicity issues if included in animal feeds (Mbagwu and Adeniji, 1988). The use of agro-industrial wastes in microalgae and duckweed cultivation as well as contamination risks and biomass safety concerns have also been studied. *L. minor* is one of the strongest candidate species for Phytoremediation technology in technologies to be established in natural environments (Ustaoğlu et al., 2015; Markou et al., 2018; Tekoğul. 2023). The results of another study revealed that duckweed could be applied as a potential plant medicine to treat industrial wastewater (Farid et al., 2022).

Duckweed is small and has the ability to grow quickly (0.16 – 0.36) (Otte, 2012). Axenic culture can be performed. With all these features, it also allows it to be used as a model in genetic studies (Cantó-Pastor et al., 2015). Lemna, with its high starch accumulation, rich in cellulose and hemicellulose, and very low lignin content, is very suitable for biofuel production (Xu et al., 2011; Xu et al., 2012; Yan et al., 2013; Kaur, et al., 2018). Studies have also been conducted on foods containing duckweed. Breads enriched with *L. minor* were extended the shelf-life of bread when compared with control group not containing *L. minor* (Tekogul et al., 2011). However, it is difficult to change people's nutritional patterns. Food acceptability traditionally requires convincing consumers that the new product is more valuable than alternatives. For example, the high protein content of the lemna is of interest (Verneau et al., 2016; De Beukelaar et al., 2019).

Bakery products are one of the largest alternative protein applications for functional and nutritional purposes. Fortifying baked goods with proteins can provide a better amino acid profile and a higher protein intake. Traditional plant proteins such as wheat and soy dominate the baked goods industry, but emerging sources such as chickpeas, faba, and peas are also gaining importance. Each protein brings its own nutritional value and unique functional properties (Boukid, 2022). The protein group called gluten, which contains two separate proteins called glutenin and gliadin in cereals, has caused these products to be included among the basic nutrients for human beings. Gluten is a normal protein that can be easily digested through the gastrointestinal tract for many people. But some people can't digest gluten. These people are gluten intolerants, which is called celiac disease. Celiac disease in recent years, the demand for gluten-free food products has increased the production and consumption of these products (Kılınç, 2021).

Emerging proteins are now a big trend, but in the future could these alternative protein sources become the healthiest, tastiest and most viable option for consumers? Therefore, this subject is not only about the development of functionally and nutritionally balanced foods, but it is also very important to analyze in depth how these alternative protein sources can be sustainably produced, processed and provided to the consumer with a full nutritional and functional potential available (Avelar et al., 2022). For this reason, duckweed is considered a promising protein source for human food products due to its environmentally friendly production characteristics, and high protein content. In order to be successfully included in the diet, duckweed should be presented to consumers in an acceptable way to increase the consumption of food products enriched with *Lemna minor* (Beukelaar et al., 2019). The aim of this study is to the replacement of wheat flour containing gluten by duckweed (*Lemna minor*) flour in bakery products against celiac disease. For this purpose, the production of various bakery products (cake, pastries, cookies) containing duckweed instead of wheat flour has been carried out.

## Environmental Conditions, Harvesting, Aquaculture of *L. minor*

Aquaculture production is under intense pressure to increase to meet the food needs from the growing global population. However, a healthy water ecosystem is essential for a sustainable food supply (Rasdi et al., 2023). Birds, poultry, fish and other animals use duckweed as food (Syampurnomo et al., 2019).

It is a cosmopolitan species. It is found in Africa, Europe, America and most of Asia. Lemna grows 0,2-2,0 cm long, at optimum temperature of 20-30°C and pH of 5.5-6.5 (Zirschky and Reed, 1988). *L.minor* can be produced in the laboratory under optimum conditions.  Outdoor tanks, ponds, canals and lakes can be used to produce large quantities.

**Picture 1.** *Lemna minor*

When duckweed covers the water surface, half the surface area is harvested. In order to achieve high yield, care should be taken to harvest daily.

Harvesting duckweed is easy. Harvesting takes place by collecting the leaves from the water surface. They grow successfully in water and convert minerals in water into protein-rich biomass (Verma and Suthar, 2014). It is more resistant to cold than water hyacinth, and even if it remains in freezing conditions, it continues to maintain its vitality until the weather warms up again (Bayhan et al., 1996; Özkoç, 2011).

## Processing of Food Products Containing *L. minor*

An increasingly stringent need for the sustainability of food consumption is forcing the search for new food sources with cheapness characteristics that maximize the usefulness of the resources (Calabrese and Ferranti, 2019). Numerous efforts have been made in the search for new and sustainable protein sources to meet the nutritional needs of a growing world population. However, these goals can only be achieved through a multidisciplinary approach that covers the entire food supply chain (from farm to fork), which needs to be addressed and reconsidered (Avelar et al., 2022). Additionally, alternative and new protein sources are increasingly being investigated for their potential to combat problems associated with traditional protein sources such as dairy products and meat. In order to be commercialized and consumed safely by humans, the potential hazards of these new ingredients must be thoroughly investigated and adequate measures taken to eliminate these hazards as well as reduce them to acceptable levels that guarantee their safe consumption and widespread use (Das et al., 2023). Very limited studies using *L. minor* have been done and some of the studies have been summarized as follows. Yahaya et al., (2022) reported that 2% dried L. *minor* containing ice cream had significantly increased the fiber, ash, and protein content, whereas the result of the formulated ice cream was determined as a small amount of bacteria (3.82 cfu/g) showed in this study that duckweed had a good candidate for future food (Yahaya et al., 2022). Breads were made by using *L. minor* extended the shelf-life of bread products up to 8 and 12 day at room temperature and cold storage, respectively. In conrast to this, the control group without containing L. *minor* was reached this non-consumable level of bread on day 3 and 8 at room temperature and cold storage, respectively (Tekogul et al., 2011).

In the research conducted in the Netherlands, tastings were organized in exhibitions and restaurants with the participation of guests. Duckweed was seen as a stylish, new and special dish that should be served especially on special occasions, conferences and high-end events. Participants were generally willing to accept it as human food. At the end of the study, it was stated that dishes such as salads, sandwiches and steaks could be suitable for duckweed dishes (De Beukelaar et al., 2019).

### Production of Bakery Products with *L. minör*

Duckweed is a plant and is naturally gluten-free, making it a potential alternative to wheat flour.

**Picture 2.** *Lemna flour*

We used duckweed flour instead of flour in baked goods. Freshly grown Lemna minor leaves are dried and then ground to a fine powder. The flour obtained is lemna minor flour. It can also be mixed with other gluten-free flours when using it. Lemna minor can pair well with ingredients like lemon, vanilla, or herbs to find the right balance for your desired flavor profile. Lemna minor flour can be incorporated into bread dough, muffin or cake batter.

**Picture 3.** Cake

**Picture 4.** chocolate cupcake

**Picture 5.** Biscuit

**Picture 6.** Cheesy pastry

**Picture 7.** Vegetable pie

## CONCLUSION

Consumers generally prefer duckweed as human food. Recently, algae species has been frequently used as human food, especially as the use of algae increases. The aquacultivation of algae is increasing. Duckweed is also a good source of protein and fiber. With this feature, bakery products made with duckweed can contribute to the production of functional products specifically for the consumption of celiac patients by meeting the deficiency of gluten protein. Although the research is promising, further studies are needed to determine the most appropriate formulations and processing techniques for using duckweed flour in baked goods.

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# CHAPTER 11

## FEED ADDITIVES AND APPLICATIONS IN AQUACULTURE

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## INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) predicts that food demand will increase by 60% by 2050. Accordingly, it is estimated that animal protein production will increase by approximately 1.7% per year between 2010 and 2050. It is also stated that in this increase, meat production will be approximately 70%, aquaculture will be 90% and dairy products will be 55% (IFIF,2023).

According to 2022 data of the United Nations Food and Agriculture Organization (FAO), it is reported that the total amount of animal aquatic products produced in the world in 2022 was 184.6 million tons, and the amount produced through aquaculture was 92.2 million tons. It is known that the consumption of aquatic products as human food is important throughout the world. Globally, aquaculture provided approximately 17 percent of animal proteins and 7 percent of all proteins in 2019. Moreover, according to FAO data, aquaculture provided at least 20 percent of the average per capita animal protein intake for 3.3 billion people (FAO,2022).

Healthy nutrition has become increasingly important as a result of the increase in overweight people and obesity-related diseases in many countries around the world. As a result, the demand for healthy and nutritious foods such as seafood has increased in recent years. Global aquaculture consumption (excluding algae) has increased at an average annual rate of 3.0 percent since 1961, compared to a population growth rate of 1.6 percent. Per capita consumption of aquatic products increased from an average of 9.9 kg in the 1960s to 20.5 kg in 2019 and decreased slightly to 20.2 kg in 2020. Rising incomes and urbanization, the development of post-harvest practices and changes in dietary trends are predicted to lead to a 15 percent increase in aquaculture consumption, projected to average 21.4 kg per capita in 2030 (FAO,2022).

FAO's 2030 forecast for fisheries and aquaculture projects an increase in production, consumption and trade, albeit at slower growth rates, with total aquatic animal production expected to reach 202 million tons in 2030, thanks to the continued growth of aquaculture, which is forecast to reach 100 million tons in 2027 and 106 million tons in 2030.

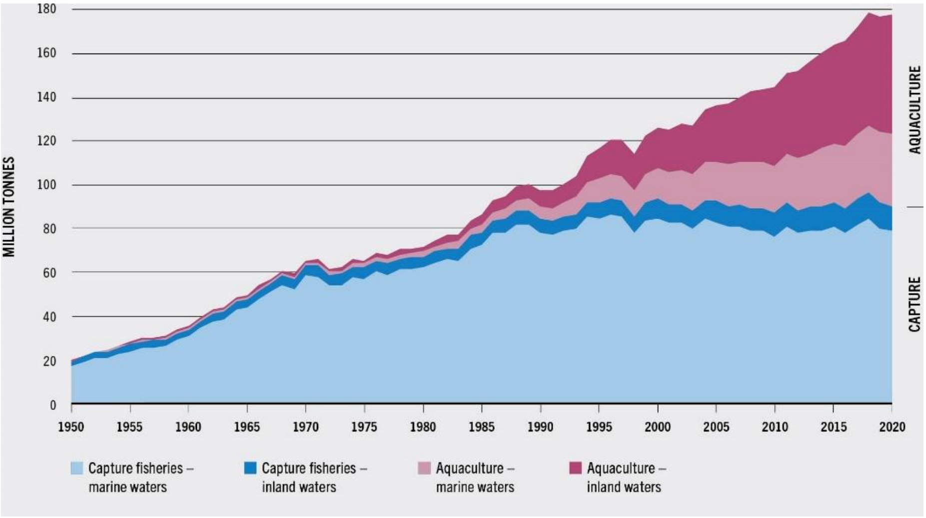


**Figure 1**. World capture fisheries and aquaculture production (FAO,2022)

Aquaculture production in Turkey increased by 6.2% in 2022 compared to the previous year, reaching 849 thousand 808 tons. While the total production through hunting was 335 thousand 3 tons, aquaculture production was 514 thousand 805 tons (TUIK, 2023). Production through aquaculture increased by 9.1% in 2022, 368 thousand 742 tons in the seas and 146 thousand 63 tons in inland waters. The most important fish species grown were trout (145 thousand 649 tons) in inland waters, sea bass (156 thousand 602 tons) and sea bream (152 thousand 469 tons) in the seas (TUIK, 2023).

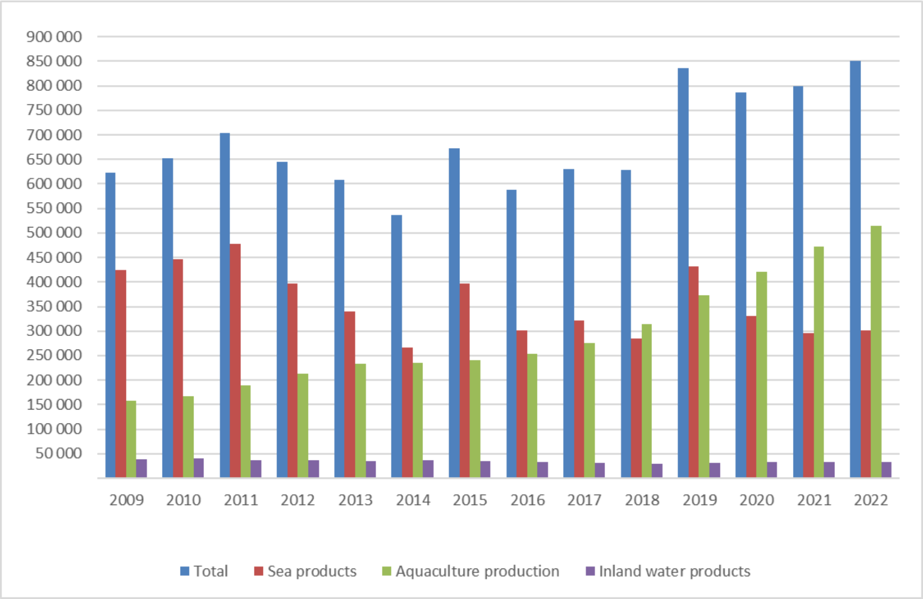


**Figure 2.** Türkiye Aquaculture production amount 2009-2022 (TUIK,2023)

When look at these data, it is observed that there has been a great increase and accompanying developments in aquaculture in our country, especially in recent years. The only animal product that is an important source of protein and can be exported especially to EU countries is aquatic products. It is a rapidly developing sector for our country and investments related to important R&D studies in this regard are also increasing. Many factors can be mentioned that affect breeding activities. However, the most important of these and the one that has the biggest impact on costs is feed. Feed is the main raw material of animal production. Feed can account for 40-60% of the total cost of fish production. Feeds used in different compositions depending on the animal type are divided into roughage and compound feed. Compound feed; It is a manufacturing industry product obtained by processing animal-based raw materials such as fish meal and meat meal, vegetable origin raw materials such as corn and soy, and products such as additives, and is an intermediate good. Roughage, on the other hand, is products consisting of plant products, the main source of which is forage plants, and used directly in animal nutrition (TAGEM, 2021).

It is reported that the feed has various definitions according to its function or content (TAGEM, 2021). The general definition of feed is that it contains organic and inorganic nutrients, and when given to certain proportions do not have a negative effect on the animal's health, development, reproduction, productivity, etc. Compound feeds, including fish feeds, can be expressed as feeds obtained by mixing more than one feed raw material consisting of organic and inorganic substances, guaranteed structure, which enables the grown creature to produce quality and abundant products (Korkut. et al. 2004).

In general, the raw materials used in making feed for aquaculture are limited, the main ones being raw materials of animal origin such as fish meal and fish oil, raw materials of plant origin such as soy products, corn and wheat products, as well as feed additives such as vitamins and minerals (Table 1).

**Table 1**. Main Raw Materials Used in Fish Feeds

| General Classification of Feed Raw Materials | | |
|---|---|---|
| RAW MATERIALS of PLANT ORIGIN | RAW MATERIALS Of ANIMAL ORIGIN | FEED ADDITIVES |
| 1. Farm Feed<br>- Seeds and Grains<br>- Oil bearing seeds<br>- Legumes and cereals | 1.Raw Materıals Of Aquatic Origin<br>(Fish, Fish silage, Fish oil, Fish meal etc. | Vitamins And Minerals<br>Antibiotics<br>Antioxidants<br>Synthetic Amino Acids<br>Emulsifier<br>Enzymes<br>.Hormones<br>Aroma And Flavor Substances<br>Colorants |
| 2.Commercıal Feed Raw Materıals<br>-Industry By-Products<br>- Milling By-Products;<br>(Wheat, corn, rice flour and bran)<br>-Starch Byproducts<br>(Wheat and corn gluten)<br>- Oil Industry By-Products<br>(Cotton meal, soybean meal) | 2.Alternatıve Raw Materıals<br>(Mealworm, Black soldier fly, etc.) | |

Total global industrial feed production in 2022 is 1.266 billion tons. 4% of this amount consists of aquaculture feeds with 41 million tons. The aquaculture sector has shown a 2.7% growth in total global feed production (IFIF,2021) (Figure 3).

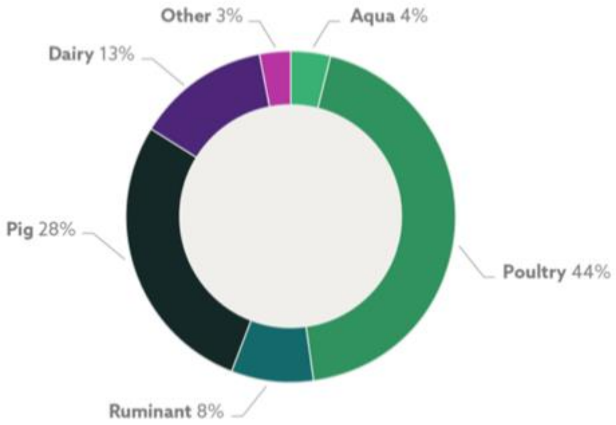


**Figure 3**. Global Feed Market as Percentage by Species 2021 Estimates IFIF 2021, (https://ifif.org/global-feed/statistics/)

According to the data announced by the Ministry of Agriculture and Forestry, our country's compound feed production increased by 3% in 2021 compared to 2020, reaching 27 million tons. Within this amount, poultry feed production was 10.2 million tons, cattle and sheep feed production was 15.5 million tons, and the production of other feeds, including cat-dog, horse and ornamental animal feed, was 681 thousand tons. Total fish feed production increased by 3% in 2021 compared to 2020 and reached 653 thousand tons (Türkiyem-bir.,2023). In our country, as of June 2023, there are 807 Enterprises Producing Compound Feed, 731 Enterprises Producing Their Own Feed, and 323 Feed Additive Premix Enterprises (GKGM, 2023).

Many studies have been conducted on feed costs, including production systems, different raw materials and additives. In recent years, these studies have focused on additives that are especially organic and economical, do not adversely affect the health of fish, and are not harmful to humans.

# 1.FEED ADDITIVES

Feed additives are substances used to improve the quantity and quality of animal products by increasing feed utilization, protecting the health of animals and thus reducing production costs. Feed additives are generally supplied abroad by feed additive premix businesses as pure, concentrated or premixed. Feed additives imported as pure or concentrated are put into use after being mixed with appropriate carrier substances in certain proportions in premix preparation facilities.

In order for animals to live healthy and have high product potential, it is necessary to use some additives in their feed. However, these substances are not sources of nutrition on their own. Although additives are added to mixed feed in small amounts, they have a positive effect on the metabolism of the animal and also have effects on protecting the feed and increasing its quality (Korkut et al., 2004). Some of these benefits can be listed as follows:

- Increases the quality of feed or animal products,
- Increases animal production and the welfare level of animals,
- Improves digestion and digestive system microflora,
- Increases the amount of nutrients in the feed,
- Contributes to the preservation of nutrients and feed,
- It reduces the environmental damage of animal production (Yıldız,D. 2021).

In the third article of the first section of the Regulation on Feed Additives Used in Animal Nutrition published by the Ministry of Food, Agriculture and Livestock in the Official Gazette dated 18 July 2013 and numbered 28711;

j) "premixtures" means mixtures of feed additives or mixtures of one or more feed additives with feed materials or water used as carriers, not intended for direct feeding to animals,

ö) "Feed additives" means substances, microorganisms or preparations, other than feed material and premixes, which are deliberately added to feed or water for the purpose of performing one or more of the functions specified in Article 5(6) in particular.”

According to the second part of the same regulation, Article 5 of the Approval Conditions,

(5) Feed additive substances must avoid the following properties;

a) not have an adverse effect on animal health, human health or the environment,

b) should not be presented in a manner which may mislead the user,

c) should not harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products.

(6) Feed additive substances should have at least one of the following properties;

a) have a favorable impact on the characteristics of feed,

b) have a favorable impact on the characteristics of products of animal origin, c) have a favorable impact on the colors of ornamental fish and birds,

ç) fulfill the nutrition requirements of animals,

d) have a favorable impact on the environmental consequences of animal production,

e) have a favorable impact on the animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs,

f) have a coccidiostatic or histomonostatic impact.

(7) Antibiotics, other than coccidiostats or histomonostats, shall not be approved as feed additives.

(8) The feed additive substances updated and approved by the European Union shall be accepted as approved within the scope of this Regulation provided that the prohibitions and restrictions indicated in the Biosecurity Law dated 18/3/2010 and number 5977 as well as the provisions of the Regulation

regarding Genetically Modified Organisms and their Products published in the Official Gazette dated 13/8/2010 and number 27671 are without prejudice.

According to Article 6 of the second part of the regulation, feed additives categories;

ARTICLE 6 – (1) A feed additive shall be allocated to one or more of the following categories, depending on its functions and properties, in accordance with the procedure set out at Articles 7, 8 and 9.

a) technological additives: any substance added to feed for a technological purpose.

b) sensory additives: any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals.

c) nutritional feed additives: additive substances indicated in article three of Annex-1 to this Regulation.

ç) zootechnical additives: any additive used to affect favorably the performance of animals in good health or used to have a favorable impact on the environment.

d) coccidiostats and histomonostats feed additive substances.

2) Within the categories referred to in paragraph 1, feed additives shall further be allocated within one or more of the functional groups mentioned in Annex I, depending on their function or functions, in accordance with the procedure specified in Articles 7 and 8.  (3) Where necessary as a result of technological progress or scientific development, additional feed additive categories and functional groups may be established.

Feed additives function groups are listed in Annex-1 of the this regulation.

Annex-1 FEED ADDITIVE SUBSTANCES FUNCTIONAL GROUPS

(1) In the category "technological additives", the following functional groups are included:

a) preservatives: substances or, when applicable, micro-organisms which protect feed against deterioration caused by micro-organisms or their metabolites,

b) antioxidants: substances prolonging the storage life of feedingstuffs and feed materials by protecting them against deterioration caused by oxidation,

c) emulsifiers: substances that make it possible to form or maintain a homogeneous mixture of two or more immiscible phases in feedingstuffs,

ç) stabilizers: substances which make it possible to maintain the physico-chemical state of feedingstuffs,

d) thickeners: substances which increase the viscosity of feedingstuffs,

e) gelling agents: substances which give a feedingstuff texture through the formation of a gel,

f) binders: substances which increase the tendency of particles of feedingstuffs to adhere,

g) substances for control of radionucleide contamination: substances that suppress absorption of radionucleides or promote their excretion,

ğ) anticaking agents: substances that reduce the tendency of individual particles of a feedingstuff to adhere,

h) acidity regulators: substances which adjust the pH of feedingstuffs,

ı) silage additives: substances, including enzymes or micro-organisms, intended to be incorporated into feed to improve the production of silage,

i) denaturants: substances which, when used for the manufacture of processed feedingstuffs, allow the identification of the origin of specific food or feed materials,

j) Substances which decrease the mycotoxin contamination of feed: substances which repress or decrease the absorption of mycotoxins, enhance excretion or change the form of impact.

k) Hygienic condition improvers: Substances or microorganisms that positively affect the hygienic properties of feed by reducing a specific microbiological contamination.( This article was added according to the Regulation On Amendments To The Regulation On Feed Additives Used in Animal Feeding from the Ministry of Agriculture and Forestry published in the Official Gazette No. 31754 on FRIDAY, February 18, 2022)

l) Other technological additives: Substances or microorganisms that are added to feed for a technological purpose and positively affect the properties of feed." (Annex: RG-18/2/2022-31754)

(2) In the category "sensory additives", the following functional groups are included:

a) colorants;

1) substances that add or regulate color in feedingstuffs,

2) substances which, when fed to animals, add colors to food of animal origin, 3) substances which favorably affect the color of ornamental fish or birds,

b) flavoring compounds: substances the inclusion of which in feedingstuffs increases feed smell and palatability.

(3) In the category "nutritional additives", the following functional groups are included:

a) vitamins, pro-vitamins and chemically defined substances having a similar effect,

b) compounds of trace elements,

c) amino acids, their salts and analogues,

ç) urea and its derivatives.

(4) In the category "zootechnical additives", the following functional groups are included:

a) digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials,

b) gut flora stabilizers: micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora, c) feed additive substances which favorably affect the environment,

ç) other zootechnical additives.

(5) ‘‘coccidiostats and histomonostats’’ category.

If the feed additive functional groups are discussed in more detail;

**1-Technological additives**

**a) Preservatives:** These are substances that protect the feed against deterioration caused by microorganisms or their metabolites. They ensure that feeds can be stored for a long time without deterioration. Organic acids (fumaric acid, lactic acid, acetic acid, propionic acid, sorbic acid and formic acid, benzoic acid, citric acid, acetic acid, malic acid and gluconic acid) and their salts are widely used to protect feeds from the effects of microorganisms.

**b) Antioxidants:** These are substances that protect feedstuffs and feeds against deterioration caused by oxidation and extend their shelf life (Yıldız, D., 2021). Similar to oxidation that causes corrosion and deterioration in metals, oxidation is also seen in feeds and feedstuffs and causes deterioration. This event occurs as a result of the combination of fats and the fatty acids that make up them with atmospheric oxygen under the catalytic effect of heat, light and some heavy metals (Çakmak, B., 2003). Oils spoiled by oxidation become bitter, color, taste and aroma disorders occur in the feed. These deteriorations are in the form of rancidity of fats, degradation of vitamins A, D, E and K, pigment substances and amino acids and decrease in the energy value of feed. Hydroperoxides formed as a result of oxidation reduce the availability of fat-soluble vitamins A, D, E and K. In addition, oxidized fatty acids react with lysine and reduce amino acid utilization (Korkut et al. 2007).

In order to prevent these deterioration in feeds, some chemical compounds are added into the feed and these substances are called antioxidants. Some conditions are required for the use of antioxidants in animal feeds. These conditions are;

1. It should be effective to protect animal, vegetable oils, vitamins and other feed quality against oxidation.

2. It should not poison humans and farm animals (chicken, fish, etc.).

3. Be effective at low concentrations.

4. Be economical.

Some of the substances used as antioxidants in feeds are synthetic and some are natural products. Natural substances used for this purpose are tocopherols (vitamin E), ascorbic acid (vitamin C), some amino acids and some aromatic plants (such as sage, rosemary, laurel, clove, thyme, cumin, mint, cinnamon), synthetics are propyl gallate (PG), butyl-hydroxy anisole (BHA), butyl-hydroxy toluene (BHT), butyl-hydroxy quinone (BHQ), citric acidethyl endyamine tetra acetic acid (EDTA), phospholipids, phenols, quinones (Korkut et al. 2004; Kırkpınar F., 2017).

**c) Emulsifiers:** Emulsifiers are substances that form a homogeneous mixture of two or more immiscible phases in the content of feeds or maintain this homogeneous mixture. They are used to ensure homogeneous distribution of the fat added to the raw material mixture during feed production. The most commonly used substance for this purpose is lecithin. Mono and diglycerides of fatty acids are also used.

**ç) Stabilizers:** Stabilizers are substances that maintain the physico-chemical state of feed materials (Yıldız, D. 2021). They are used to increase the durability of vitamins, essential amino acids and some drugs added to the feed at very low levels. For this purpose, substances such as antioxidants and gelatin can be used (Kırkpınar F. 2017).

**d) Thickeners:** Substances that increase the viscosity of feedstuffs.

**e) Gelling agents:** These are substances that give consistency to feeds with gel formation. In the Registration List of Feed Additives Permitted for Use According to Article 16 of the Regulation on Feed Additives Used in Animal Nutrition of the Ministry of Agriculture and Forestry, the substances under the title of emulsifiers, stabilizers, thickeners and gelling agents are Sodium Alginate, Agar Agar, Carrageenan, Carob Gum, Tamarind Seed Flour, Guar Gum, Gum Arabic, Xanthan Gum, Pectin, Gelatin, Sodium Carboxymethyl Cellulose (CMC), etc.

**f) Binders:** Substances that increase the binding tendency of feed material particles. Binders are used during pelletizing of feeds. The purpose of these additives is to ensure that feed raw materials and other additives are easily bonded during pelletization. In this way, the pellets take shape more easily. In addition, their resistance to breakage and dusting increases in stages such as processing, transportation and sieving applied in feed production. Lignosulfonate, Molasses, Bentonite (Clay), Bolus alba, Carboxymethylcellulose, Polymethylocarbamide etc. can be given as examples of the substances used for this purpose.

**g) Substances used for the control of radionuclide contamination:**

Substances that suppress the absorption of radionuclides or increase their excretion. Ferric (III) ammonium hexacyanoferrate (II) and Bentonite are used.

**ğ) Anti-caking agents:** Calcium Carbonate, Silicon Dioxide, Tricalcium Phosphate are substances that reduce the tendency of feed material particles to bind to each other.

**h) Acidity regulators:** Substances used to regulate the pH of feed are Sodium bisulfate, Sodium malate, Benzoic acid, Potassium dihydrogen orthophosphate, Dipotassium hydrogen orthophosphate, Tripotassium orthophosphate, Ammonium dihydrogen orthophosphate, Diammonium hydrogen orthophosphate, Sodium sesquicarbonate, Potassium hydrogen carbonate, Ammonium carbonate, Ammonium hydrogen carbonate, Hydrochloric acid, Ammonium chloride, Sulfuric acid.

**ı) Silage additives:** They include enzymes, microorganisms and chemicals prepared to be added to the feed to improve silage formation; Amylase (from Aspergillus oryzae), Alpha-amylase from Bacillis amyloliquefaciens, Alpha-amylase from Bacillus subtilis, Beta-glucanase from Aspergillus niger, Cellulase from Aspergillus niger, Cellulase from Trichoderma longibrachiatum, Xylanase from Trichoderma longibrachiatum, Microorganisms; Bacillus subtilis, Enterococcus faecium, Lactobacillus brevis, Lactobacillus buchneri, Propionibacterium acidipropionici, Lactobacillus casei etc. , Chemical substances include Propionic acid, Sodium propionate, Ammonium propionate, Formaldehyde, Hexamethylene tetramine, Sodium benzoate, Sodium nitrite, Sodium bisulfate.

**i) Denaturants:** substances which, when used for the manufacture of processed feedingstuffs, allow the identification of the origin of specific food or feed materials,

**j) Substances which decrease the mycotoxin contamination of feed:** substances which repress or decrease the absorption of mycotoxins, enhance excretion or change the form of impact. Bentonite and DSM11798 strain microorganism from Coriobacteriaceae family are used within the scope of these substances.

**k) Hygienic condition improvers:** Substances or microorganisms that positively affect the hygienic properties of feed by reducing a specific microbiological contamination (Annex: RG-18/2/2022-31754). Chemical substances of acid or alkaline origin that prevent the density of pathogenic microorganisms in the digestive systems of animals.

**l) Other technological additives:** Substances or microorganisms that are added to feed for a technological purpose and positively affect the properties of feed." (Annex: RG-18/2/2022-31754)

**2- Sensory additives**

**a) Colorants;** Color Substances are pigments. Color is an important quality factor in aquaculture. Under this heading, Carotenoids and xanthophylls, Other colorants, Colorants are classified as substances that increase or maintain the color of feed.

1) substances that add or regulate color in feedingstuffs; Patent blue V, Acid Brilliant Green BS / (Lissamine green) ; Acid brilliant green BS, Canthaxantin Allura Red, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Caramel Colors, Carmoisine, Chlorophyllin Copper Complex, Erythrosin, Indigotin, Iron Oxide Red, Black & Yellow; Patent Blue V; Ponceau; Quinoline Yellow; Titanium dioxide (anatase & rutile structure); Vegetable Carbon; Tartrazine; Sunset Yellow, Azorubin or Carmoisine (Disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)naphthalene-1-sulfonate.

2) substances which, when fed to animals, add colors to food of animal origin; Capsanthin, Cryptoxanthin, Beta-apo-8'-carotenal, Ethyl ester of Beta-apo-8'-carotenoic acid, Lutein, Canthaxanthin, Zeaxanthin, Citranaxanthin, Astaxanthin, Phaffia rhodozyma rich in Astaxanthin, Astaxanthin dimethyldisuccinate, Paracoccus carotinifaciens rich in red carotenoids.

3) substances which favorably affect the color of ornamental fish or birds, Canthaxanthin, Astaxanthin, Astaxanthin-rich Phaffia rhodozyma, Acid Brilliant Green BS / (Lissamine green), Astaxanthin dimethyldisuccinate, Red carotenoid-rich Paracoccus carotinifaciens, Beta-carotene, Canthaxanthin, Tartrazine, Sunset Yellow FCF - Sunset yellow, Ponceau, Erythrosine, Indigotine, Chlorophyll Copper complex, Carbon black, Bixin, Iron oxide.

**b) Aromatic substances:** These are substances that increase the smell and flavor of feed when added to feedstuffs. Some substances with or without nutritional value, containing special odor and taste are added to the feed to ensure that the feed is consumed with appetite. Some of the substances used for this purpose are; anise, anise oil, saccharin, vanillin, lemon acid etc. It has been observed that shrimps consume the feed better when animal materials such as cuttlefish, shrimp head, etc. are added to shrimp feeds. Under this heading, aromatic compounds, natural or corresponding synthetic chemically defined aromatic substances, natural products - botanically defined natural products - non-herbal aromatic substances are defined as a large number of additives to be used in feeds for all animal species. They constitute the largest class of additives. Acer spicatum Lam: Spiked maple extract, Achillea millefolium L.: Pure Yarrow, Aesculus hippocastanum L.: Chestnut extract, Agaricus bisporus L.: Mushroom extract, Allium cepa L.: Onion, Allium porrum L.: Spray-dried

Leek, CAS No. 100-09-4 / p-Anisic acid / Flavis No. 08.071, CAS No. 101-97-3 / Ethyl phenylacetate / Flavis No. 09.784, CAS No. 109-60-4 / Propyl acetate / Flavis No. 09.002, Viola tricolor L.: Pansy / Pansy extract / Pansy tincture, Vitex agnus-castus L.: Chaste herb / Chaste herb oil, Saccharomyces cerevisiae Hansen : Yeast extract/ Yeast oil can be given as examples.

**3- Nutritional additives**

**a) Vitamins:** provitamins and substances with defined chemical properties and similar effects; fat-soluble vitamins such as Vitamins A, D, E and K, water-soluble vitamins such as Vitamin B1 / Thiamine, Vitamin B2 / Riboflavin, Vitamin B6 / Pyridoxine, Vitamin B12 / Cyanocobalamin, Vitamin C and well-defined chemical substances with similar biological effects as Vitamins; Carnitine / L-Carnitine, Betaine, Taurine, Omega-3 Essential Unsaturated Fatty Acids, Niacin, Niacinamide, Folic acid and Choline chloride are substances used for this purpose.

**b) Compounds of trace elements;** Other feeds that are at least as important as other feedstuffs in animal nutrition are mineral feeds. These feeds are necessary for animal growth, reproduction, fulfillment of body functions, etc. It is possible to classify mineral substances under two groups. The first one is "Macro Elements" which are needed in large amounts by animals and must be taken daily. Calcium, phosphorus, magnesium, potassium, sodium, chlorine and sulfur are included in this group. The other group is the so-called "Trace Elements", which are both necessary for animals and have a low rate of presence in feed. These include Iron, Manganese, Copper, Cobalt, Zinc, Molybdenum, Iodine and Selen.

**c) Amino acids, their salts and analogues**; They are used against amino acid deficiency in animal nutrition. Methionine, Lysine, Threonine, Tryptophan, L-arginine, L-valine, L-valine, Guanidinoacetic acid, L-isoleucine are given for all animal species, while L-histidine monohydrochloride monohydrate 98%, amino acid produced from Escherichia coli (ATCC 9637) is reported especially for Salmon.

**ç) Urea and its derivatives;** Biuret, Urea-phosphate and Diureidoisobutane are used in all animal species.

**(4) Zootechnical additives**

When used in animal nutrition, they are substances that increase the digestion of feed by acting on feed raw materials. For this purpose, enzymes, probiotics, prebiotics (oligosaccharides), essential oils and plant extracts are used and these are substances with multifunctional effects (Kırkpınar, F.2017). This category includes the following functional groups:

**a) Digestibility enhancers;** When used in animal nutrition, they are substances that increase the digestion of feed by acting on target feed ingredients, *Saccharomyces cerevisiae*, Alpha-galactosidase, Endo-1,4-betaxylanase, 6- Phytase are used for various animal species. Among these, 6-phytase *Aspergillus oryzae* DSM 14223 is specifically indicated for use in Trout feeds.

**b) Gut flora stabilizers**; Microorganisms or other substances with defined chemical properties that have a positive effect on the intestinal flora when used in animal nutrition, Bifidobacterium animalis SC20, *Bacillus subtilis*, *Clostridium butyricum*, *Pediococcus acidilactici* are examples of substances used in all animal species.

1) Enzymes are additives used to increase the utilization of feeds that are difficult to digest. For this purpose, proteases, lipases, phytases, pectinase, amylase, cellulase, pectinase, amylase, cellulase produced from various fungi such as *Trichoderma reesei*, *Trichoderma viride* and *Aspergillus niger* or *Bacillus subtilis* are used to contribute to digestion and improve performance. Endogenous enzymes of bacterial or fungal origin are insufficient for the breakdown of compounds that are difficult to digest for aquatic species. Therefore, exogenous enzymes are used to increase the digestibility of feeds (Yiğit and Koca 2011; Yıldırım et al. 2014). Especially in the feeding of carnivorous species, the application of phytase enzyme positively affects digestibility (Zheng et al., 2020)

2) Probiotics are the beneficial microorganisms including bacteria and yeast which confers a health benefit to the host when administered in adequate amount (WHO, 2002). The most commonly used microorganisms in probiotic production are lactic acid-producing Lactobacillus and Streptococcus bacteria.

The main probiotics used in aquaculture are *Lactobacillus rhamnosus* and Carnobacterium. While these protect fish against pathogens, probiotics obtained from *Lactobacillus sakei* and *Scutellaria baicalensis*, *Pediococcus parvulus*, *Candida parapsilosis* (yeast) and herbal extracts such as *Echinacea purpurea* and *Uncaria tomentosa* have positive effects on the growth rate and feed evaluation rate of aquatic organisms (Peraza-Gomez et al., 2014).

3) Prebiotics (Oligosaccharides); They are non-digestible non-living feed additives that increase the number and activity of beneficial bacteria living in the intestines and have a positive effect by improving the health of the animal. The most commonly used prebiotics are mannanoligosaccharides, fructooligosaccharides, chitosan oligosaccharides and betaglucans. The most commonly used prebiotics in fish are inulin fructooligosaccharides (FOS, short-chain fructooligosaccharides (scFOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS), xylooligo-saccharides (XOS), arabinoxylooligosaccharides (AXOS), isomaltooligosaccharides (IMO) and GroBiotic-A (Ringø et al., 2010)

4) Herbal Extracts; Herbal extracts or essential oils are natural substances with antimicrobial, growth promoting and feed utilization improving properties. For this purpose, aromatic plants such as sage, rosemary, laurel, mustard, clove, thyme, cumin, coriander, mint, garlic, cinnamon, ginger, garlic extracts and oils are used as additives (Kırkpınar, F.2017). In aquaculture, aloe (*Aloe vera*), almonds, basil flowers (*Ocimum sanctum*), cumin seed meal, cinnamon (*Cinnamomum zeylanicum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), green tea (*Camellia sinensis* L.), olive tree leaf (*Olea europaea*), mint (*Mentha piperita*) (Mousavi et al., 2011), seaweeds (*Sargassum spp.*) are the plants reported to be studied (Van Hai, 2015).

**c) Feed additive substances which favorably affect the environment**, Substances used to reduce methane gas formation in ruminants and the enzyme phytase, which increases the utilization of phytin phosphorus in poultry and fish. Lanthanum carbonate octahydrate used to reduce urinary phosphorus excretion in dogs and ammonium chloride used to reduce urine pH are examples.

**ç) Other zootechnical additives**; *Pediococcus acidilactici*, benzoic acid, sodium benzoate, preserved citric acid, sorbic acid, thymol and vanillin preparation, caraway oil, lemon oil, spices and dried plants are used to improve zootechnical parameters, to improve performance parameters, to increase live weight gain or feed conversion ratio, to positively affect the growth of animals. Potassium diformate (KDF) (Kakavand et al., 2021), thymol and carvacrol (Frota et al., 2022) are examples of additives used in aquatic organisms.

1)Toxin Binders; They are used to prevent molds growing in feeds. For this purpose, organic acids (propionic, sorbic, benzoic and acetic acids), organic acid salts (such as calcium propionate and potassium sorbate), organic dyes, compounds such as copper sulfate and ammonia, adsorbents such as activated charcoal, hydrate sodium calcium aluminosilicate, bentonite, perlite, diatomaceous earth and zeolite are used as toxin binders in feeds (Kırkpınar, F.2017).

2) Buffer Substances; These types of substances are generally used to prevent decreases in rumen pH that occur as a result of high intensive feed intake in ruminants. For this purpose, buffer substances such as sodium bicarbonate, various salts of volatile fatty acids, phosphate salts, ammonium chloride and sodium sulfate are also used (Kırkpınar, F.2017).

3)Chitosan; Chitosan is a linear aminopolysaccharide obtained by deacetylation of chitin. Chitin is a non-toxic and bioavailable substance similar to cellulose found in the shells of arthropods such as crabs and shrimps. In various studies, chitosan is thought to be effective on digestibility, growth performance, energy and protein utilization (Goiri et al., 2009; Kırkpınar, F.2017).

4)Beta-glucans; Beta glucan (β-glucan) is a water-soluble polysaccharide formed by the combination of glucose molecules, which can be obtained from oat and barley grains as well as mushrooms. It strengthens the immune system and shows antitumor and antimicrobial effects (Leung et al., 2006). When lignin, a phenolic compound with high molecular weight, is purified and used as an additive in animal feed, it shows similar effects to prebiotics and has positive effects on animal health (Baurhoo et al., 2008).

**(5) Coccidiostats and histomonostats category**.

They are substances used to protect animals from coccidiosis (bloody diarrhea) caused by Eimeria species protozoa that settle in the intestines of poultry (Kırkpınar, F.2017). Decoquinate, Robenidin hydrochloride, Halofuginon, Narasin, Salinomycin sodium, Diclazuril etc. substances are used.

## 2. APPLICATIONS OF FEED ADDITIVES IN AQUACULTURE

In recent years, in parallel with the increase in aquaculture production, the use of feed additives in fish feeds has become widespread in order to increase the digestibility of feed, to reduce the effects of anti-nutritional factors, and to increase production by providing fish resistance to diseases. For this purpose, probiotics and prebiotics, enzymes, amino acids, herbal feed additives are widely used in feeds. There are valuable scientific studies and reviews on the use of these substances (Yıldırım et al., 2014; Hai 2015; Yılmaz and Koç, 2016; Dawood et al., 2018; Shefat 2018; Toledo et al., 2019; Zheng et al. 2020; Guo et al., 2020; Maas et al., 2021; Zarantoniello et al., 2022; Aly et al., 2023; Liu et al., 2023) The results of some scientific studies carried out in our country and in the world in the 2000s are compiled in the form of a table and presented below. This subject is quite broad. There are many substances used and/or recommended as feed additives, as well as variables such as species, body weight, application amounts of additives. Although there are very valuable studies on each additive group, it is not possible to evaluate all of them here. For this reason, innovative studies with different characteristics conducted in recent years are included here.

**Table 2.** Feed Additives Applications from Turkey and the World

| Additives | Species | Utilization Rates | Conclusion | Reference |
|---|---|---|---|---|
| Enzyme<br>Phytase | *Onchorhynchus mykiss*<br>800 g | 4000 IU/kg feed | Decreased phytic acid in feces and increased digestibility of vegetable feed raw materials | Vielma, 2004 |
| Probiotics, Biomos<br>(Yeast *Saccharomyces cerevisiae*) | Gilthead Sea bream (*Sparus aurata*)<br>90,77±1,5 g | 0% - 10% - 20% Proportion in total ration | No difference in growth performance, mortality rate lower with higher use. | Kop, A.et al., 2005 |
| Enzyme<br>Protease | *Onchorhynchus mykiss*<br>106.16 ±16.73 g | 250 g/kg feed | Increased protein and dry matter digestibility | Drew et al., 2005 |
| Probiotic<br>Bacillus sp. | Common Carp *Cyprinus carpio*<br>6,49 ± 0,24 (in aquarium) | 1 g $kg^{-1}$ feed | It was determined that probiotics significantly increased growth performance and digestive enzyme activity and decreased FCR. | Yanbo, W.,and Zirong, X., (2006). |
| β-carotene | Cichlid (*Cichlasoma severum sp*) 0,62± 0,01 g | 50 mg/kg feed | There was a significant difference in the coloration of the fish compared to the control group. | Kop, A and Durmaz, Y. 2008 |
| L-Carnitine | Gilthead Sea bream (*Sparus aurata*) | 0 , 0.8 , 1.6 l/100 kg feed | As a result of the control group (Commercial feed 44/18) and 2 different | Demirtaş, N., 2009 |

| | | | | |
|---|---|---|---|---|
| (L-β-hydroxyl-γ-N,N,N,N-trimethylaminobutyric acid) | 198±1,5 g<br>(In a net cage environment) | | dose applications, growth increased and fatty liver decreased in the high dose application. It is positive for FCR. | |
| Sangrovit<br>(Sanguinarine Benzophenanthridine alkaloid) | Gilthead Sea bream (*Sparus aurata*)<br>10±1,07 g<br>(In a net cage environment)<br>Sea bass (*Dicentrarchus labrax*)<br>17,03±0,43 g | 500 g/ton and 750 g/ton feed<br>500 g/ton and 1000 g/ton feed | Mortality rate was less in high dose use and growth was observed positively. There was a difference in adiposity compared to the control group. FCR resulted positively.<br>Faster growth, lower mortality, less visceral adiposity (VSI) and improved FCR compared to control feed. | Korkut, A.Y., Kop, A., 2012 |
| Amino acid<br>Trypthophan | Gilthead seabream (*Sparus aurata*) | | Enhanced innate immune response | Cuesta et al. (2008) |
| Glutamine | Channel catfish (*I. punctatus*) | | Improve immune response | Pohlenz et al. (2012) |
| Instead of antibiotics<br>Selenium | Nile tilapia (*Oreochromis niloticus*) | 1, 3 and 5 mg Se/kg. | dietary supplementation with Se is useful for improving growth, | Wangkahart<br>2022 |

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| | | | antioxidant status, immune response, and disease resistance. | |
| Phytogenic feed additives:<br><br>Garlic and cinnamon | O. *niloticus*,<br><br>25 ± 5 g | 1.5 and 2 g/kg of garlic powder<br><br>8.5 and 10 g/kg of Cinnamomum powder | Garlic and cinnamon can serve as valuable immunostimulants for managing fungal infections in fish. Their inclusion in the diet enhances the immune response, consequently bolstering the fish's resistance to fungal infections. | Aly et al.,2023 |
| Butyric acid (butyrate) | *Sparus aurata*<br><br>15.06±0.26<br><br>Rainbow trout *Oncorhynchus mykiss*<br><br>8.86 ± 0.36 g | 0.3% of GUSTOR AQUA BP70®<br><br>NaB levels of 0.20% | Fish receiving butyrate showed a significant increase in weight, while increasing the availability of several essential amino acids and nucleotide derivatives.<br><br>The dietary NaB levels of 0.20% improved the growth of juvenile O. mykiss and reduced the severity of anti-nutritional factor-induced enteropathy by enhancing digestive enzymes and improving intestinal morphology. | Robles et al.,2013<br><br>Liu et al.,2023 |

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| Betain | Nile Tilapia<br>60.03±0.02 g<br>outdoors in concrete tanks<br><br>*Penaeus monodon*<br>(0.23 ± 0.00) g | d diets supplemented with 0, 0.25, 0.5, 0.75, and 1% of the dietary mixture of betaine, lactic acid bacteria, and exogenous digestive enzymes..<br><br>0.60% | , the growth performance of Nile tilapia was markedly enhanced while the feed conversion ratio (FCR) was reduced ($P<0.05$). The lipase amylase and protease activity, was significantly higher<br><br>Dietary betaine supplementation positively influenced intestine histology, lipid metabolism, and immune gene expression in the intestine. | Magouz et al.,2023<br><br>He et al.,2023 |
| Natural bioantiooxidants of flavonoid natüre<br><br>antioxidant-dihydroquercetin and<br>immunostimulator-arabinogalactan | tilapia hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*) | 25.0-50.0 mg/kg and<br>50.0 mg/kg | It was found that the addition of dihydroquercetin (25.0-50.0 mg/kg) and arabinogalactan (50.0 mg/kg) to the composition of production feeds allowed to increase productivity by 26.0 %, as well as to have a positive effect on the physiological state of fish. | Ponomarev et al.,2023 |

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| Pineapple waste crude extract (PWCE) | Pacific white shrimp (*Litopenaeus vannamei*)<br><br>0.51±0.04 g | 90, 170, and 250 ml/1000 g feed | he results showed that shrimp fed with the PWCE 250 ppt supplementation provided the highest growth rate and the best feed utilisation and yield | Klahan et al.,2023 |
| *Achyranthes aspera* L. leaves and seeds (Amaranthaceae), | Asian catfish, *Clarias batrachus* (0.352±0.008 g) | 0.25% leaves (EFL1), 0.5% leaves (EFL2), 0.5% seeds (EFS) | A. aspera seeds and leaves showed significant immunostimulatory properties in Asian catfish fry. | Kumar, et al.,2023 |
| *Ceratophyllum demersum*<br><br>Coontail | common carp (*Cyprinus carpio)*<br><br>18.89 ± 0.14 g | 5%, 10%, and 15%<br><br>*C. demersum* meal | Increasing algae levels in diets improved protein levels in body compositions while decreasing lipid. The results showed that instead of soybean meal, 5.75% and 6.07% as natural phytoremediation, C. demersum optimized the best growth and feed utilization performance in carp diets | Kiziloğlu et al.,2023 |

## 3. USE OF FEED ADDITIVES AND LEGAL REGULATIONS

Additives must comply with the following conditions in order to be used:

1. They must be completely safe for human and animal health and must have no toxin and carcinogenic effects,

2. It should not adversely affect the composition and technological properties of animal products,

3. The amounts in feed and in products derived from animals consuming this feed should be analytically detectable,

4. Improve performance effectively and economically,

5. Be environmentally safe, biodegradable and non-polluting,

6. Efficacy and stability must be established,

7. Do not cause cross-resistance with other additives,

8. It should not corrode or corrode equipment,

9. It should be easily available (Kırkpınar, F.2017).

As a result of the re-evaluation of feed additives in recent years, the use of some additives has been limited or completely eliminated. Antibiotic growth promoters have been found to accelerate the development of antibiotic-resistant bacteria and as a result put the health of both animals and humans at risk. In addition, according to the 2018-2019 EU Regulations, the use of additives such as formaldehydes, ethoxyquin, Cassia gum has been abolished (Yıldız, D,2021).

The use of feed additives is subject to certain legal regulations and approvals. Additives authorized for use in feed can only be placed on the market and used for the purpose specified in the authorization certificate. These legal regulations and approvals vary from country to country. For example, all feed additives offered for sale in the European Union must be approved according to Regulation (EC) No 1831/2003. Today, in many countries of the world and in our country, the legal regulations for feed additives are based on the European Union regulation.

The revision of the EU Feed Additives Regulation (EC) No 1831/2003 aims to support the transition to more sustainable food chains and the "Farm to Fork" strategy has been established. The aim, as explained by the EU Commission, is to facilitate market access to feed additives, in particular to reduce the environmental impact of livestock farming.

To this end, reducing the burden of proof of efficacy of additives, granting some market protection such as cost sharing to encourage generic feed additive manufacturers to apply for authorization, and modernizing labeling are the main issues to be considered by the EU Commission.

FEFAC (the European Feed Manufacturers' Federation) describes its main expectations from the revision of feed additives legislation in its 2020/21 annual report as follows;

1) the need to secure access to generic feed additives, 2) stimulate innovation in areas of strategic importance such as reducing the need for antibiotics, animal welfare or reducing environmental impact, and 3) reduce dependence on third countries for the supply of feed additives

In Turkey, the Feed Law and Feed Regulation were harmonized with the EU in 2005. In accordance with the Law No. 5996 on Veterinary Services, Plant Health, Food and Feed and its regulations, compound feed legislation was harmonized with the EU legislation in 2010. When we look at the developments with feed additives in the compound feed industry and legislation in Turkey chronologically;

- Prohibition of the use of some growth stimulant feed additives of antibiotic origin in broiler feeds in 1999,
- Regulation on Feed Additives Used in Animal Nutrition 2013,
- Communiqué on Preparation and Submission of Application File for Evaluation and Approval of Feed Additives 2016, (TAGEM 2021)

- It is seen that the regulation on the amendment of the regulation on feed additives used in animal nutrition was realized in 2022.

In our country, the mixed feed additives and importers sector is represented by the Feed Additives Manufacturers, Exporters and Distributors Association (Yem Katkıları Üreticileri, İhracatçıları ve Dağıtıcıları Derneği TÜYEKAD).

## 4. MARKET STATUS

The global animal feed additives market is segmented by additive type, livestock, form, function, and region.

By additive type, the market is classified into amino acids, antioxidants, feed enzymes, feed acidifiers, vitamins, minerals, binders, antibiotics, and others. The amino acids segment is further classified into methionine, lysine, threonine, tryptophan, and others. The antioxidants segment is sub-segmented into BHA, BHT, ethoxyquin, and others. Feed enzymes are further subdivided into phytase, non-starch polysaccharides, protease, xylanase and others. Feed acidifiers are subdivided into formic acid, butyric acid, fumaric acid, acetic acid and others. The vitamins segment is subdivided into water soluble and fat soluble. Minerals are further segmented into zinc sources, iron sources, manganese sources, and copper sources. Binders are further broken down into calcium lignosulfate, guar (Arabic) gum, and others. The antibiotics segment is sub-segmented into tetracycline, penicillin and others.

Based on livestock, the market is classified into pigs, ruminants, poultry, aquatic animals and others (horses, pets and birds).

By form, it is classified as dry, liquid and others.

By function, it is divided into single-function and multifunction.

The region is analyzed across North America (USA, Canada, Mexico), Europe (Spain, Russia, Germany, France, France, Netherlands, Italy, UK, rest of Europe), Asia-Pacific (China, India, Japan, Indonesia, South Korea, rest of Asia-Pacific) and LAMEA (Latin America, Middle East, Africa) (Shankar B., 2019).

When the results of various reports on global animal feed additives are evaluated, it is projected that the market size will reach an average of USD 35.50 billion in 2022, reaching a compound annual growth rate of 4.6% by 2030, with an average market size of USD 50 billion.

The global feed additives market is driven by the continuous increase in the global population. The world population is expected to reach 8.6 billion by 2030. Increasing global food demand will also boost the feed additives market. In addition, rising incomes, changing lifestyles and increasing health awareness have led consumers to reduce the amount of carbohydrates in their diets and adopt a high-protein diet. This has increased the global demand for meat, seafood, milk and eggs. Some of the other factors driving the feed additives market include industrialization of meat production, increasing consumer awareness regarding diseases occurring in livestock, growing concerns regarding the quality of livestock products, etc.

As a result of the use of antibiotics as growth promoters in livestock production, the European Union banned the use of antibiotics as feed additives for growth due to the threat of foodborne diseases such as salmonella and E.coli to public health and problems such as cross-resistance in drugs. Restrictions on antibiotic use in the US and Europe have increased the use of substitutes such as enzymes, probiotics, acidifiers and amino acids. (Grand view research 2023; Research and Markets,2023)

Poultry production had the largest share in the sector with 37.00% of total revenue in 2022. Aquatic feeds are prepared by mixing various raw materials and additives, taking into account the age and species of various aquatic animals such as salmon, cod, tilapia, shrimp, oysters. In addition, rising incomes and consumer awareness are increasing the demand for seafood. Asia Pacific had the largest share of the sector, accounting for more than 33.30% of total revenue in 2022. This is attributed to the presence of diverse agricultural economies and abundant livestock populations. Analyzing the sector by product, Amino acids accounted for the largest share of 34.70% of the global total revenue in 2022. Its high share is attributed to its ability to build immunity and support animal growth. It helps prevent hoof and skin problems in animals. It also plays an important role in preventing brain dysfunctions that can cause muscle coordination disorders in animals. Therefore, amino acids are predicted to have a high demand from the product industry as a feed supplement and pet food ingredient.

Competition in the global industry largely depends on the quality of the product, the number of manufacturers and distributors and their geographical locations. In addition to this, key companies are conducting research and development activities to provide consumers with efficient products that are expected to fuel market growth. Some of the prominent companies in the global animal feed additives market are: Cargill Inc, Hong Ha Nutrıtıon, Basf Se, Kemin Industries, Inc., Anova Group, Biomın Holding Gmbh, Olmix Group, ADM, Evonik Industries, Chr. Hansen Holding, Alltech, Inc.

Increasing demand for processed meat, which increases the consumption of feed additives, and hence the demand for additives in the animal nutrition and feed industry. However, different government regulations for the use of some synthetic additives and increasing production costs of effective feed additives restrain the market growth

The production amount of compound feed additives in Turkey in 2019 was 276,565 (tons/year). The import amount was 608,343 tons and $1,370,000 was paid. In the same year, the export amount was 1,280,509 tons and $ 677,419 was obtained. Additives are the second most imported group after raw materials with 608 thousand tons. Kaolin, chemical products, acids and premixes are important products of the group in terms of import volume. In addition, vitamins and yeasts are also reported to be products with a high level of demand in the sector. The most important product group in exports is premixes and bentonite with 1.3 million tons. In terms of foreign trade balance, Turkey has been a net exporter of additives and prepared feeds after 2015. It is also stated that the share of exports of additives by transforming imported raw materials into premixes is very important (TAGEM, 2021).

## 5. PROBLEMS AND RECOMMENDATIONS:

Adopted in 2003, the Feed Additives Regulation lays down the rules for the authorization of feed additives and their placing on the market. The Commission has established the European Union Register of Feed Additives, which is regularly updated. Since its entry into force in October 2003, the feed additives regulation has been updated in line with scientific and technological developments. Most recently, two new functional groups for feed additives have been introduced: "Other technological additives" under the Technological category and "Physiological state stabilizers" under the zootechnical category. As of April 5, 2023, the new register in PDF format has been replaced by an online version.

The European Commission is carrying out a study to assess whether the current legislation covering feed additives for use in animal nutrition is fit for purpose. The current EU Feed Additives Regulation (EC) No 1831/2003 is in serious need of revision to ensure that it meets some of the key objectives set out in the EU FARM TO FORK strategy. The Commission's assessment has identified factors that are hindering the achievement of some of the objectives.

In line with the action plan of the 'farm to fork' strategy, the objective of the proposal is to contribute to a more sustainable food production system by establishing new criteria to promote the authorization of feed additives with positive effects on animal welfare and animal welfare. In particular, it is to facilitate market access to feed additives to reduce the environmental impact of livestock farming. Mechanisms will be established to incentivize innovations in feed additives, in particular those that contribute to reducing the use of antibiotics and mitigating climate change impacts. Feed additives and veterinary medicines used in medicated feeds should be assessed for safety and used under specified conditions of use as pre-approved by the competent authorities.

Veterinary medicines used in medicated feed should comply with the provisions of the Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programs for the Use of Veterinary Medicines in Food Producing Animals. The boundaries between feed additives and veterinary medicines used in medicated feeds can be set to prevent misuse. Feed additives should be received, used and stored to maintain their integrity and minimize misuse or unsafe contamination. Feed containing them should be used in strict accordance with clearly defined instructions for use. Antibiotics should not be used in feed for growth-promoting purposes unless there is a public health safety assessment.

Additional objectives include speeding up risk assessment processes and reducing the administrative burden of permit holder applications to bring innovative feed additives to the market earlier. In particular, it is emphasized that all authorizations for candidate products that can be used as feed additives should be proprietary. Only authorizations for zootechnical feed additives and coccidiostats can benefit from this status. All authorizations for nutritional, technological and sensory feed additives are general, meaning that once one of these products is approved, any other company can sell the same product as long as it meets the specifications set out in the authorization. Therefore, due to the financial burden of generating data and then receiving no market protection once authorized, companies are not investing in new products that fall into these 'generic' categories of feed additives. In addition to granting some market protection to encourage generic feed additive producers to apply for authorization (cost sharing), other objectives are to reduce the burden of proving efficacy, modernize labelling and reduce dependence on third countries for the supply of feed additives. (IFIF,2021).

For Turkey, feed additives are a product group for which production is insufficient despite high demand. Therefore, external dependency in these products is an important situation. The fact that the imports of these products are over 1 billion US dollars reveals the importance of this product group. In addition, some difficulties in international markets and exchange rates can increase the cost pressure of imports on the product.

In the sector report prepared by TAGEM to compensate for these situations, it is reported that it is important to support feed additives in order to increase domestic production as much as possible, but it does not seem possible to minimize this ratio in the short term. For this reason, it is suggested to prioritize and support R&D studies for the production of important inputs such as additives and vitamins in animal feeds with domestic resources and to prioritize and support R&D studies for the production of important inputs such as additives and vitamins in animal feeds with domestic resources, as well as contracted production in Turkey or abroad, putting into effect practices that facilitate imports and reduce costs (fixed exchange rate, logistics and stock management, etc.) (TAGEM, 2021).

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# CHAPTER 12

## USING STORED-PRODUCT PEST IN FEEDING FISH LARVAE CULTURE: A FOCUS ON MEALWORM

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## INTRODUCTION

The world population is increasing rapidly and these bring environmental degradation and global climate change with them. According to the estimates of the International Feed Industry Federation (IFIF), there will be more than 10 billion people worldwide by 2050. The biggest problem in the future will be finding sustainable protein production methods with minimal environmental impact due to the increasing global population and protein demand.

Insects have become more commonly accepted as a significant source of sustainable raw materials for animal feed, particularly in fish, poultry, and swine. (Bessa et al., 2020; Sogari et al., 2019). Many insect species are known to plant pests that result in significant losses. On the other hand, insects are considered beneficial when they carry out beneficial activities like pest management and pollination or when they are produced specifically for human use. *Tenebrio molitor* is an insect species commonly known as mealworm and yellow mealworm. It is a member of the family Tenebrionidae, a group of insects that includes beetles from the order Coleoptera. Due to its adaptability, simplicity in rearing, and use as a model organism for research in entomology, aquaculture, biochemistry, and nutrition, *T. molitor* has been the subject of extensive studies.

*T. molitor* has traditionally been regarded as a pest because it consumes or degrades the quality of stored foods and grain products. This tiny creature is a significant problem in terms of plant protection. However, They can be used as food due to their high nutritional value (Chen et al., 2010). For the purpose of evaluating the inclusion of *T. molitor* larvae in monogastric animals such as fish diets, several studies have been carried out. Fresh mealworms have a protein content that is nearly equal to that of fish and higher than that of milk, eggs, pigs, cattle, and mutton. Additionally, larvae have higher concentrations of nutrients like vitamins, inorganic salts, fats, and carbohydrates (Hong et al., 2020; Oonincx et al., 2012; Zhao et al., 2016). In this situation, *T. molitor* might be considered either a pest or a beneficial insect (Bessa et al., 2020; Sogari et al., 2019). They are also suggested as a source of human nourishment and provide an alternative to protein-rich animal feed. *T. molitor* larvae may also be helpful because they can effectively break down plastic and polystyrene waste (Brandon et al., 2018). Because of these factors, *T. molitor* is currently being thought about for industrial-scale production.

Certain nations have authorized the use of commercially produced *T. molitor* larvae or protein concentrate products as protein supplements. The insects will be a significant part of future protein sources for animal feed because of their nutritional value as well as their effects on the environment. For instance, compared to other animal species, insects produce less greenhouse gas, use less water, and use less arable land. Also, *T. molitor* is frequently used to biodegrade organic waste into proteins (Miglietta et al 2015; Hong et al., 2020; Oonincx et al., 2012)

From this point of view, fish and other aquatic products are the first to come to mind as a cheap, nutritious and healthy source of animal protein. Animal proteins play an important role in a healthy and balanced diet. In this respect, fish meat, which is rich in protein and unsaturated fatty acids, is essential for human health as a source of animal protein (Özuğur et al., 2019). Fish is considered as an important protein source today by examining the nutritional content of fishery products and knowing the effect of nutrients on human health (Turan et al., 2006).

The success of the early stage feeding protocol in the fish species culture is very important in terms of reducing larval losses. For this reason, it is necessary to produce and use live food organisms that suitable for the immature digestive system and first mouth opening size of fish larvae. In this regard, some zooplankton species such as *Brachionus plicatilis*, *Artemia* sp. are commonly used in fish farms. However, the fact that rotifer production is difficult and costly and the Artemia supply and enrichment costs are high, make it necessary to evaluate alternative live food organisms. The use of zooplankton is essential not only in the production of table fish species, but also in the larval rearing protocols of most economically important ornamental fish species. Similarly, this process creates a significant cost in hobbyist or commercial ornamental fish farming. So, feeding live baits in freshwater and marine fish hatcheries creates high economic costs. This makes it necessary to transition to artificial powder feeds as soon as possible. For example, Person Le Ruyet et al. (1993), reported in a study that making the transition to artificial feeding 15 days earlier in sea bass (*Dicentrarchus labrax*) larval rearing could result in 80 percent savings in artemia usage rates. In general, the first food is live baits with the larvae absorbing the yolk sac (mouth and anus opening) and after a certain period of time, the transition to artificial powder feeds takes place. Reducing the usage of live baits and the successful use of artificial feeds are factors of species-specific digestive system development pattern and digestive enzymes. Determining and applying the most appropriate feeding procedures for the larvae are very important in terms of reducing larval losses and terminating the use of zooplankton as soon as possible.

Considering all these reasons, it is very important that aquatic products, which are an important source of animal protein, can be produced at lower costs. The most important determining factor in achieving this situation is reducing larval feeding costs.

## 1. MATERIAL AND METHOD

As commercial aquaculture expands, marine fish culure depends on the supply of live food organisms. Compound diets are considered an alternative to live prey in rearing fish larvae (Cahu and Zambonino-Infante, 2001; Tovar-Ramırez et al., 2004). Microparticle foods and other types of micro-pellet baits have expensive and difficult production procedures. In addition, there are no particles that can completely replace live feed in larval rearing today. However, there are practices that reduce the cost of live feed by mixing it in certain proportions. When the importance of live feed in the larval rearing protocol is examined, the financial benefit of reducing usage rates will be clearly understood. For instance, it has been determined that the use of artemia, which accounts for a large share of feed costs in rearing sea bream larvae, can be reduced by 25% by substitution (Gamsız and Alpbaz, 2006). This situation makes it necessary to find creatures such as brine shrimp and rotifers that can be alternatives to expensive zooplankton. For this reason, the search for alternative living species in early life stage feeding studies in fish has become more important in recent years.

The most decisive parameters in choosing the species planned to be used as live bait include; their fast life cycle, rapid reproduction, and the easy availability and low cost of nutrients to be used in survival and reproduction. Apart from these characteristics, when maximum stock density is reached, mortality should be low and the culture should be continuous.

One of the important problems in the use of live feed is the risk of disease contamination. Since live food directly feeds fish larvae, it is more susceptible to possible disease agents. Vinegar eels are an important example in terms of their easy and rapid reproduction and the fact that the nutrients used to increase the stocks are cheap. In feeding and reproduction of these live organizms creatures as a culture medium, a glass jars containing fifty percent apple cider vinegar and fifty percent apple juice by volume are sufficient. And also when the sizes of vinegar eels are examined, the thickness of body region average was determined as 57 µm (Önalan and Sepil, 2019). Its size is suitable for feeding early stage larvae and its propagation in a very low-cost medium reveals its potential to be used as live bait.

Similarly, in this study, mealworms were evaluated as a living organism that can reproduce quickly and easily. After mealworm broodstocks were caught from their natural environment, they were subjected to the process of shaping them for fattening and breeding purposes in containers consisting of a mixture of flour and oats (Figure 1). Breeding conditions were observed by supplementing these nutrients at regular intervals to the broodstock placed in plastic containers with small holes in the lid at appropriate stocking density (approximately 15-20 individuals/L).

**Figure 1.** Nutrient medium prepared for mealworm.

Once the eggs were detected, the larvae were raised in other containers with the same nutritional content. It was aimed to determine the sizes of meal worm larvae examined daily and their usability in different ontogenic processes, from early stage larval feeding to the feeding of broodstock fishes. Fort this aim, in the photographs obtained by imaging with a stereo microscope (Nikon SMZ 745T) at daily intervals, a software (ImageJ 1.46) was used and some external morphometric measurements of worms were measured. In order to determine the acceptability of worms to the larvae, mealworm larvae produced in culture medium were tested in some fishes in the post-larval stage and with different mouth sizes (*Puntius tetrazona*, *Danio rerio* and some Malawi cichlid species). Additionally, the survival time of the worms in water column, their sinking speed and mobility in the water were monitored.

## 2. RESULTS AND CONCLUSION

When the sizes of mealworm eggs and larvae were evaluated, it was determined that were not suitable for the first mouth gap sizes of altricial fish larvae. Data shows that mealworm larvae cannot be used instead of rotifers, but can be used as a substitute in the post-larval stage and starting from the transition to powdered baits (Figure 2). Reducing the time or amount of feeding on artemia will provide a significant advantage at this stage, as it will reduce the cost. From this perspective, it can be thought that the role of mealworm larvae in fish feeding is similar to that of water fleas (*Daphnia* sp.) in terms of their size (Figure 3). In the trials, it was determined that mealworm larvae remained alive in the water column for more than 1 minute (80-85 seconds) and their movements in the water were fast and lively, creating attraction for the larvae.

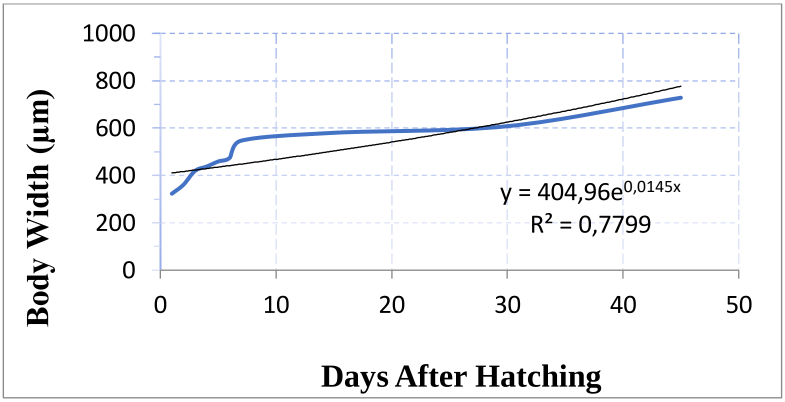


**Figure 2.** Graph of body width development of mealworm larvae.

Although the worms that died in the water varied depending on their size, it was determined that they descended to the tank floor in an average of 9 s ± 3 s in the 50 cm water column. Apart from this, it has been determined that worms with a diameter of 0,5-1 mm can also be used in broodstocks of some fishes (zebra, tetrazone and cichlid fishes).


**Figure 3**. Mealworm 3 days old eggs and larvae from 2th to 7th days.

The mealworm species used in the study is a creature that should be evaluated in commercial fish production and has a high potential in this regard, as it has an easy production procedure and rapid reproduction without requiring labor. The fact that the nutrients used as media (flour, oats, etc.) are easily and cheaply available also increases the importance of the species. It can be used for feeding from the post-larval stage, as well as being a live feed that can be used to increase the fecundity of brodstock fishes. Creating intensive stocks and producing them in the form of continuous cultures will make a great contribution to aquaculture production. Mealworms which reaches different sizes in the larva and pupa stages (Figure 4), can also be used for different purposes in fish feeding (larval feeding, increasing fecundity in broodstock).

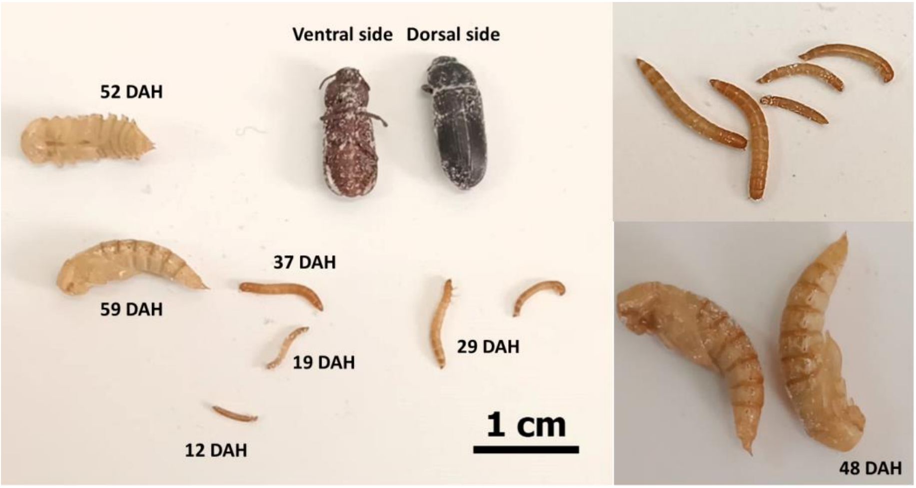


**Figure 4.** Different larval-pupal stages and adults.

*DAH: Day after hatching

It is important to determine the nutritional content of this species and to establish intensive culture conditions in future studies. Determining the morphometric properties and nutritional contents of mealworms and different alternative species and evaluating their usability in fish larvae are very important in creating low-cost feeding protocols for fish culture.

In addition, it is ecologically very important to evaluate these and similar insects, which are considered harmful to various agricultural nutrients, and to use them as feed for other living creatures.

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# CHAPTER 13

## FEED MICROSCOPY (INNOVATIONS AND APPLICATIONS)

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## INTRODUCTION

FAO's 2030 forecast for fisheries and aquaculture projects an increase in production, consumption and trade, albeit at slower growth rates, with total aquatic animal production expected to reach 202 million tons in 2030, thanks to the continued growth of aquaculture, which is forecast to reach 100 million tons in 2027 and 106 million tons in 2030.

FAO's 2030 forecast for fisheries and aquaculture projects an increase in production, consumption and trade, albeit at slower growth rates, with total production of aquatic animals expected to reach 202 million tons in 2030, thanks to the continued growth of aquaculture, which is estimated to reach 100 million tons in 2027 and 106 million tons in 2030. The reason for this increase is not only the increase in the human population but also the fact that fish oils and fish products are healthier than other food sources. As a result of the increase in overweight people and obesity-related diseases in many countries around the world, healthy nutrition has become increasingly important, and especially in recent years, the demand for healthy and nutritious foods such as seafood has increased. Studies on the production of new aquatic species are ongoing. In order to ensure the sustainability of valuable and nature-dependent raw materials such as fish meal and oil, trials of feeds produced using new / alternative raw materials are also continuing (FAO, 2023).

Feed is an important expense in aquaculture and livestock production. Feed accounts for 40-60% of the total cost of fish production. Nutritionally complete diets are necessary to optimize the growth of livestock by increasing feed efficiency. This type of feed can also reduce disease-related costs due to its healthy and nutritious properties. When disease problems are encountered, questions about feed quality are inevitable.

When raw materials arrive at the feed mill, they must be checked for quality characteristics before storage. Laboratory analyses are the analyses that evaluate feed and feed components in terms of nutrient adequacy, quality and contamination risks. These analyzes are classified as chemical, physical and biological analyzes. Chemical analyzes determine the nutritional properties of feeds and feed raw materials such as protein, fat, cellulose, etc. Physical analyses determine changes in structural properties such as pollination, breakage, and the presence of foreign substances such as stones, metals or insects. Biological analyzes include the stages of testing the effectiveness of feed or feed raw materials on living organisms. Breeders and feed producers must monitor the quality of feed and raw materials to ensure the quality of the product they buy and sell. Similarly, knowing the source of the raw material is an advantage for the feed producer to produce quality feed. Microscopic examination of feeds is an ideal method to determine the quality of raw materials and feed. Feedstuffs should be examined microscopically if the source, purity, or contaminants and other specific characteristics of feedstuffs are of interest. In microscopic examination, these substances cannot be stored. Therefore, microscopic examination is known as a quality program (QA) for the examination of aquatic feeds and feed raw materials. The separation power and speed of these analyzes is the biggest advantage (Bates, 2018).

Microscopic examination of feeds and feed raw materials is more important than the examination of their nutrient content. This is because many aquatic species require high levels of protein. For this reason, protein sources of animal origin are used in high proportions in the feeds of aquatic animals, especially carnivorous animals. However, the prices of raw materials such as fish meal, which has high nutritional properties and a balanced amino acid profile, are quite high. The use of such raw materials at high rates increases feed prices and therefore the cost of production. For this reason, a wide variety of animal-derived flours with similar appearance but lower quality and price are used in feeds.

In addition, the processing of all feed raw materials and especially the various processes in the production stages of raw materials of aquatic origin directly affect the quality. For example, during the production of fish meal, mechanical effects such as transportation of the product before processing, temperature of cookers and dryers, drying method, temperature of the final product going to storage, grinding process affect the color, appearance and nutritional quality of the final product. Microbial changes can occur in some raw materials made from fish processing residues and problems such as disease can increase. Aquatic raw materials are expensive and deteriorate very quickly. For this reason, the most common bad practices are to add protein-like substances to feeds or to add imitation substances that will give protein value in analyzes. One such additive, ammonium nitrate, is cheap and readily available as fertilizer. It used to be widely available in feeds. However, with the Nessler marker, it can be chemically detected, seen with a microscope and its effect can be stopped. Animal feces, fermented plants, low quality flours are other substances that impair purity in feeds. These adversely affect the amino acid balance in the feed (Bates, 2018). Raw materials can be damaged in unshaded or unprotected storage areas. Increased bacterial or fungal activity in products stored in too humid or inadequate conditions causes very rapid deterioration. The high drying process to minimize the moisture problem that causes mold accelerates oxidation, which reduces the quality of the raw material.

Quality control starts before the materials are unloaded at the factory. Natural changes, dirt, additives and contaminants in the raw materials can be easily observed under a microscope and compared to acceptable standards. Acceptance or rejection decisions are made by the Quality Assurance manager based on the result of appropriate comparisons against established standards or internal defect action levels. Each ingredient in finished feeds is verified by qualitative identification and checked against the feed label. Quantitative verifications of ingredients are checked against the feed label ingredient list as well as chemical analysis (Bates, 2018).

However, light microscopy is so far the only method officially recognized by the European Commission for the detection of animal proteins. The regulation on this subject was first published in 1970. It was subsequently updated in various years and most recently in 2020.

## 1. TYPES OF MICROSCOPIC ANALYSIS

Microscopic examination of feeds is based on the comparison of unknown substances with substances in known standards. Microscopic examination of feeds is divided into 2 main groups as Qualitative and Quantitative. Qualitative examination is the detection of foreign substances and raw materials in the mixture or alone, the examination of surface properties or cell and internal particle properties. The proportion of contaminants, degradants in the raw material or the proportion of each raw material in the prepared feed is quantitative examination. Polarized light, density, particle separation and chemical spot tests are added to the qualitative and quantitative tests. PCR and DNA extraction methods were added to these tests by the European COMMISSION REGULATION (EU) No 51/2013 of 16 January 2013.

There are two general methods for examining the substances contained in feeds. In one of the methods, an 8x - 50 x stereomicroscope is used and the substances examined are separated according to their superficial (external) and physical properties. In this method, small samples are needed, feed items are examined in terms of odor, brightness, structure, solidity, softness, particle size, color, shape.

Another method is to examine the cellular or histological structure of feed raw materials using a compound microscope. It is used to examine finely ground raw materials that cannot be distinguished by their structural appearance. This method requires more technical skills. The raw materials are usually immersed in immersion oil, then examined at magnification from 40x to 400x depending on the type of microscope. 100x magnification is the most commonly used type (Bates et al., 1992).

In the absence of a specialized microscopist, routine screening of incoming raw materials can be done at the receiving point of the mill using a 6x or 8x hand lens in the hands of a trained supervisor. At this point about 90% of potential problems can be avoided or corrected (Bates, 2018). Better results will be obtained if these 3 methods are used together.

## 1.2. Materials And Methods Used in Microscopic Analysis

The list of materials given below may vary according to personal needs, technological developments, the characteristics of the samples to be examined and the budget.

**a) Apparatus**

- Known raw material samples and raw material mixtures (Reference sample sets)

- Airtight, containers or glass vials.

- Stereomicroscope with wide-angle, zoom objective with 8x - 50x magnification for fast work.

Stereomicroscopes consist of 2 identical ocular systems. There is a 16 degree angle between the ocular axes. This ensures that the two axes intersect on the object to be examined. A prism is used to obtain a flat image of the observed object. The lowest power stereomicroscope requires natural lighting. The most suitable light source is halogen systems.

- A 40x - 400x magnification compound microscope with adequate illumination. They are designed for clear-transparent, small and thin objects. An adjustable condenser with filter carrier and field diaphragm is also required. They have an illumination system.

- Illuminating (double light), cheap high intensity reading lamps can be used, but the light needs to be filtered. Top and bottom lighting should be used depending on the condition of the sample.

- Ocular micrometer

- Stainless steel or plastic sieves with pore sizes from 20 µm to 125 mm.

- Mechanical or electronic balance (0.1 - 0.01 gr. error margin).

- Hand tools : Clean, steel forceps; straight and curved separator needles; microspatula with clean, steel end, straight and curved; small brush; plastic ruler; straight knife and box for storage of all tools.

- Small plates: Each white porcelain and black coated glass.

- Glass petri dishes (150 mm), porcelain dishes, small bottle (50 ml), aluminum pan and polypropylene cup or paper.

- Filter paper, quartz (Whatman No 1) or coffee filters.

- Syringe, brush, lens paper, cotton sponge with paper body, methanol for cleaning instruments (Bates et al., 1992; Ergen 2007; Bates 2018)

**b) Microscopic Analysis Methods**

The collected samples should be stored in tightly closed small glass bottles or plastic containers in dark and cool environments to avoid mixing with each other, microbial contamination and insects. It is very important that sampling is done well in order to obtain accurate results from the analyzes.

*Sample Preparation*

First, a group of samples should be taken and the type of analysis should be decided. The samples must first be mixed thoroughly. In manual mixing, a wide spatula should be used to spread the materials diagonally on the paper.

1. Grinding and Separation; It is applied to reduce the particle size of the samples. A mortar or pestle is used to break the large pieces of all samples, thus ensuring homogeneous particle size. If the samples are pellet feed, they should be crushed in a mortar and pestle before mixing. Raw material can be directly sieved to be examined under a microscope (Bates et al., 1992; Khajarern & Khajarern 1999). If the samples are to be analyzed with a stereomicroscope, they should be examined after passing through 420-840-2000μ sieves without disturbing the original structure. The grinding process may cause the analysis to give erroneous results. In normal microscope analysis, the samples should be ground after passing through a 420μ sieve in order to clearly see the histological structures of the samples. After 1-2 g of the sample is taken, it is heated in 50 ml of 8% KOH solution for 30-45 minutes. It is cooled at room temperature and then examined under a microscope. If the sample is not clear enough, it is gently heated in acidic chloral hydrate solution for 10-15 minutes (Khajarern & Khajarern 1999).

2.Flotation and removal of oils; In order to determine the mineral substances in pellet feeds, these substances must first be purified from organic substances. For this purpose, organic solvents such as chloroform, petroleum ether, acetone, hexane, benzene and carbontetra chloride are used. The sample is liberally wetted with these organic solvents in the evaporation container. The part that decomposes and settles to the bottom contains inorganic and the floating part contains organic substances. This process is called flotation. The liquid is taken into a separation vessel and the settled part and the floating part are separated. The settled part is kept in a container and at room temperature until it dries. The sample is then analyzed for the type of mineral matter with the help of stereomicroscope and chemical spot tests (Khajarern & Khajarern 1999).

**Stereomicroscope Analysis**

The first thing to do before starting the analysis is to make an assessment of the color, smell, taste, texture and appearance of the raw material or pellet feed to be examined and the opinions should be noted. Then the prepared samples should be spread neatly on pieces of paper. The sample should first be divided into two parts and then these parts should be divided again and 4 groups should be obtained. A certain amount of these groups should be taken and spread on petri dishes and the examination should be started from one place and proceed straight ahead. The samples should be examined in this way with a 10x - 20x stereomicroscope. Separation of the examined material from the unexamined material should be done with a needle or clamp. Sharp, pointed forceps are recommended for this purpose.

If the sample is a mixture, each raw material should be checked and marked in the raw material list. Raw materials not listed should be recorded. If none of the raw materials are identified, the sample should be re-examined.

The processes described above are used for many recognized raw materials, and mineral substances are also added to feeds as salt, calcium and phosphorus. The presence of trace minerals (in small amounts) can be detected by chemical spot tests (Bates et al., 1992)

**Compound Microscopy Analysis**

Stereomicroscopic examinations by experienced persons are sufficient to identify spoilage, contaminants and raw materials in feed. If the raw materials are in small quantities or in very thin layers, it is very difficult to examine with a stereomicroscope. The sample must be recognized by its histological structure. For this reason, a compound microscope should be used. Usually 100x magnification is sufficient for examination.

A small amount (0.1 g) of the finely ground sample is taken and placed on the slide. A drop of carrier liquid is added, the sample is thoroughly mixed and spread evenly over the entire slide and examined with a compound microscope. Avoid using too much sample and liquid. They limit light transmission and make the examination difficult. For wetting the sample: pure water; glycerol; mineral oil; chloral hydrate and glycerol; several liquids such as 100 g of crystalline phenol plum in 20 ml of glycerol. The examination starts from the upper left edge and continues to the right. Then the width of the slide is reduced to a lower area and the examination is continued from the right corner to the left corner. The examination is continued in this way until the entire slide area is covered.

**Spot and Other Chemical Tests**

Although the microscopic analyst uses both the physical surface properties of the particles (color, gloss, irregularities, etc.) and cellular structures (cell walls, hairs, starch grains, etc.) to identify components, chemical tests are an important aid to identification. This test method is used to quickly check correct formulation and mixing for the presence or absence of drugs and other feed additives. In these analyses, precipitation, color formation, color change of the indicator and gas formation with some chemical reactions are used as determining criteria. It is an economical and rapid analysis (Bates et al., 1992; Khajarern & Khajarern 1999).

In spot tests, samples with markers for color separation react with chemicals. As a result of the reaction, the color change or the formation of blistering/blistering can be seen with the naked eye or under a stereomicroscope. The results are compared with previously prepared charts (Tables 1). In some analyses, 1-2 tests are sufficient, while in others it may be necessary to use several markers. The amount of marker, temperature and freshness are important here (Bates et al., 1992; Ergen D., 2007).

## 2. CHRONOLOGICAL LIST OF THE IMPLEMENTING REGULATION OF THE COMMISSION OF THE EUROPEAN UNION AND ITS AMENDMENTS BETWEEN 2003 AND 2020

The regulation for the identification of animal proteins by the European Commission was first published in 1970

First Commission Directive 71/250/EEC of 15 June 1971 establishing Community methods of analysis for the official control of feedingstuffs (2),

Second Commission Directive 71/393/EEC of 18 November 1971 establishing Community methods of analysis for the official control of feedingstuffs (3),

Third Commission Directive 72/199/EEC of 27 April 1972 establishing Community methods of analysis for the official control of feedingstuffs (4),

Fourth Commission Directive 73/46/EEC of 5 December 1972 establishing Community methods of analysis for the official control of feedingstuffs (5),

Fift Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feedingstuffs (6),

Seventh Commission Directive 76/372/EEC of 1 March 1976 establishing Community methods of analysis for the official control of feedingstuffs (7),

Eight Commission Directive 78/633/EEC of 15 June 1978 establishing Community methods of analysis for the official control of feedingstuffs (8),

Ninth Commission Directive 81/715/EEC of 31 July 1981 establishing Community methods of analysis for the official control of feedingstuffs (9),

Tenth Commission Directive 84/425/EEC of 25 July 1984 establishing Community methods of analysis for the official control of feedingstuffs (10),

Eleventh Commission Directive 86/174/EEC of 9 April 1986 fixing the method of calculation for the energy value of compound poultry-feed (11), Twelfth Commission Directive 93/70/EEC of 28 July 1993 establishing Community methods of analysis for official control of feedingstuffs (12),

Commission Directive 93/117/EC of 17 December 1993 establishing Community methods of analysis for official control of feedingstuffs (13),

Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC (14),

Commission Directive 1999/27/EC of 20 April 1999 establishing Community methods of analysis for the determination of amprolium, diclazuril and carbadox in feedingstuffs and amending Directives 71/250/EEC, 73/46/EEC and repealing Directive 74/203/EEC (15),

Commission Directive 1999/76/EC of 23 July 1999 establishing a Community method of analysis for the determination of lasalocid sodium in feedingstuffs (16),

Commission Directive 2000/45/EC of 6 July 2000 establishing Community methods of analysis for the determination of vitamin A, vitamin E and tryptophan in feedingstuffs (17),

Commission Directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs (18),

Commission Directive 2003/126/EC of 23 December 2003 on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs (19).

**COMMISSION DIRECTIVE 2003/126/EC OF 23 DECEMBER 2003** on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs consist of the following headings (https://eur-lex.europa.eu/eli/dir/2003/126)

1. Objective and field of application

2. Sensitivity

3. Principle

4. Reagents

5. Equipment and accessories

6. Procedure

7. Calculation and evaluation

8. Expression of the result of the examination

9. Optional protocol for analysing fat or oil

**COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009** consist of the following headings:. (https://eur-lex.europa.eu/eli/reg/2009/152/)

1. Purpose and scope

2. Definitions

3. General provisions

4. Apparatus

5. Quantitative requirements as regards number of incremental samples

5.1. Quantitative requirements as regards incremental samples in relation to the control of substances or products uniformly distributed throughout the feed

5.1.1. Loose solid feed

5.1.2. Loose liquid feed

5.1.3. Packaged feed

5.1.4. Feed blocks and mineral licks

5.1.5. Roughages/forage

5.2. Quantitative requirements as regards incremental samples in relation to the control of constituents or substances likely to be distributed non-uniformly in feed

5.3. Quantitative requirements as regards the incremental samples in the case of very large lots

6. Quantitative requirements as regards aggregate sample

7. Quantitative requirements as regards final samples

8. Method of sampling for very large lots or lots stored or transported in a way whereby sampling throughout the lot is not feasible

9. Instructıons for takıng, preparing and packaging the samples

10. Sampling record

**COMMISSION REGULATION (EU) NO 278/2012 of 28 March 2012** consist of the following headings: https://eur-lex.europa.eu/eli/reg/2012/278/

B. Determination of the levels of dıoxins (pcdd/pcdf) and pcbs

CHAPTER I;Methods of sampling and interpretation of analytical results

CHAPTER II; Sample preparation and requirements for methods of analysis used in official control of the levels of dioxins (PCDD/PCDF) and dioxin-like PCBs in feed

CHAPTER III; Sample preparation and requirements for methods of analysis used in official control of the levels of non-dioxin-like PCBs (PCB # 28, 52, 101, 138, 153, 180)

**COMMISSION REGULATION (EU) No 51/2013 of 16 January 2013** consist of the following headings (https://eur-lex.europa.eu/eli/reg/2013/51/)

ANNEX VI Methods of Analysis for The Determination of Constituents Of Animal Origin for the Official Control of Feed

1.Purpose And Scope

2. Methods

2.1. Light microscopy

2.1.1. Principle

2.1.2. Reagents and equipment

2.1.3. Sampling and sample preparation

2.1.4. Microscopic examination

2.1.5. Expression of the results

2.2. PCR

2.2.1. Principle

2.2.2. Reagents and equipment

2.2.3. Sampling and sample preparation

2.2.4. DNA extraction

2.2.5. Genetic amplification

2.2.6. Interpretation and expression of results

**COMMISSION REGULATION (EU) No 691/2013 of 19 July 2013**, amending Regulation (EC) No 152/2009 as regards methods of sampling and analysis consist of the following headings (https://eur-lex.europa.eu/eli/reg/2013/691)

Annex I

Methods Of Sampling

1. Purpose And Scope

2. Definitions

3. General Provisions

4. Apparatus

5.Quantitative Requırements As Regards Number Of Incremental Samples

6. Quantıtatıve Requırements As Regards Aggregate Sample

7. Quantıtatıve Requırements As Regards Fınal Samples

8. Method Of Samplıng For Very Large Lots Or Lots Stored Or Transported In A Way Whereby Samplıng Throughout The Lot Is Not Feasıble

9. Instructıons For Takıng, Preparıng And Packagıng The Samples

10. Samplıng Record

Annex Iı

General Provısıons On Methods Of Analysıs For Feed

A. Preparatıon Of Samples For Analysıs

1. Purpose

2. Precautions to be taken

3. Procedure

4. Storage of samples

B. Provısıons Relatıng To Reagents And Apparatus Used In Methods Of Analysıs

C. Applıcatıon Of Methods Of Analysıs And Expressıon Of The Results

1. Extraction procedure

2. Clean-up procedure

3. Number of determinations

4. Reporting of the method of analysis used

5. Reporting of the analytical result

6. Measurement uncertainty and recovery rate in case of analysis of undesirable substances

**COMMISSION REGULATION (EU) No 709/2014 of 20 June 2014,** amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls consist of the following headings (https://eur-lex.europa.eu/eli/reg/2014/709)

ANNEX

In Annex V to Regulation (EC) No 152/2009, Part (B) 'DETERMINATION OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND PCBs' is replaced by the following:

'B. DETERMINATION OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND PCBs

CHAPTER I

Methods of sampling and interpretation of analytical results

1. Purpose and Scope

2. Compliance of the lot or sublot with the maximum level

3. Results exceeding action thresholds as laid down in Annex II to Directive 2002/32/EC

CHAPTER II

Sample preparation and requirements for methods of analysis used in official control of the levels of dioxins (PCDD/PCDF) and dioxin-like PCBs in feed

1. Field of application

2. Background

3. Quality assurance requirements

4. Requirements for laboratories

5. Basic requirements to be met by analytical procedure for dioxins (PCDD/PCDFs) and dioxin-like PCBs.

6. Specific requirements for GC-MS methods to be complied with for screening or confirmatory purposes

7. Specific requirements for bioanalytical methods

8. Reporting of the results

CHAPTER III

Sample preparation and requirements for methods of analysis used in official control of the levels of non-dioxin-like PCBs (PCB # 28, 52, 101, 138, 153, 180)

1. Field of application
2. Applicable detection methods
3. Identification and confirmation of analytes of interest
4. Demonstration of performance of method
5. Limit of quantification
6. Quality control
7. Control of recoveries
8. Requirements for laboratories
9. Performance characteristics: criteria for the sum of the six indicator PCBs at the maximum level
10. Reporting of the results

**COMMISSION REGULATION (EU) 2017/645 of 5 April 2017**,correcting the Latvian language version of Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed (https://eur-lex.europa.eu/eli/reg/2017/645)

**COMMISSION REGULATION (EU) 2017/771 of 3 May 2017**,amending Regulation (EC) No 152/2009 as regards the methods for the determination of the levels of dioxins and polychlorinated biphenyls (https://eur-lex.europa.eu/eli/reg/2017/771)

In Annex V to Regulation (EC) No 152/2009, Part B 'DETERMINATION OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND PCBs' is replaced by the following:

**COMMISSION IMPLEMENTING REGULATION (EU) 2020/1560 of 26 October 2020**, amending Annex VI to Regulation (EC) No 152/2009 laying down the methods of analysis for the determination of constituents of animal origin for the official control of feed (https://eur-lex.europa.eu/eli/reg_impl/2020/1560/).

## 3. APPLICATION EXAMPLES OF MICROSCOPIC ANALYSIS IN FISH FEED

Samples were taken from various raw materials and pellet feeds used in fish feed production and microscopic analyzes were performed. Samples were kept in airtight plastic film protectors. Similarly, the samples were stored in airtight containers.

The samples were examined under a stereomicroscope with a wide-angle, zoom lens capable of 20x - 80x magnification and the photographs were taken with a Nikon Coolpix 5000 camera. The findings obtained as a result of microscopic analysis of some raw materials used in fish feeds and some pellet feeds kept under different pelletizing conditions are summarized as follows (Kop et al.,2006).

Maize Products (*Zea mays*)

Maize is a raw material given as feed to livestock, either ground or whole (Figure 1). When its macroscopic structure is examined, it is seen that the kernel is serrated and covered with a husk. Maize is a raw material whose characteristics cannot be changed when processed and broken down.

Corn gluten meal

When examined stereocroscopically, it is seen that corn gluten meal is the part remaining after bran separation and extraction of most of the seed and starch. Corn gluten meal has no cellular structure. It is recognized by its golden sphere shape with a single, round surface (Figure 1).

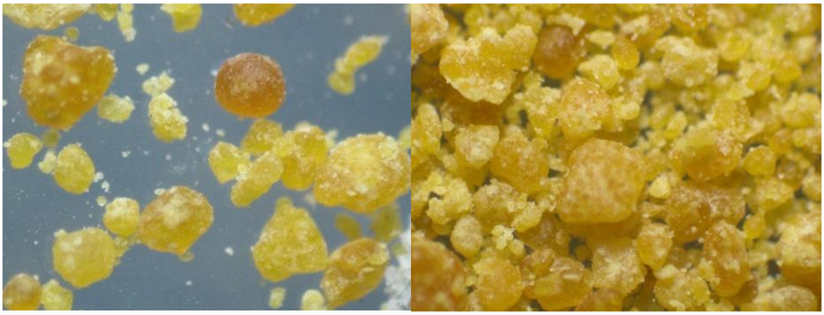

**Figure 1**. Corn gluten meal (80 x) (Kop et al.,2006).

Soy Products (*Glycine max*)

Soy Soy products are a widely used raw material in all feeds. Soy flour (extract or full fat) and protein concentrates are rich sources of protein (Figure 2-3). Soybean is a typical legume. Macroscopic examination shows that the seeds are elliptical and about 6 - 10 mm in diameter. They range in color from greenish to black. For example, the species cultivated in America are yellow. The hilum is egg-shaped and measures 3 mm x 1 mm.

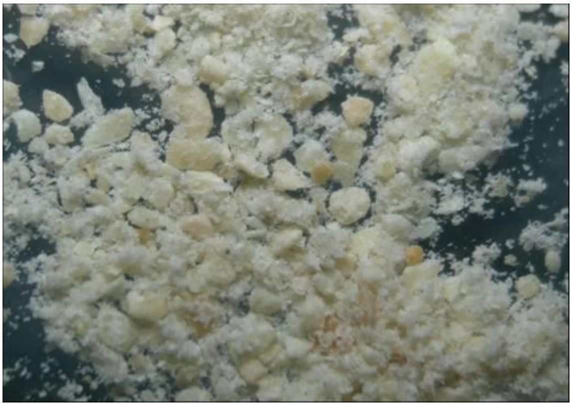

**Figure 2.** Soy bean meal (40 x) (Kop et al.,2006).

In stereoscopic views, many soybean products can be recognized by their shell structure at large magnification. The hilum is black or dark brown. Straight to thin slit and oval edges are distinguished. The outer surface of the shell is mottled. The particles are transparent and range in color from cream to pale red brown.

**Figure 3.** Soy bean meal (80 x) (Kop et al.,2006).

Wheat Products (Tricutum spp.)

Milled wheat is used as mechanically separated seed endosperm and bran in the feed industry. Macroscopically, wheat is a raw material that can vary in color from light brown to dark brown. The seed grain is 6 - 8 mm long, hairy and irregularly rounded to heart-shaped (Figure 4).

Steroscopically, bran particles are present in many wheat products and are used for identification. Color varies from white to reddish brown. White starch granules are crystalline, adhered to the inside of the bran surface and are distinct diagnostic structures. Milled wheat granules are recognized by their adhesion to the bran (Figure 5-6).

**Figure 4.** Whole Wheat (20 x) (Kop et al.,2006).

**Figure 5.** Wheat meal (40 x) (Kop et al.,2006).

**Figure 6.** Red Dog / Bonkalit Flour (40 x) (Kop et al.,2006).

Fish Meal

Fish meal is produced by grinding and drying the unseparated tissues of whole or slaughtered fish. It can be fatty or non-fatty. On macroscopic examination, fishmeal is brownish yellow in color. The bones are shiny and contain an acceptable amount of flakes. Many samples are oily and have a strong fishy odor.

Fishmeal is scaly and bony. Some bones are cylindrical and mottled. Scales are flat or curved, concentric and transparent. Similar to meat meal but lighter in color. Teeth, eyes and otoliths are frequently observed (Figure 7-8).

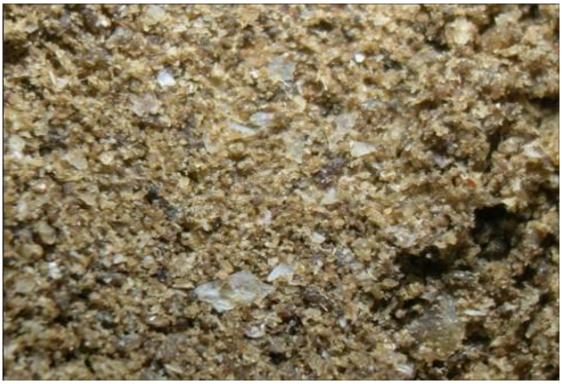

**Figure 7.** Fish meal (20 x) (Kop et al.,2006).

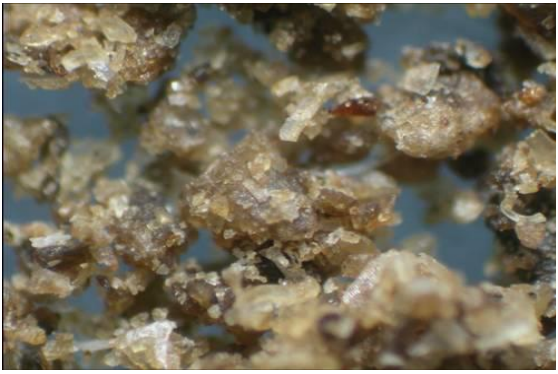

**Figure 8.** Fish meal (40 x) (Kop et al.,2006).

Shrimp Meal

Shrimp meal is obtained by grinding and drying whole and/or partial shrimp. The shrimp meal is characterized by numerous amounts of fine mica-shaped shells. There are small black spots (eye fragments) in the flour. Some flours contain only the shell. Shrimp meal, transparent chitin layer and fused antennal structures (the 1 mm long antenna contains 4 segments). The leg segments are also fused (Figure 9-11).

**Figure 9.** Shrimp meal (20 x) (Kop et al.,2006).

**Figure 10**. Shrimp meal (40 x) (Kop et al.,2006).

**Figure 11.** Shrimp meal (80 x) (Kop et al.,2006).

Pellet Feeds

The difference between the deterioration of pelleted feed (Figure 12) and the pellets conforming to the quality criteria (Figure 13) can be observed quite well with this method when the pelletizing conditions are not fully realized.

**Figure 12.** Poor quality pellet (20 x) (Kop et al.,2006).

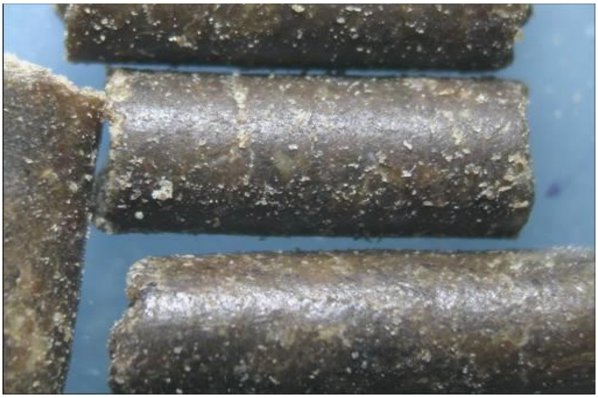

**Figure 13.** Quality pellets (20 x) (Kop et al.,2006).

Granular feeds are shown in Figure 14, powdered feeds in Figure 15 and aquarium feeds in Figure 16. Figures were obtained at 40 x magnification.

**Figure 14.** Granular Feed (20 x) (Kop et al.,2006).

**Figure 15.** Powder feed (20 x) (Kop et al.,2006).

**Figure 16.** Aquarium fish feed (20 x) (Kop et al.,2006).

Feed Additives

Substances such as vitamins, minerals and binders are also present in the feed. The appearance of such substances under the microscope is shown in Figures 17, 18, 19 and 20.

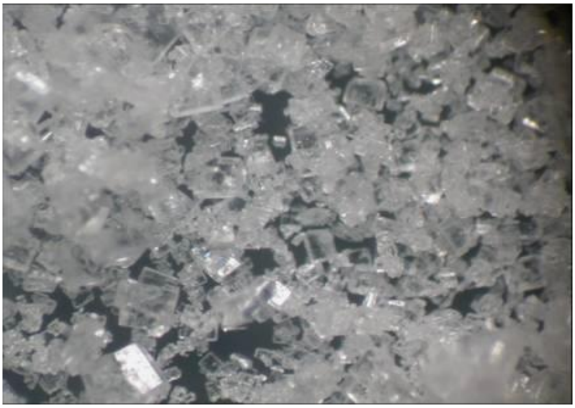

**Figure 17.** Vitamin C (40 x) (Kop et al.,2006).

**Figure 18.** Vitamin mixture (20 x) (Kop et al.,2006).

**Figure 19.** Mineral mixture (20 x) (Kop et al.,2006).

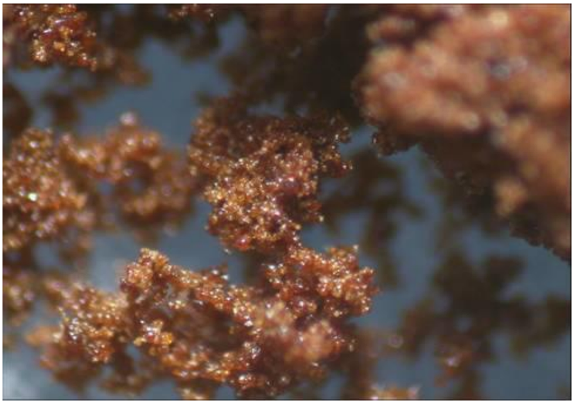

**Figure 20.** Binding (80 x) (Kop et al.,2006).

**Table 1**. Spot tests of various substances (Bates et al., 1992)

| | Sakaguchi Reagent | Silver Nitrat in Alcohol | 2 N NaOH | HCl | Glacial Acetic Acid & Water | Bromine Water & Starch Paper | Potassium Ferrocyanide | Nitroso-R-salt |
|---|---|---|---|---|---|---|---|---|
| Drugs | | | | | | | | |
| Chlortetracycline HCl | Blue violet | - | Brownish yellow | | | | | |
| Furazolidone | Pale yellow | Pale yellow | Pale yellow | | | | | |
| Nitrofurazone | Brownish yellow | | Brownish yellow | | | | | |
| Oxytetracycline HCl | Red | | | | | | | |
| Vitamin | | | | | | | | |
| Vitamin A | Turn blue then Brown (crushed sample) | | | | | | | |
| Vitamin C | | Blue-Black | | | | | | |
| Menadione | Red Brown | Brick red | Brown | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Riboflavin | Orange tailing to yellow | | Pale yellow | | | | | |
| Thiamine | Effervesces | | Pale yellow | | | | | |
| Minerals | | | | | | | | |
| Cobalt | | | | | | | Green (Turquoise) | Pink |
| Copper | | | | | | | Reddish Brown | Brown |
| ıron | | | | | | | Blue | Bluish Green |
| Manganese | | | | | | | Milky white | |
| Zinc | | | Fuchsia | | | | | |
| Diğer | | | | | | | | |
| Bone | | | | Slow action | | | | |
| Gelatin | | | | | Soft and swollen | | | |
| Horn | | | | | Hard | | | |
| Iodide | | | | | | Blue purple | | |

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