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Analysis of Production Capacity, Profitability, and Constraints in The South African Aquaculture Industry: Case of Gauteng Province Food Fish Aquaculture

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ABSTRACT

Gauteng province's aquaculture was grossly underdeveloped and very little was known about its profitability and the possible constraints faced by its operators. Hence, this study was conducted to assess the production capacity, and profitability of existing aquaculture projects and to identify the constraints responsible for the underdevelopment of the industry in the province. To achieve these, data was collected from five fish farms located in the province using structured questionnaires and interviews. Gross margin analysis and gross profit margin ratio were used to determine the profitability of aquaculture production. The fish farmers in the study used 36% of the capacities of the established aquaculture projects leading to lower tonnage of fish per cycle of production in all the farms. This is an indication of the underutilization of the production capacities of the established fish farms. The profit margins were greater than 40% in all the projects surveyed, proof that aquaculture has good potential as a business enterprise in the province. However, the study identified several constraints which include lack of skilled workforce, poor access to funding, and lack of established input suppliers within the province as being responsible for the underdevelopment of the sector.

Keywords: Aquaculture, profitability, production capacity, constraints, underdevelopment

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Introduction

Aquaculture is the fastest-growing food production sector in the world (Edwards et al. 2019), and Africa is an aquaculture destination of choice due to the continent's favorable environmental conditions, with approximately 43% potential area for farming tilapia, African catfish, and carp (Adeleke et al. 2021). Several African countries including Egypt, Nigeria, Uganda, and Ghana are making significant contributions to global aquaculture by serving as the continent's main aquaculture destinations (Adeleke et al. 2021).

Although South Africa has one of the most well-developed infrastructural networks, and conducive environmental conditions suitable for aquaculture development in the continent (Britz and Venter 2016), however, aquaculture development in the country is still at developmental stages. The country's contribution to global aquaculture production is insignificant compared to the leading African contributors (Adeleke et al. 2021; FAO, 2020). According to Ortega et al. 2021, the South African aquaculture sector made an annual contribution of about 6400 tonnes of fish to the total South African fisheries industry in 2018, while the country placed tenth among the African 10 aquaculture-producing countries, contributing 0.28% of the total African farmed food fish production (Adeleke et al. 2021).

South Africa's aquaculture industry is separated into marine and inland aquaculture. The sector is geographically diversified across all nine provinces that comprise the country. The province of the

Western Cape is the economic center for aquaculture output. It is also the country's most major contributor to GDP in terms of aquaculture exports. Other significant contributing provinces include Eastern Cape and Mpumalanga (DAFF 2018a).

Gauteng province is one of South Africa's warmest provinces. While winter temperatures can range between 5º and 19º celsius, bringing frost and frigid mornings, summer temperatures can range between 17º and 28º celsius and can last up to 8 months out of the year (Moja Media 2015). The province is ideal for warm water fish, particularly tilapia and catfish, with temperatures ranging from 20º to 30º celsius (Hecht et al. 1988; FAO 2011).

DAFF (2014) rated the province fourth in terms of overall freshwater fish production and the number of fish farms operating in the province in 2013 (DAFF 2019). In 2011, Gauteng province was in a distant third in the export of fish and aquatic invertebrates. The province exported 105,312 tonnes which made 2.99% of total national exports, trailing behind Western and Eastern Capes respectively (DAFF 2014). These export products were not only provincial products, but also products brought into the province from other parts of the country, indicating the province's poor aquaculture production (DAFF 2014).

Despite its current output, the aquaculture business in Gauteng province has a bright future. Because the province has a huge population, including expatriates from fish-eating countries across the world, it has a large potential market for aquaculture products. However, due to the non-commercial character of the aquaculture industry in the province, production estimates for the sector are underreported just like for the rest o the country (DAFF 2013). And despite the high market potential, nothing is known about the province's aquaculture venture's profitability. Little or no thought has been given to the business's viability or the elements that influence it. Rather, research has concentrated on issues such as fish biology, production, growth processes, and nutrition. However, aquaculture production involves more than just fish growth processes; it also entails paying attention to the financial aspects of production, which aids in better decision-making and progress. Aquaculture financials are important for promoting commercialization, obtaining capital, and convincing investors of the enterprise's profitability (Mwangi 2007; Kaminski et al. 2018). The lack of knowledge on aquaculture profitability affects not just the farmers who produce fish, but also all stakeholders who may be interested in engaging in the sector.

The purpose of this study therefore was to assess the production capacity and profitability of existing aquaculture projects, as well as to identify the constraints causing the underdevelopment of the sector in Gauteng province, despite the availability of strong support structures and enabling environment.

Materials And Methods

Study Area

The research was carried out in South Africa's Gauteng area. Gauteng province is the smallest of South Africa's provinces, accounting for around 1.5% of the country's total geographical area (Dlamini et al. 2022). However, it is the most populous of South Africa's nine provinces and has been named the country's economic center and gateway to the country (Gauteng City-Region Observatory [GCRO] 2016; Dlamini et al. 2022). The province has the highest migrant population, with a combined total migrant population that exceeds the total of all other provinces (Dlamini et al. 2022).

The aquaculture industry in Gauteng province is underdeveloped compared to provinces like Western and Eastern Capes or Mpumalanga of South Africa. The province was ranked fourth by DAFF (2014) in terms of the total freshwater fish production and the number of fish farms operating in the province in 2013 (DAFF 2014).

Gauteng province is mainly an urban province. However, its agricultural sector makes up a small share of the economy providing the cities and towns with daily fresh produce (GCIS 2004; Makiti Guides and Tours 2009). In terms of climatic conditions, the province falls within the areas with hot summer and cold winter making it generally too cold for warm water fish and too hot for cold water fish (GCIS 2004).

Tilapia is the most common farmed species in the province. *Oreochromis mossambicus* was a predominantly farmed species in the past because it is indigenous to South Africa. However, most farms have replaced *O. mossambicus* with the fast-growing *Oreochromis niloticus* which is listed as an alien invasive species in the country (DAFF 2018b). Tilapia species is embraced in Gauteng province because of its white flesh for which it has been dubbed "aquatic chicken", and also due to the presence of foreign African nationals who are from countries where fish consumption is valued as well as South Africans who are imbibing the art of healthy eating.

The African sharptooth catfish, *Clarias gariepinus*, is another food fish which are being farmed in the province but whose production is not very high because they are difficult to sell due to their red color, meaty texture, and distinct muddy flavor (Stander 2007). Local buyers found its appearance unappealing and the cost price often too high. However, African catfish is sought after by some

immigrants from some African regions where it is regarded as a delicacy (Britz 2015). Other fish species produced in the province include carp, prawns, and ornamental fishes (DAFF 2019).

Sampling

Primary data for the study were collected using snowball and convenience sampling methods. The snowball sampling method is a referral-based non-probability sampling approach. The method is to find someone who is a good fit for the study and then have that person recommend other people (Kirchherr and Charles 2018). Each respondent was contacted by phone prior to the formal survey. After the respondent accepted the survey, the purpose of the study was explained, and a formal appointment was scheduled. According to Gauteng Department of Agriculture and Rural Development database, Gauteng province had 14 operable food fish aquaculture farms. However, as of the time of this study, only six of the listed farms remained in operation while three were partially functional. The remainder had either closed or the owner had discontinued fish production for a variety of reasons. Therefore, acquainting with one project led to the other projects by recommendation and referral. All the identified project owners were contacted. Only five, however, volunteered to be interviewed.

Data Analysis

All obtained data was coded and recorded into a Microsoft Excel 2010 spreadsheet before being uploaded to the Statistical Package for Social Sciences version 24 (SPSS 24). The descriptive features of the respondents, such as frequencies, percentages, mean, and standard deviation, were examined using Microsoft Excel and SPSS 24. In terms of profitability, a range of management strategies for assessing profitability in agricultural and other sorts of enterprises have been established. Many studies have employed techniques such as cost-benefit analysis (CBA), net present value (NPV), internal rate of return (IRR), gross margin, and net revenue. Each strategy is determined by elements such as investment size, investment period, and business objectives (Kamangira et al. 2014). The gross margin was used as an analysis method in this article to determine the profitability of aquaculture ventures in the province.

Gross Margin and Gross Margin Ratio Analyses

The gross margin is the overall income generated by an enterprise less the variable costs incurred by the enterprise while the gross margin ratio which is a measure of a company's efficiency measures the ratio of the gross margin and total revenue generated. The bigger a company's profit margin, the more efficient it is. Because of its accuracy in profit assessment, gross margin has been employed in numerous research and appears to be the method of choice in profitability calculation (Nkadimeng et al. 2021; Sambo et al. 2021; Ogundari and Ojo 2009).

The gross margin and the gross margin ratio were denoted by:

$G.M = TR - TVC$

$GMR = GM/TR$

Where:

G.M. stands for gross margin, *G.M.R* stands or gross margin ratio, *TR* = Total revenue (from fish sales), *TVC* is the Total variable cost (which includes feed costs, labor costs, electricity costs, maintenance expenses, and so on).

Results

Farm Production Capacity and Current Production

The production capacities of each participated project and the current capacity utilization are presented in Figures 1, 2, and 3. Figure 1 shows the farm capacity while Figure 2 shows the actual usage of each farm that participated in the study. The outcome shows that none of the farms are operating to full capacity.

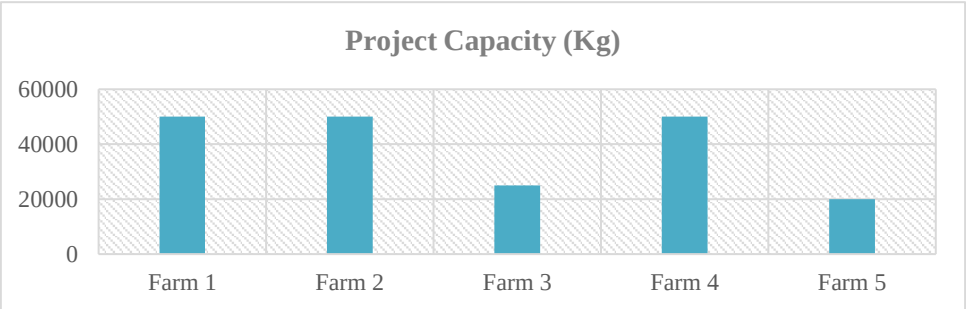

Figure 1. Aquaculture project capacity

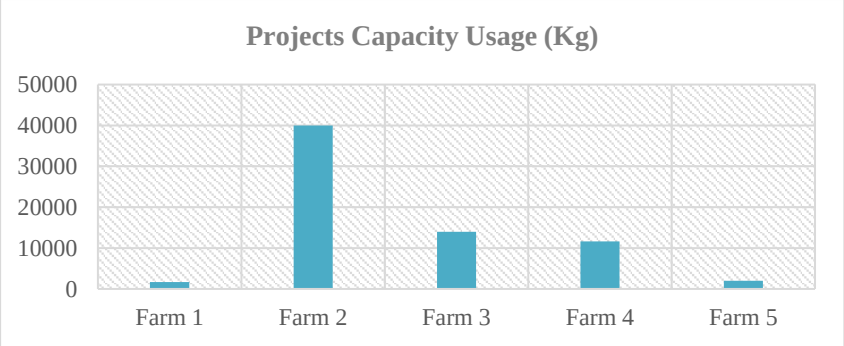

Figure 2. Aquaculture project active capacity

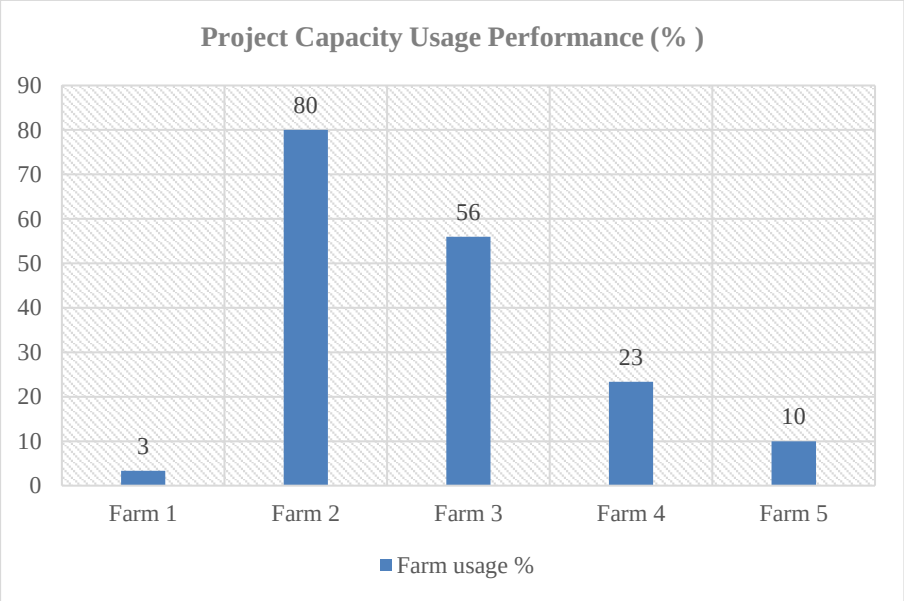

Figure 3. Aquaculture project capacity usage performance

Figure 3 indicates that only farms 2 and 3 are being utilized at above 50% of their total capacities, all the other farms are greatly underutilized.

Productivity and Profitability of Aquaculture Operations Investigated

To produce fish, either in table-size, juvenile, or fingerling sizes, many inputs are necessary. Such inputs include fixed and variable inputs. Fish farming is one of the agricultural activities undertaken by responding farmers in the same location. This is done in the same place as other farm practices such as vegetable cultivation, piggery, and cow breeding. As a result, the endeavors share the same inputs. And because no fixed input could be ascribed to the aquaculture firm due to the interlaced usage of the inputs, aquaculture productivity could not be established but inferred from production and utilization. A gross margin analysis was used to determine profitability.

Gross Margin and Gross Margin Ratio Analyses

Table 1 summarizes the gross margin ratios from the farms studied. The least gross profit for the production cycle analyzed was R33 334 for the five farms on which the gross margin was calculated, while the maximum was R840 000. The disparities in profit between farms were considered to be attributable to the scale of production as well as the amount of management used throughout production, which included timely and efficient feeding as well as water quality management.

Constraints Limiting Aquaculture Development in Gauteng Province

Table 2 summarises the issues that farmers face. According to the survey results, there are numerous restrictions impeding the growth of aquaculture in the province. However, a lack of qualified labor in aquaculture seems to be a problem shared by all of the farmers who responded to the poll. Concerning financing, all of the farmers complained about not being able to obtain funds from the government or banks. Farm expansion is unfeasible due to a lack of funds.

Other obstacles highlighted by respondents included a ban on the cultivation of other species of tilapia other than Mozambique tilapia, as well as government laws and regulations governing the subsector, notably around fish importation.

Table 1. Gross margin output of fish production

Variables	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5
Total production per cycle (Units)	5 000	120 000	42 000	35 000	6 000
Unit production cost (Rand/Kg) *	25	25	26	26	22
Total production cost (Rand)**	41 666	1 000 000	364 000	303 333	44 000
Selling price per kg (Rand)	45	46	46	45	45
Total revenue per cycle (Rand)	75 000	1 840 000	644 000	525 000	90 000
Gross margin (Rand)***	33 334	840 000	280 000	221 667	46 000
Gross margin ratio	44	46	43	42	51

*Unit production cost includes the cost of fingerlings, labour, feed, electricity etc.
**Total production cost = Unit production cost multiplied by number of fish produced in kilogram
***Gross margin = Total revenue – Total production cost

Table 2. Constraints to the growth of aquaculture as indicated by fish producers

Farmers	Constraints experienced by farmers				
Farm 1	Lack of skilled worker	Limiting government policies	Lack of labor knowledge of fish		
Farm 2	Poor legislation	Erratic power supply	Poor extension	Underdeveloped market	
Farm 3	Lack of knowledge of system design	Oxygen control	High cost of heat generation	Unavailability of fish seed	Unavailability of fish feed
Farm 4	Poor funding	Lack of skilled worker	Limiting policies		
Farm 5	Unavailability of Broodstock	Lack of passionate workers	Lack of skilled worker	Erratic electricity supply	

Discussion

Human capital is one of the variable components employed in aquaculture production, as is electricity in the case of the Recirculation Aquaculture System (RAS), which is the type of aquaculture production system used in the province. Water, feed, and oxygen are also variable inputs. All the participated fish producers were unable to assign costs to the numerous inputs utilised in their farms for fish production. They do, however, have an evaluation system which is the production of a kilogram of fish at a particular cost. As a result, the farmers were onlyable to give the price of producing one kilogram of fish per cycle of production. This production cost was used to determine the gross margin of each farm surveyed. All the analysed farms had a positive gross margin, indicating that the aquaculture industry in Gauteng Province is earning a healthy return on sales while keeping overhead expenses under control.

Based on the estimated gross margin ratios from the aquaculture operations in this study, it can be concluded that fish farming in Gauteng Province is profitable. Findings from several agricultural studies using gross margin and gross margin ratio have yielded similar results and conclusions: Southwest Bangladesh aquaculture (Siddiqa et al. 2018), tilapia farming in Southern China (Matlala 2014), shrimp culture in Song Cau district, Vietnam (An 2012), Nguni cattle farmers in Limpopo (Nkadimeng et al.

2021) and Menchum River Valley rice production in Cameroon (Bime et al. 2014). However, Boyd et al. (2020) on the other hand, emphasised the importance of good planning and operation by aquaculture farmer for sustainable growth and profitability.

Due to the industry's limits, none of the farmers interviewed were working their farms at full capacity. Only one farm was using more than 50% of its complete capacity, while the remainder were significantly underused. According to farmers, the main restrictions affecting the underutilization of farm facilities include a lack of funding, higher input costs, and a shortage of qualified manpower. The farmers were all aware of the government's intention to fund aquaculture. However, the procedures for obtaining the funds are time-consuming. All except one farmer claimed to have been able to access government funding at one time. All the other farmers claimed to run the farms out of their personal funds. The inability to obtain finance is a significant barrier. As a result, because intense commercial aquaculture is costly to create and run, fish farming in Gauteng Province has stayed on a limited scale. This finding is consistent with the findings of Sebola M.P (2018), Madibana et al. (2020). The investigations revealed that the majority of fish farmers were unable to obtain funding and hence could not grow as planned.

Another restraint was the prohibition on the production of other tilapia species other than Mozambique tilapia. However, due to the slow growth of Mozambique tilapia, farmers favored Nile tilapia, which grows quickly and is commercially viable. According to the farmers, getting a government permit to cultivate Nile tilapia, was a difficult task. Britz et al. (2009) identified this constraint as a major impediment to the development of aquaculture enterprises in South Africa as a whole, and Madibana et al. (2020) repeated the same opinion as a key point that the government should investigate to promote the expansion of aquaculture in South Africa.

Other obstacles mentioned by the farmers include government policy as well as subsector regulation. On a personal level, some other challenges that farmers face regularly include power outages, which always entail the need for alternate sources of power supply, resulting in a rise in production expenses as well as the cost of fish products.

Another obstacle faced by farmers, particularly those planning to enter the industry of producing fish fingerlings and young fish, is the issue of brood stock. A major barrier is the country's lack of sources for purchasing brood stock. The government's regulation of fish importation is a major impediment to the subsector's expansion. Only one of the five farmers who responded can grow fingerlings for table-size fish production. As a result, the other farmers had to rely on him for their fingerlings and juvenile fish. The remedies to the province's limits to aquaculture expansion are unique to the country as a whole. These limits are mentioned in the works of Hecht et al. (1988), and Madibana et al. (2020).

Conclusion

According to the study's findings, aquaculture projects in Gauteng province are producing below capacity. This is evident from each project's output capacity and actual present use. At maximum capacity utilization, the combined total production should have been 195 tonnes of fish. For this survey, only roughly 70 tonnes of total fish production were documented. The current use equals 36% of the overall capacity of the farms.

Several constraints in aquaculture productivity were identified. Nonetheless, despite the sector's problems and underproduction, fish farming in the area is profitable. While one of the farms had a gross margin ratio greater than 50%, all the other farms had a gross margin ratio greater than 40%. This demonstrates that aquaculture is a viable business. The initiative has the potential to contribute to the government's socioeconomic goals of job creation and poverty eradication.

To aid the growth, development, and profitability of aquaculture in Gauteng province, government agencies, and non-governmental groups must raise youth awareness in order to stimulate fish farming in the province. In addition, legislation for aquaculture practices is needed to overcome current rules, particularly those that do not promote aquaculture in the province. Furthermore, there is a need to develop a full-fledged department of aquaculture management in institutions around the province to support aquaculture study and research. It is also critical for the government to make it easier for farmers to access the funds set aside to encourage aquaculture in the province.

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Toxic Blue-Green Algal Blooms in Keban Dam Lake and Lake Hazar

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ABSTRACT

The blue-green algae are main members of summer phytoplankton in Keban Dam Lake and to some extent in Lake Hazar. Sporadic occurrences of *Microcystis aeruginosa* blooms were noticable in Keban Dam Lake whilst blooms of *Nodularia spumigena* was characteristic in Lake Hazar. Three variants of cyanotoxin namely *microcystin*-RR, *microcystin*-YR and *microcystin*-LR were analyzed in Keban Dam Lake only during *M.aeruginosa* blooms. Their concentrations were determined in the range of *microcystin*-RR 0.27-1.12 µg L^{-1}; *microcystin*-YR 0.12-0.54 µg/L and *microcystin*-LR 0.27-1.15 µg/L respectively. Chlorophyll *a* concentrations were found to range 16-20 µg L^{-1} and 32-36 µg L^{-1} during two blooms of *M. aeruginosa. Nodularia spumigena* appeared in the first week of june (2010) with a biomass of 1.368 mg L^{-1} and it proliferated rapidly giving rise to a bloom within two weeks in Hazar Lake. The bloom lasted nearly four weeks and maximum biomass was recorded as 28.4 mgL^{-1}. Chlorophyll *a* concentration was found to be 12-14 µg L^{-1} in the first two weeks of the active multiplication period and increased up to 21-24 µg L^{-1} at the maximum bloom level. *Nodularin* (toxin of *N. spumigena*) was detected in the samples with concentrations varied between 42.3 and 607 µgL^{-1}.

Keywords: Toxic algal blooms, *Microcystis aeruginosa*, *Nodularia spumigena*, Keban Dam Lake, Lake Hazar.

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Keban Baraj Gölü ve Hazar Gölü'nde Toksik Mavi-Yeşil Alg Çoğalmaları

Öz: : Mavi-yeşil algler, Keban Baraj Gölü ve bir ölçüde Hazar Gölü'ndeki yaz fitoplanktonunun başlıca üyeleridir. Keban Baraj Gölü'nde *Microcystis aeruginosa* bloomları dikkat çekerken, *Nodularia spumigena* bloomları yalnızca Hazar Gölü'nde gerçekleşmiştir. Keban Baraj Gölü'nde *M. aeruginosa* bloomları esnasında alınan örneklerde siyanotoksinin üç varyantı olan mikrosistin-RR, mikrosistin-YR ve mikrosistin-LR analizleri yapılmıştır. *Mikrosistin*-RR konsantrasyonu 0,27-1,12 µg/L; *mikrosistin*-YR konsantrasyonu 0,12-0,54 µg/L ve *mikrosistin*-LR konsantrasyonu 0,27-1,15 µg/L aralığında belirlenmiştir. *M. aeruginosa* bloomları esnasında klorofil *a* konsantrasyonları 16-20 µg/L ve 32-36 µg/L aralığında bulunmuştur. Hazar Gölü'nde *Nodularia spumigena*'nın ilk çoğalmaları, 1.368 mg/L'lik bir biyokütle ile haziran (2010) ayının ilk haftasında ortaya çıkmış ve hızla çoğalmaya devam ederek iki hafta içinde bloom oluşturmuştur. Bloom yaklaşık dört hafta sürmüş ve maksimum biyokütle 28,4 mg/L olarak kaydedilmiştir. Aktif çoğalma periyodunun ilk iki haftasında 12-14 µg/L olarak analiz edilen klorofil *a* konsantrasyonu, maksimum çoğalma seviyesinde 21-24 µg/L'ye kadar yükselmiştir. *Nodularin* (*N. spumigena* toksini) göl suyunda 42,3 ile 607 µg L^{-1} arasında değişen konsantrasyonlarda tespit edilmiştir.

Anahtar kelimeler: Toksik alg çoğalmaları, *Microcystis aeruginosa, Nodularia spumigena,* Keban Baraj Gölü, Hazar Gölü.

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Introduction

Cyanophyta (also refered as cyanobacteria) is conspicuous and common member of phytoplankton of many freshwater ecosystems. Under suitable conditions, they can proliferate rapidly and form blooms. In fact, blooms of blue-gren algae were reported by many authors throughout the world (Paerl et al. 2001; Philips 2007). However one of the major concerns with these blooms is that some of the blue-green algal species have the ability to produce toxins (cyanotoxin) that pose a risk for aquatic

ecosystems and to human health (Corus and Bartram 1999). In addition, eutrophication and apperance of cyanobacterial bloom, have become a world-wide problem that can cause serious problems when bloom-forming species release watersoluble toxins (Watanabe and Oishi 1980; Carmichael 1994). There are about 30 species of cyanobacteria that can be associated with toxic water blooms (Skulberg et al. 1993). Reports are available for lakes in at least 44 countries as well as for seas (e.g. the Baltic Sea, Caribbean Sea, Atlantic, Pasific and Indian Oceans) and oceans (Carmichael 1994).

One of the more common and widespread bloom-forming blue-green alga associated with toxin production is *Microcystis aeruginosa* (Kutzing) Lemmerman. However, *Aphanizomenon flos-aquae* Ralfs ex Bornet and Flahault, *Dolichospermum spiroides* (Klebhan) Wacklin, L.Hoffmann and Komárek (*Anabaena spiroides* Klebahn) and *Nodularia spumigena* Mertens ex Bornet and Flahault are also included in the list of toxin producing blue-green algae (Carmichael 1994; Paerl et al. 2001). Toxic algae produce two main types of toxin; alkoloid neurotoxins and peptide hepatotoxin (Cood 1994). Contact with water containing toxic blue-green algae can cause various harms to human health such as vomiting, diarrheria, weakness, liver damage and hepatitis (Cood 1994). The occurrence of toxic cyanophytes can also become a major concern for drinking water when they form blooms in water reservoirs (Rositano et al. 2001).

Toxin of *M. aeruginosa* is called microcystin, a hepatotoxin that can negatively affect aquatic animals and human health (Chorus and Bartram 1999; Zurawell et al. 2005). As a result of this, blooms of toxic *M. aeruginosa* has been reported to be responsible for mass mortalities of aquatic animals thus giving rise to destabilization of food web in aqauatic ecosystems (Zurawell et al. 2005). Consumption of microcystin contaminated drinking water was also reported to pose potential human health risks (Chorus and Bartram 1999).

Aphanizomenon flos-aquae was also reported to produce a cyanotoxin called cylindrospermopsin (CYN) (Preussel et al. 2006). This is a tricyclic guanidine alkaloid associated with the production of harmful metabolites and inhibition of protein synthesis (Froscio et al. 2003). It has been linked to gastrointestinal distress, damage to liver in a variety of aquatic animals. CYN has also been shown to be genotoxic, causing DNA strands breakage (Shen et al. 2002).Concerning human health, 148 people were reported to be hospitalized with hepatitis-like symptoms after exposure to CYN-contaminated water (Bourke et al. 1983).

Nodularia spumigena is a flamentous, heterocystous nitrogen-fixing blue-green alga mostly occurring in saline and brakish waters. Filaments are easily recognizable by the short, compressed/disclike cells and heterocysts. The sheath is rather thin and close the filament. Blooms of *N. spumigena* are usually toxic due to hepatotoxin that they produce. Their toxin is called nodularin that is a cyclic pentapeptide. Its structure and biological activity is similar to that of microcystin. This toxin is reported to be hazardous to aquatic animals when dense blooms occurred that can give rise to acute cases. It may cause death through liver failure (Carmichael 1994).

The first report of cyanotoxic bloom of *Nodularia spumigena* was observed in Lake Alexandria (Australia) as early as in 1980s (Hobson et al. 1999). The lake had the characteristic of estuarine salinity at that time. In recent years prominent blooms of *N. spumigena* were reported to have occurred in Baltic Sea (Kahru et al. 1994; Mazur-Marzec 2006). Blooms of *N. spumigena* are usually toxic due to hepatotoxin that they produce. Their toxin is called *nodularin* that is a cyclic pentapeptide. Its structure and biological activity is similar to that of microcystin. These toxins are reported to be hazardous to aquatic animals when dense blooms occurred that can give rise to acute cases. They may cause death through liver failure (Carmichael 1994).

Microcystin and other cyanotoxins have been identified in many freshwater resources in various countries. To our knowledge, the first report related to occurrence of toxic blue-green algal bloom and cyanotoxin (microcystin) production in freshwater ecosystems in Turkey belongs to Albay et al. (1998). Their work on *Microcystis aeruginosa* bloom was performed in Sapanca (meso-oligotrophic) and Taşkışla Lake (eutrophic). They reported that *microcystin-RR* was dominant cyanobacterial microcystin in Lake Sapanca whilst *microcystin*-LR was determined with higher concentration than other types in Taşkışla Lake. *M. aeruginosa* bloom was also observed in Ömerli Dam Lake by the same authors. Several thosound of fish mortalities were reported due to this bloom. In the same geographical region, occasional blooms of *N. spumigena* were observed in Lake İznik (Akçaalan et al. 2008) and more recently a toxic cyanobacteria bloom was reported from Lake Uluabat (Apolyont) (Ulcay et al. 2010).

Keban Dam Lake and Lake Hazar are two significant freshwater resources for Elazığ and neighbouring provinces. The former is a man-made lake whilst the latter is naturally originated. Lake Hazar is known as the second deepest lake in Turkey after Lake Van. The mean depth is calculated as 90 m (Şen et al. 1999).The lake is tectonic in origin and has elopsoid shape with 4 km width and 20 km

length. Lake Hazar is categorized as alkaline lake due to its high alkalinity and pH values is 9 or over 9 in most of the year (Koçer and Şen 2014). This features make Lake Hazar as a specialized/extreme ecosystem.

Irregular occurrence of blue-green algal blooms were observed in lakes situated in Elazığ Province from year to year. Occurrence of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* blooms were noticable in Keban Dam Lake whilst *Nodularia spumigena* bloom was characteristic only in Lake Hazar. These species have been known to produce toxin under suitable conditions. The cells of these algae contain gas vacuoles (pseudovacuoles) which give them great buoyancy. This naturally accounts for the fact that profuse growths become concentrated at the surface of the lakes where floating scums (constituted by mass of colonies) result.

The present paper emphasizes some significant/interesting findings concerning blooms of toxic blue-green algae *Microcystis aeruginosa* in Keban Dam Lake and *Nodularia spumigena* in Lake Hazar. It is noteworthy that Lake Hazar is the second ecosystem in which the toxic bloom of *N. spumigena* was recorded in our country after Lake İznik.

Materials and Methods

Blue-green algal blooms occurred in summer and early autumn. *Microcystis aeruginosa* blooms occurred in july (2015) and august (2019) in Keban Dam Lake, *Nodularia spumigena* blooms were observed in Lake Hazar during autumn (september) in 2014 and 2016. Blooms of *Aphanizomenon flos-aquae* were also observed in Keban Dam Lake. Blooms were recorded in 2018 (mid september) and 2020 (late september). Toxin analysis were carried out both during *M. aeruginosa* and *N. spumigena* blooms. However such analysis could not be performed for *A. flos-aquae* bloom due to lack of laboratory facilities.

Blooms occurred in littoral regions in both lakes and samplings were carried out only at one station (from the center region of the blooms). Water and algal samples were collected from under the surface of the lakes. Plankton net (20 μm mesh size) and water botttle were used for qualitative and quantitative algal and water collections. Water temperature and pH were measured *in situ* using a multi parameter analizer (YSI 63). Biomass of algae was determined according to method described by Rott (1981). Nitrate and orthophosphate concentrations were analysed following the spectrometric method outlined in APHA (1989). Chlorophyll *a* was determined spectrometrically through aseton method described by Wetzel and Likens (1991) and calculated through monokromatic method (Lorenzen 1967). Toxin analysis was performed at Fisheries Faculty of Istanbul University according to method described by Lawton (1994). The ISO 20179 method was used for microcystin analysis and results were given as Microcystin-LR eq. Micrographs of algae were taken with Nikon Eclipse 80i research microscope and algae were identified from relevant references (Wehr and Sheath 2003; John et al. 2003).

Results

The blue-green algae are main members of summer phytoplankton in Keban Dam Lake and to some extent in Lake Hazar. Blooms of blue-green algal species known potentially to be toxic were observed to occur in these lakes from time to time. When blooms occurred, colonies and/or filaments of algae formed visible thick and wide bands on surface of the lakes. The highest dentisities of filaments and colonies were usually found at first 0.1-1.5 m depth. However filaments and colonies were also found to be suspended in deeper water columns.

Occurrence of *Microcystis aeruginosa* was noticable in Keban Dam Lake whilst occurrence of Nodularia spumigena was characteristic only in Lake Hazar.

Microcystis are characterised as notoriously and overwhemlingly dominant species in many tropical, subtropical and temperate lakes (Fogg et al. 1973; Chorus and Bartram 1999). Sporadic occurrence of *Microcystis aeruginosa* (Figures 1, 2) blooms was also noticable in Keban Dam Lake. When blooms occurred, scums of the alga covered a large part of the lake surface (Figure 3). During the bloom, scums formed visible thick oily surface layers/bands particularly in the littoral region (Figures 4-6). The highest densities of filaments and colonies were usually found at first 0.1-1.0 m depth. However they were also found suspended in smaller numbers up to 1.5-2.0 m. Great density of colonies also changed the colour of lake water to blue-greenish.

Microcystis aeruginosa blooms in Keban Dam Lake did not occur at regular intervals. First bloom was recorded in July 2015 and the second one occurred in August 2019. Density of *M.aeruginosa* during the blooms was estimated as high as 90-95% in overall phytoplankton population. Chlorophyll a concentration was found to range 25-28 μg/L and 32-36 μg/L during the first and the second bloom of the

alga respectively. The blooms particularly occurred in Uluova Region of the lake. This region is the most polluted part of the dam lake as city sewage is being discharged into the lake in this part. Thus, high nutrient contents available in Uluova Region could be considered as one of the main reasons for blooms to occur in this part of Keban Dam Lake since no blooms were observed in other parts of the lake.

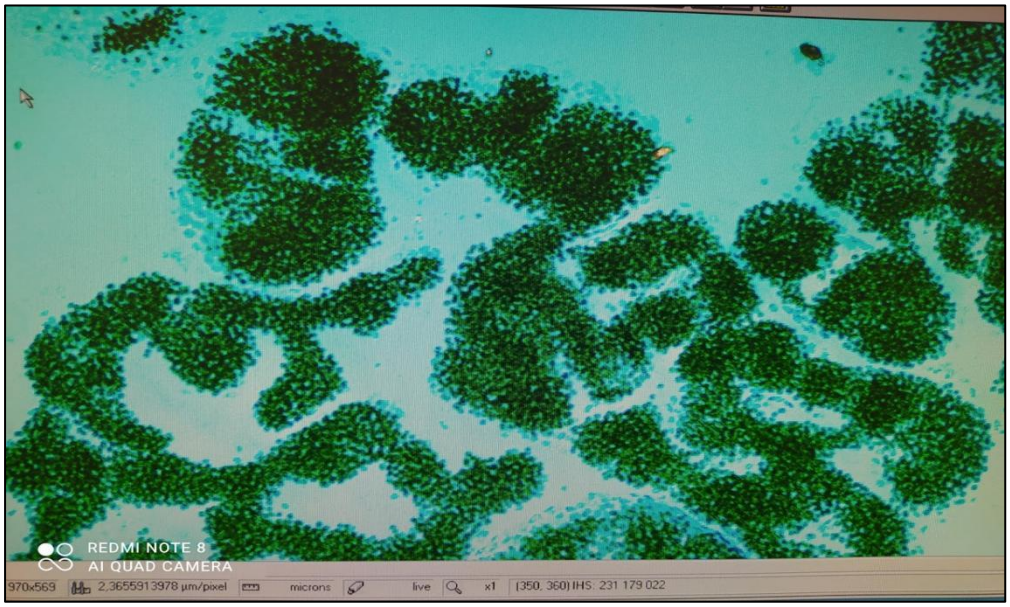

Figure 1. The general appearance of *Microcystis aeruginosa* colonies under the microscope.

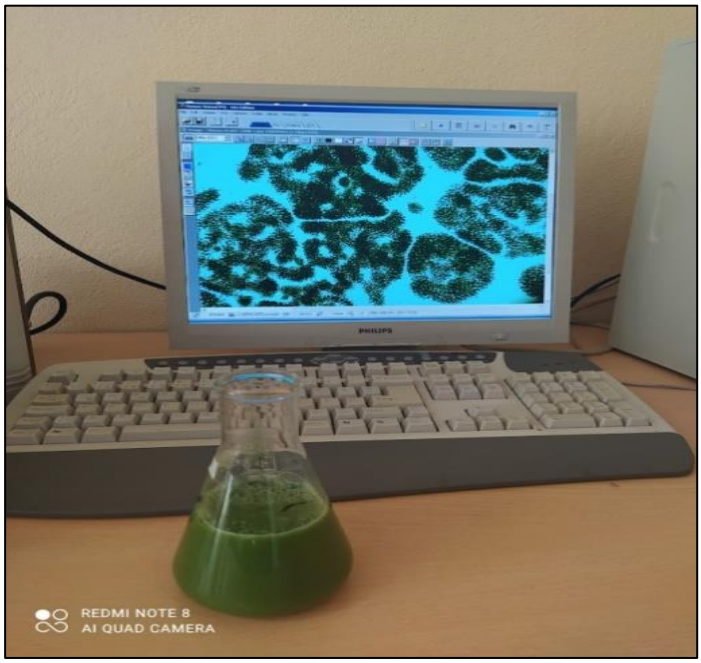

Figure 2. The microscobic appearance of *Microcystis aeruginosa* colonies as stored in a computer attached to microscope. **Note:** Notice the thick, dense mass of *M. aeruginosa* colonies in the conical flask collected from Keban Dam Lake.

Figure 3. General view of *Microcystis aeruginosa* bloom on the surface of Keban Dam Lake. **Note:** Notice how scums covered almost the whole surface of the lake.

Figure 4. The bloom-band of *Microcystis aeruginosa* visible on the surface of Keban Dam Lake

Figure 5. Thick oily scums of *Microcystis aeruginosa*

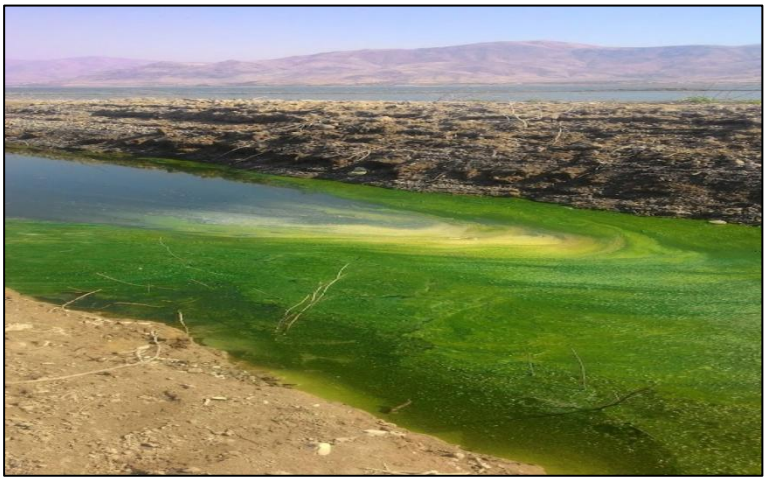

Figure 6. Blue-greenish oily scums of *Microcystis aeruginosa* in a indentation of Keban Dam Lake.

Scums of *M. aeruginosa* constituted thick oily layer on the surface water particularly near the shore (Figures 6, 7) in Keban Dam Lake. This region is better protected from strong wind effect therefore it offers a calm/unwindy water conditions for the colonies to accumulate and proliferate.

Naturally this gives rise to the formation of thick-oily scums. Blooms of the alga usually lasted 3-4 weeks with varying densities. Colonies proliferated and spreaded rapidly to form massive scums on the surface of the lake. They usually spreaded slowly from littoral region towards open water part. However thicker scums were always in the littoral. It was also possible to observe blue coloured remains of scums on the shore after water level decreased towards the end of summer (Figure 7).

Figure 7. Conspicuous blue coloured remains/residues of *Microcystis* scums on the lake shore after water level decreased.

Toxin of *M. aeruginosa* is called microcystin. Three variants of microcystin namely ***Microcystin-RR***, ***Microcystin-YR*** and ***Microcystin-LR*** were analyzed in lake water during the blooms. Their concentrations were determined in the following range; microcystin-RR 0.27-1.12 µg/L, microcystin-YR 0.12-0.54 µg/L and microcystin-LR 0.27- 1.15 µg/L (Table 1).

Table.1 Microcystin values determined in Keban Dam Lake during *Microcystis aeruginosa* blooms.

Bloom 1	Microcystin-YR	0.12-0.52 µg/L
	Microcystin-LR	0.27-1.13 µg/L
Bloom 2	Microcystin-RR	0.27-1.12 µg/L
	Microcystin-YR	0.12-0.54 µg/L
	Microcystin-LR	0.27-1.15 µg/L

Table.2. Mean values of some physical and chemical parameters of Keban Dam Lake during *Microcystis aeruginosa* blooms.

	Littoral	**Open water**
pH	8.1	8.3
Water temperature (°C)	25.6	28.4
Dissolved oxygen (mg O_2/L)	7.8	8.3
Electrical conductivity (µS/cm)	290	284
Orthophosphate (µg/L)	110	80
Nitrate (µg/L)	2100	1120
Total hardness (mg$CaCO_3$/L)	144	142

(table continues…)

Chlorine (Cl)	24.1	21.9

In recent years blooms of *Aphanizomenon flos-aquae* was also observed in Keban Dam Lake. Blooms were recorded in 2018 (mid september) and 2020 ((late september) that never coincided with the bloom years (2015, 2019) of *M. aeruginosa.* During the bloom, *A. flos-aquae* filaments constituted the almost 90 % of the whole phytoplankton population. Many individual filaments of *A. flos-aquae* gather together to form free-floating flakelike bundles (Figure 8) which were easily visible scattered/spreaded on surface of the lake at first 0.1-1.5 m depth. Toxin analysis during the bloom of *A. flos-aquae* could not be performed due to lack of laboratory facilities.

Figure 8. General appearance of filaments of *Aphanizomenon flos-aquae* under the microscope. **Notice** flakelike bundles of the alga.

Blooms of *Nodularia spumigena* (Figure 9) are usually toxic due to hepatotoxin called nodularin. First bloom of *N. spumigena* in Lake Hazar was reported by Şen et al. (2010). The high densities of filaments were usually found at 0.1-0.5 m water column. The first apperance of the alga was observed in the first week of june (2010) with a biomass of 1.368 mg/L (1368 µg/L) and it multiplied rapidly giving rise to a bloom within two weeks. The bloom lasted nearly 4 weeks with varying densities. The maximum biomass was recorded as 28.4 mg/L (28400 µg/L). At this stage, a thick band caused by dense filaments of the alga was visible on the surface of the lake (Figure 10). Chlorophyll a concentration was found to range 12-14 µg/L in the first two weeks of the active multiplication period and increased up to 22 µg/L at the maximum bloom level. During the first bloom of *N. spumigena* (2010), the concentration of the toxin of the alga (nodularin), was detected to vary in a range of 42.3 - 607 µg/L. On the onset of the bloom, concentration of nodularin was determined as 42.3 µg/L and it increased up to 607 µg/L at maximum bloom level.

Figure 9. Micrograph of *Nodularia spumigena* observed in Lake Hazar.

Figure 10. Thick bloom-bands of *Nodularia spumigena* spreading on the surface of Lake Hazar.

The second bloom was observed during summer of 2016. Biomass of the alga showed similarity to that of the first bloom. On the onset of the bloom (mid July), biomass was determined as 1.204 mg/L (1204 µg/L) and finally reached to a maximum biomass of 19.2 mg/L (19200 µg/L) in August. Chlorophyll a concentration was 17.2 µg/L at maximum bloom level. Concentration of nodularin was determined to vary minumum 34.6 µg/L (on the onset) and maximum 544 µg/L. No fish mortalities were observed during both blooms.

Concentrations of nitrate (NO3) and orthophosphate (PO4) generally ranged 0.361- 0.581 mg/L (361-581 µg/L) and 0.008- 0.017 mg/L (8-17 µg/L) respectively during the summer seasons in Lake Hazar (Table 3).

Table.3 Summer physical and chemical water properties of Lake Hazar

Parameters	Minimum	Maksimum
pH	9.0	9.2
Water Temperature (°C)	24.1	26.0
Electrical Conductivity (µS/cm)	2161	2439
NO_2 (µg/L)	48	61
NO_3 (µg/L)	361	581
TN (µg/L)	740	1010
PO_4 (µg/L)	8	17
TP (µg/L)	14.1	21.4
Cl (mg/L)	231	314

N. spumigena has never been observed and/or was reported from Keban Dam Lake. On the contrary, blooms of *Microcystis aeruginosa* and Aphanizomenon flos-aquae never occurred in Lake Hazar. Higher contentrations of nodularin in Lake Hazar compared those of microcystin detected in Keban Dam Lake was noticable.

Discussion

The blue-green algae are main members of summer phytoplankton in Keban Dam Lake and to some extent in Lake Hazar. Occurrences of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* were noticable in Keban Dam Lake whilst occurrence of *Nodularia spumigena* was characteristic only in Lake Hazar. These species have been known to produce toxins under suitable conditions.

Microcystis is characterised as notoriously and overwhemlingly dominat species in many tropical, subtropical and temperate lakes. Sporadic occurrence of *Microcystis aeruginosa* blooms was also noticable in Keban Dam Lake. The blooms of the toxic blue-green alga *M. aeruginosa* occurred particularly in Uluova Region of Keban Dam Lake. This region is the most polluted part of the dam lake. City sewage is discharged into this part and there are many agricultural lands in the vicinity where fertilizers are used extensively. Thus one may think that, main reason for blue-green algal blooms to occur in this part of the dam lake could be the high nutrient concentrations available for the algae due to nutrient loading. This finding is in harmony with that of Roxas and Salgados (2014). Uluova Region of the lake has moderately alkaline and moderately hard water characteristics. Thus, it may be posssible to suppose that these properties of the lake water may also favour the cyano-blooms to occur in the region.

Blue-green algae (cyanobacteria) are widespread. Fogg et al. (1973) reported that the major factors influencing their growth are light, temperature, chemical composition of lake water and dissolved oxygen concentration.

Planktonic species such as *Aphanizomenon, Anabaena, Coelosphaerium, Gloetrichia, Microcystis* and *Oscillatoria* were shown to have a preference to warm water condition (temperature ranging 17-20 °C) for their growth (Fogg et al. 1973; Walsby 1975). In addition, it is also considered that higher temperatures over 20 °C seem to favour the formation of blooms. Reynolds (1984) emphasized that 20 °C is the most suitable water temperature for the optimal growth of most blue-green algae occurring/known in lakes of temperate regions.

Present study supported above findings since cyanobacteria blooms in Lake Hazar and Keban Dam Lake usually occurred during summer when water temperaturee were high. *M. aeruginosa* and *N. spumigena* bloom showed a clear relation with water temperature since occurrence of blooms always coincided with temperatures over 20 °C (usually 20-26 °C). It is also worth to mention that Albay et al. (2005) reported that microcystin production in Lake Küçük Çekmece occurred between 16-25 °C and maximum production was recorded at 24-28.5 °C. Considering most of the blooms occurring at high temperatures, it may be possible to suggest that summer water conditions, particularly high water temperatures, also favour the growth and bloom formation of cyanobacteria both in Lake Hazar and Keban Dam Lake. However, it should be noted that active multiplication of *Aphanizomenon* also occurred at lower (16-18 °C) water temperatures.

Mature mode of nutrition of blue-green algae is autotrophic. They have ability to survive the extreme light conditions of summer season at the water surface (Mur and Beijsdorf 1978). Present study supported this as cyanoblooms both in Keban Dam Lake and Lake Hazar occurred in summer when high-light conditions were prevaling. Oppositely, cyanobacteria are also reported to be able to sustain biomass under low-light conditions better than eukaryotic algae. The main reason for that was explained with their low maintenance energy requirements at low light levels (Mur and Beijsdorf 1978). Occurrence of *A. flos-aquae* in Keban Dam Lake supported this finding since the alga also grew well in late-winter and spring. However it is noteworthy that the alga never formed a bloom in this period of the year.

Many cyanobacteria cannot survive high light intensities over long period. This may limit their distribution to more turbid eutrophic ecosystems. However *Microcystis* spp. are less sensitive to high light intensities because buoyancy regulation enables them to find light conditions that are optimal for their growth (Fogg et al. 1973; Walsby 1975). This means that the presence of *Microcystis* spp. cannot be related strictly to the level of eutrophication. This genus therefore can be found in mesotrophic, eutrophic and hypertrophic waters. However, it is logical to consider that amounts of biomass that this species attain depends on the level of eutrophication. In Keban Dam Lake, *M. aeruginosa* and *A. flos-aquae* blooms only occurred in the most polluted part (Uluova Region) of the lake where nutrient concentration is possibly higher than in other parts. It is evident that high nutrients contents naturally favours the cyanotoxic algal blooms. It is also noticable that no blooms were observed in other parts of the lake probably due to insufficient nutrient concentrations. Thus, occurrence of cyanotoxic species only in polluted part of Keban Dam Lake that has eutrophic water properties clearly supports the findings of Chorus and Bartram (1999).

Most *Microcystis* blooms are found in lakes with summer chlorophyll *a* concentration of 20-50 µg /L (Chorus and Bartram 1999). The present study is in harmony with the finding of these authors as chlorophyll *a* concentration in Uluova region of Keban Dam Lake was found to range 16-36 µg/L

during *Microcystis aeruginosa* blooms. In addition, chlorophyll *a* concentration during the bloom of *Nodularia* in Lake Hazar was found to range 12-14 µg/L in the first two weeks of the active multiplication period and increased up to 22 µg/L at the maximum bloom level. In addition *Nodularia spumigena* blooms in Lake Hazar coincided with chlorophyll *a* concentrations over 20 µg/L.

Blue-green algae are sometimes predominant in waters poor in nutrients. Maxima tend to occur some weeks after the nutrients decreased (Fogg et al. 1973). These algae may store previously available nitrogen that they use under nitrogen-limiting conditions. Akçaalan et al. (2008) determined the concentration of nitrogen (NO_2 + NO_3) as 81.9 µg/L (0.081 mg/L) during the bloom *of Nodularia* in Lake İznik. Concentrations of nitrate (NO_3) and orthophosphate (PO_4) generally ranged 361-372 µg/L (0.361-0.372 mg/L) and 8-12 µg/L (0.008-0.012 mg/L) respectively during *Nodularia spumigena* blooms in Lake Hazar. Occurrence of *Nodularia* bloom at low nitrogen concentrations in lake İznik and Lake Hazar appears to support the finding of Fogg et al. (1973). However, concentrations of nitrate were always high (ranged 2.1-4.6 mg/L) to support the rapid multiplication of *Microcystis aeruginosa* in Keban Dam Lake where nitrate and orthophosphate concentrations were 5-6 folds of those recorded in Lake Hazar and Lake İznik.

Cyanotoxic blooms of *Nodularia spumigena* were observed in lakes (Hobson et al. 1999) as well as in seas (Kahru et al. 1994; Mazur-Marzec 2006). However toxic bloom of the alga was reported only from a freshwater lake (Lake İznik) in Turkey so far. Lake Hazar is the second ecosystem in which the toxic bloom *N. spumigena* was recorded in our country.

Akçaalan et al. (2008) reported that maximum filament concentrations of *N. spumigena* occurred in August in Lake İznik. Present study is in harmony with these authors since first bloom of *N. spumigena* in Lake Hazar occurred late-July and the second one was observed in early-August. Therefore it may indicate that blooms of *N. spumigena* tend to occur in summer like that of *M. aeruginosa* in this geographical region.

Akçaalan et al. (2008) analysed chlorophyll *a* concentration as 11.4 µg/L during the bloom of *Nodularia spumigena* in Lake İznik. Similarly, chlorophyll *a* concentration was found to range 14-18 µg/L during the blooms of the same alga in Lake Hazar. Level of pH in lake İznik was reported to be 8.99 (Akçaalan et al. 2008) that is almost as high as that of Lake Hazar.

A direct reletionship between *N. spumigena* blooms and high phosphate and low nitrogen content in brakish waters was found by Lehtimaki et al. (1994). However, the present study is partly in harmony with this finding since active growth of *N. spumigena* coincided with low concentrations of both nutrients in Lake Hazar.

Salinity was reported to be one of the main factors for blooms of toxic cyanobacteria to occur (Kahru et al. 1994; Mazur-Marzec 2006; Akçaalan et al. 2008). However effect of salinity in the present study was obscure since chlorine concentration were too low to affect strongly the occurrence of cyanotoxic blooms in Keban Dam Lake. However this may hold true for the bloom of *Nodularia spumigena* in Lake Hazar that is a characteristic lake with high alkalinity and pH level. In fact, chlorine (Cl) concentration was found to range 200-300 mg/L in Lake Hazar (Koçer and Şen 2014) that is almost 10 folds of that recorded (21.9-24.1 mg/L) in Keban Dam Lake.

It is noteworthy to emphasize that *N. spumigena* was never observed and/or reported from Keban Dam Lake. On the contrary, blooms of *M. aeruginosa* and *A. flos-aquae* never occurred in Lake Hazar. This is most probably due to different water quality characteristics of the two lakes. In fact, it is possible to consider that specialized environmental features of Lake Hazar (such as high alkalinity and pH) may prevent *Microcystis* and *Aphanizomenon* from occurring in in this lake. This may be true as it was reported that considerably high and low degree/concentration of any environmental variables may become a restricting factor for algal growth in the ecosystems (Rai and Gaur 2001).

Concentrations of cyanotoxins produced by *Nodularia spumigena* and *Microcystis aeruginosa* were considerably different although both algae formed thick bloom bands when blooms occurred. In fact, higher concentration of *nodularin* in Lake Hazar was noticable compared to that of *microcystin* detected in Keban Dam Lake. Different water properties of the two lakes may be taken as one of the reasons for this. It is a well-known fact that dam lakes have different water properties and dynamics than those of highly alkaline and less dynamic lakes like Lake Hazar. Different trophic status of these lakes should also be taken into consideration to explain the difference related to toxin production.

Albay et al. (1998) studied *M. aeruginosa* bloom occurred in Sapanca (meso-oligotrophic) and Taşkışla Lake (eutrophic). They reported that *microcystin-RR* was dominant cyanobacterial microcystin in Lake Sapanca whilst *microcystin*-LR was determined at higher concentration than other variant of microcystin in Taşkışla Lake. All three variants of microcystin were also determined in Keban Dam Lake during *Microcystis aeruginosa* bloom and amount of microcystin-RR and microcystin-LR were also higher than that of

microcystin-YR. Albay et al. (2005) reported that highest microcystin production occurred when ratio of TN/TP was over 7. High concentration of nitrate in Keban Dam Lake also appeared to support the cyanotoxin production.

Blooms in Keban Dam Lake and Lake Hazar usually commenced to occur in the littoral regions. Nutrient concentrations in littoral regions are always higher than those in open water parts of the lakes. This may be one of the the main reason for blooms to occur in the littoral regions. In addition, wind and water movements push the filaments and colonies of the blue-green algae to accumulate in a much smaller volume of water to form dense populations. Naturally, this may give rise to an increase in number of individuals/colonies in the littoral.

Fortunately, fish mortalities were not observed during the blooms of both *M. aeruginosa* and *N. spumigena*. This is most probably due to the low concentrations of the cyanotoxins in these lakes. Both lakes have enormous water volume that most probably contribute a rapid dilution of cyanotoxins. In addition, fishes probably prefer to gather in colder deep water rather than staying in warm surface water during summer. This creates a good alternative for fishes to keep themselves away from the effect of harmful toxic blooms that always occurred on the surface waters (at 0.1-1.5 m water column). However, it was reported that the longevity of the effects of toxic *M. aeruginosa* in different ecosystems depend on factors that can accelerate the degradation or dilution of the toxin, such as ultraviolet radiation, strong oxidizers, naturally occurring bacteria which deactivate or otherwise eliminate microcystin (Watanabe et al. 1992). Hydrologic consideration such as tidal flushing and water residence time should also taken into consideration to define the rate of dilution of both toxic cells and extracellular toxin.

Conclusions

Abundance of cyanobacteria is usually closely linked to eutrophication. In fact, it is a significant observation considering the fact that cultural eutrophication of surface waters resources has increased with the world-wide occurrences of putatively toxic algal blooms. The same threat is also valid for Keban Dam Lake and to some extent Lake Hazar. It should always be noted that continous nutrient loading from catchment area will definitely give rise to a change in the trophic status of these lakes in the long term. It is a well-known fact that, shifting trophic status of the lakes from oligomesotrophy to eutrophy with increasing nutrients will be inevitable in future. At that stage, unfortunately it may be possible to witness more frequent and more intensive toxic blooms of cyanophyta/cyanobacteria in Keban Dam Lake and Lake Hazar, possibly resulting in fish deaths.

Because of potential harmful effects of *M. aeruginosa*, *A. flos-aquae* and *N. spumigena*, occurrence of these algae in Keban Dam Lake and Lake Hazar should be monitored requlary. In addition external nutrient loads of diffuse and point resources in these lakes from their vicinity should be estimated and controlled carefully. It should be an urgent issue for local govermental authorities that, these algae should become a focus of efforts to control harmful algal blooms in these ecosystems. Preventing bloom of harmful algae is extremely significant to maintain their sustainable use for fisheries, irragation and recreational purposes. It should always be remembered that toxic algae naturally cause a serious damage to the sustainable use of the lakes.

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Understanding How Parasites from Farmed Fish May Influence Wild Fish Declines Using Epidemiological Modelling

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ABSTRACT

Beside various fields of its applications, in this study epidemiological modelling was used to understand how parasites from farmed fish may cause wild fish declines. Two separate strategic models were constructed addressing the transmission of micro-parasites and macro-parasites between farmed and wild fish: A SIR (Susceptible-Infective-Removed) model for micro-parasite infections and a compartmental density-dependent model for macro-parasite infestations. The results indicated that parasites originated in wild fish populations, after infecting farmed fish can cause epizootics. Subsequently, these parasites can be transmitted from farmed to wild fish and might have negative impact on the dynamics of wild fish populations. Sensitivity analysis of the basic model parameters in both models showed that model parameters, which are influenced by abiotic factors and allow passive manipulation, such as pathogen specific transmission rate (β), pathogen specific transmission rate between infected farmed and susceptible wild fish (δ), the rate of production of infective stages by an adult parasite (λ) and transmission rate between host and parasite infective stages (β) are more sensitive compared to model parameters which encompass chemical control and fallowing. This emphasizes the importance of the preventive medicine rather than intervention procedures in aquaculture aiming at eradicating epizootics caused by parasites and protecting wild fish stocks.

Keywords: Aquaculture, parasites, epidemiology, modelling, wild fish

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Kültür Balıklarından Kaynaklanan Parazitlerin Nasıl Yabani Balık Azalmalarını Etkileyebileceğini Epidemiyolojik Modelleme Kullanarak Anlaşılması

Öz: : Çeşitli uygulama alanlarının yanı sıra, bu çalışmada epidemiyolojik modelleme kültür balıklarından kaynaklanan parazitlerinin nasıl yabani balık azalmalarına yol açabileceğinin anlaşılması için kullanılmıştır. Kültür ve yabani balıklar arasındaki mikro-parazitlerin ve makro-parazitlerin yayılımına yönelik iki ayrı stratejik model geliştirilmiştir: mikroparaziter enfeksiyonlar için SIR (Sağlam-Enfektif-Geçiren) modeli ve makro-paraziter enfestasyonlar için bölümlü ve yoğunluğa bağlı model. Sonuçlar, başta yabani balık popülasyonlarında çoğalan parazitlerin kültür balıklarını enfekte ettikten sonra belirli yetiştiricilik şartları altında epizootiklere neden olabileceklerini işaret ettiler. Akabinde, bu parazitler kültür balıklarından yabani balıklara yayılabilirler ve yabani balık popülasyonlarının dinamikleri üzerinde olumsuz etkiye sahip olabilirler. İki modeldeki temel model değişkenlerinin duyarlılık analizleri, abiyotik etmenler tarafından etkilenen ve edilgen yönetime izin veren değişkenler, örneğin mikroparaziter modelde patojene özgü yayılım hızı (β) ve enfektif kültür balıkları ile sağlam yabani balıklar arasındaki patojene özgü yayılım hızı (δ) ve makroparaziter modelde erişkin parazitlerin enfektif evre üretim hızı (λ) ile parazitin enfektif evreleri ile konakçısı arasındaki yayılım hızı (β), kimyasal kontrol ve üretim alanının boş bırakılmasını içeren model parametreleri ile kıyasla daha duyarlı olduklarını göstermiştir. Bu, parazitlerden kaynaklanan epizootiklerin yok edilmesinde ve yabani balık soyunun korunmasını amaçlayan su ürünlerindeki müdahale yöntemlerinden çok koruyucu hekimliğin önemini vurgulamaktadır.

Anahtar kelimeler: Su ürünleri yetiştiriciliği, parazitler, epidemiyoloji, modelleme, yabani balıklar

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Introduction

There has been a steady growing trend in the world fish production in the last few decades, paralleled with world population growth and respectively fish consumption. According to FAO, total world fisheries production in 2020 was estimated as 177.8 million tonnes, from which aquaculture accounted for 87.5 million tonnes (FAO 2022). Whereas capture fisheries shows a relatively steady state, aquaculture production is the true contributor of growth in world fish production with a continuous growing pace of roughly 3.3% annually (FAO 2022). This expansion of the world aquaculture production can be truly regarded as a "Blue Revolution". However, the revolution can be regarded as a reality only if aquaculture growth is sustainable and does not negatively impact and endanger wild fish populations. In this respect, not only demand for fish oil and fishmeal of aquaculture industry must be considered, but also interchangeable status of disease between wild fisheries and farmed fish.

Disease in aquaculture limits the expansion of the sector with different pathogens constraining its growth (Murray and Peeler 2005). Previously existing disease in wild populations can be exacerbated in artificially reared fish due to high population densities and other stresses (Reno 1998; Murray and Peeler 2005). The problem can also be formulated as a double-edged sword where pathogens transmission from wild to farmed and vice versa takes place. Such transmission of pathogens between wild and farmed fish populations named spill-over and spillback can be true cause of emergence of infectious diseases (Krkošek 2010; Reno 1998). Moreover, disease agents which exist in wild populations, if no control measures are taken, might be magnified in farmed populations and itself become a source of pathogens for wild populations, with negligible to significant impact (Miossec et al. 2005; Murray 2009).

A substantial amount of the epizootics, which occur in wild and farmed fish populations with sometimes devastating impact, are parasitic in their nature. Parasitic disease outbreaks account for much of the economic losses in aquaculture and they might also be a cause for wild fish decline elsewhere. Understanding their establishment mechanisms is essential prerequisite for eradicating them (Guo and Woo 2009; Tokşen and Çilli 2010; Costello 2009; Munday et al. 2001; Morris 2011).

Vital tools for understanding the establishment mechanisms of parasitic disease in wild and farmed fishes are risk analysis and epidemiology. Analysing and identifying the risk factors associated with parasitic diseases and conducting epidemiological surveys is the way for prevention of mortality (Soares et al. 2013). Furthermore, focusing on risk analysis, biosecurity and supportive epidemiological studies can improve understanding of the causes of parasitic disease and leads to better management of these diseases in aquaculture and wild fish populations, subsequently informing decision making authorities and policy makers (Rodgers and Peeler 2012). Because aquaculture health issues are mostly based on population medicine, epidemiological methods are used when tackling parasitic diseases in farmed and wild fish (Georgiadis et al.2001; Beaglehole et al. 1993) and effectively implemented in order to passively reduce the risk of parasitic disease occurrence (Murray 2013). Nevertheless, epidemiology is one of the most important tools for identifying risk factors that increase the probability of parasitic disease occurrence and for optimizing the cost efficacy of any intervention or control strategy (Turnbull et al. 2011).

One of the crucial subject areas of veterinary epidemiology is theoretical disease modelling. These models offer solutions to aquatic animal health problems, estimate the impact of the parasitic disease on population level and can be appropriate where there is lack of experimental data (Peeler and Taylor 2011; Murray et al. 2011). However, epidemiological modelling has its constraints as well. Models, which are based on mathematical simulations, vary in their ability to reflect the real world where the onset of disease is a multiplicative process rather than additive process (Reno 1998). For example epidemiology of sea lice *Lepeophtheirus salmonis* (Krøyer 1838) has been regarded as multifactorial in origin with many abiotic and biotic factors interacting in a rather complex way (Revie et al. 2005). In contrast, many epidemiological models assume that any change observed in the output is solely due to the single variable change in the basic input variables and does not take into account the correlation between them (Anonymous 2015). Even the most complex models for many diseases are oversimplified, where many "guesstimated" basic disease parameters make in long run quantitative disease predictions impossible (Roberts and Heesterbeek 1993). Despite these limitations models can be used efficiently because they point important underlying relationships and hypothesises such as basic reproduction ratio (R_o), simulate thought experiments where practical experiments are impossible, highlight the importance of parameters with critical influence on onset of the diseases and eventually they are beneficial for building control strategies. For example, the evidences for farm and wild parasite sea lice exchanges are indirect because infective stages cannot be traced physically but modelling can help predict that exchange (Todd

2007). Besides, against many parasitic diseases there are no effective drugs (Guo and Woo 2009; Munday et al. 2001) and prevention is the best choice, which makes epidemiological modelling inevitable.

In this study, strategic epidemiological models were constructed to reflect spreading of more easily establishing micro and macroparasites, with direct life cycles, between farmed and wild fish populations. This study was completely theoretical one and was based on hypothetical parameter values. The latter were subsequently evaluated through sensitivity analyses in order to reveal important parameters underlying the basic transmission mechanisms of parasitic diseases between wild and farmed fish populations. This allowed different scenarios to be derived, which might lead to management policy changes.

The present study is not concerned with revealing the true cause and effect relationship responsible for the decline of wild fish populations in European seas, nor is it concerned with parameterising the specific parasitic diseases, which are very complex tasks on their own. The aim of the study is simply to build strategic models in order to understand the basic underlying facts in transmission of parasitic diseases between wild and farmed fish and whether there is any possibility of negative impact on wild fish populations from fish farming activities.

Materials and Methods

The models in the present study were based on the basic principles and differential equations developed in the epidemiological models by Anderson and May (1979a, 1979b). Because both micro and macroparasites have much shorter life span than their respective fish hosts and the duration of the epizootic is relatively shorter compared to the life duration the of the fish, the natural birth rate and death rate of the wild and farmed fish were neglected and omitted from the models in this study. Respectively, the original differential equations by Anderson and May (1979a, 1979b) were changed and simplified (Krkošek 2010).

In addition, it was also preliminary assumed that wild and farmed populations were closed populations, contacts between individuals were random (Anderson and May 1979a), the density of fish was fairly constant in time with negligible addition of new susceptible individuals to both farmed and wild fish populations and eventually farmed fish not being harvested at time of epizootics (Roberts and Heesterbeek 1993).

In both models, densities of farmed hosts were adopted as five times greater than their wild counterparts (Heuch et al. 2005). This had implications on the calculations of the basic reproduction ratio (R_o) of both models. Only densities of farmed fish populations were considered because it was assumed that farmed fish are the real focal point for epidemics.

Another basic assumption was that the physical milieu where epizootics took place was semi - closed sea bay, fjord or loch where transmission of pathogens is enhanced (Amundrud and Murray 2009; Penston et al. 2008; McKenzie et al. 2004). The contact structure of the pathogens was considered to be via water column with the aid of the flowing sea water currents (Krkošek et al. 2005; Salama and Murray 2013), escaped fish (Green et al. 2012; Costello 2006) and presence of feeding wild fish around the sea cages (Esat Çilli`s personal observation).

Estimation of the basic model parameters such as pathogen specific transmission rate, infection removal rate, the rate of production of infective stages of the macroparasites and the mortality rate of the macroparasite infective and adult stages was another sensitive issue due their multifactorial nature. For example, the disease transmission coefficient (=pathogen specific transmission rate) is one of the most difficult parameters to be estimated in any epidemiological model (McCallum et al. 2001). Attempts to calculate it have been concentrated on preliminary knowledge on host-disease behaviour, controlled experiments and deduction of the parameter by observation of real epidemics (McCallum et al. 2001). Reno (1998) proposed that the factors affecting transmission coefficient of a particular disease (=pathogen specific transmission rate) are host resistance factors such as species, age, natural immunity, induced immunity; pathogens factors such as ability to infect species, dose, vertical transmission and finally environmental factors such as population density, temperature, water flow and water chemistry.

In the models presented below, attempts were made where possible to deduce the disease model parameters from existing experimental data (Amundrud and Murray 2009; Munday et al. 2001; Morrison et al. 2004; Wagner et al. 2008; Costello 2006). However, due to the difficulties of parameter estimation mentioned above and relative lack of real epidemiological data about transmission of parasites between farmed and wild fish population, most model parameters were completely arbitrary in order to prevent numerical instability of the models. In each model arbitrary time steps were used, such that they do not denote for particular time period as hour, day or month.

The first epidemiological model attempted to explain the transmission behaviour and onset of epidemics between wild and farmed fish caused by protozoan parasites with direct life cycles such as the causative agent of amoebic gill disease

Neoparamoeba spp., microsporidian parasite such as *Loma* spp. and metazoan parasites with direct life cycle such as *Gyrodactylus* spp.

The second epidemiological model had the main aim to elucidate the epidemiology of macroparasites with direct life cycle such as the copepod parasites *L. salmonis* (Krøyer, 1838) and *Caligus elongatus* (Nordmann, 1832). According to Murray (2009), the first model in this study can be regarded as a direct density-dependent transmission model, respectively the second model as a constant open recruitment model. However, due to lack of enough mathematical information in the study of Murray (2009), for construction of the model for macroparasites differential equations developed in the epidemiological models by Anderson and May (1979a, 1979b) were used.

Microparasite Model

A compartmental density-dependent SIR (Susceptible-Infective-Removed) model consisting of series of differential equations was developed to investigate the epidemiology of microparasitic infections between farmed and wild fish. The overall schematic representation of the model is provided in Figure 1 and the description of all model parameters along with their dimensional analysis are summarised in Table 1.

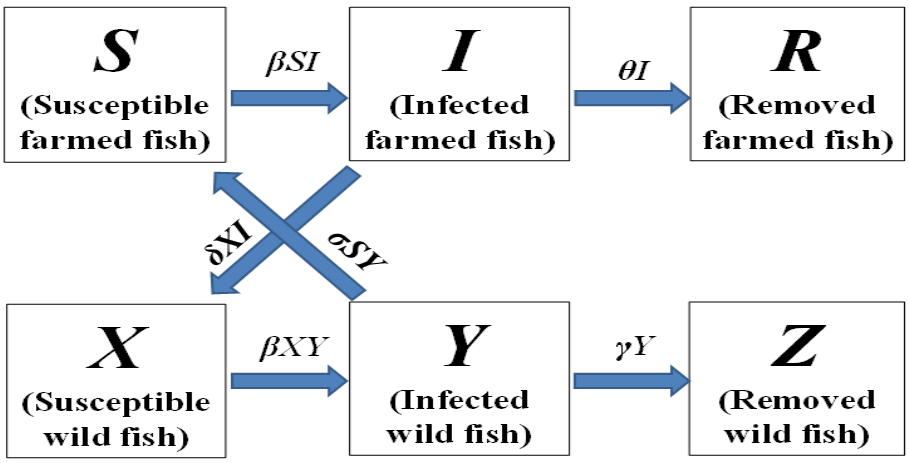

Figure 1. A schematic representation of the compartmental density-dependent SIR (Susceptible-Infective-Removed) model of microparasitic infections between farmed and wild fish (all model parameters are described in Table 1)

Table 1. Description of the model parameters and variables used in the compartmental density-dependent SIR (Susceptible-Infective-Removed) model of microparasitic infection between farmed and wild fish

Parameter symbol	Description	Dimension
S	Susceptible farmed fish density	[M]
I	Infected farmed fish density	[M]
R	Removed farmed fish density	[M]
X	Susceptible wild fish density	[M]
Y	Infected wild fish density	[M]
Z	Removed wild fish density	[M]
β	Pathogen specific transmission rate	$[M]^{-1}[T]^{-1}$
γ	Infection removal rate of wild fish	$[T]^{-1}$
θ	Infection removal rate of farmed fish	$[T]^{-1}$
δ	Pathogen specific transmission rate between infected farmed (I) and susceptible wild (X) fish	$[M]^{-1}[T]^{-1}$
σ	Pathogen specific transmission rate between infected wild (Y) and susceptible farmed (S) fish	$[M]^{-1}[T]^{-1}$

For parameter dimensions, T represents time and M represents host density.

Susceptible, infective and removed compartments of both farmed and wild fish were represented as host densities. In contrast to the viral and bacterial diseases, where hosts acquire long-lasting immunity to reinfection, in the present model it was assumed that there was no recovery from the disease. In parasitic diseases fish do not build significant immune response which can confer them

with immune resistance to microparasites (Guo and Woo 2009). The basic model parameters described in Table 1 were used to formulate the following differential equations reflecting the dynamics of the epizootic:

$$\frac{dS}{dt} = -\beta SI - \sigma SY \quad (1)$$

$$\frac{dI}{dt} = \beta SI + \sigma SY - \theta I \quad (2)$$

$$\frac{dR}{dt} = \theta I \quad (3)$$

$$\frac{dX}{dt} = -\beta XY - \delta XI \quad (4)$$

$$\frac{dY}{dt} = \beta XY + \delta XI - \gamma Y \quad (5)$$

$$\frac{dZ}{dt} = \gamma Y \quad (6)$$

Subsequently, the differential equations above on which the model was based were used for the writing the model code. The Microparasite model as it was schematically outlined in Figure 1 represented two distinct fish populations, wild and farmed denoted in the model as S (susceptible farmed fish density) and X (susceptible wild fish density). Infection started in farmed fish with gradual accumulation of infected farmed fish (I) depending on the pathogen specific transmission rate (β) between S and I. Infection spread to wild fish proportionally to the contact rate between infected farmed fish (I) and susceptible wild fish (X) which in the model was shown as δ. Epizootics in wild and farmed fish populations proceeded by cross infections between both populations (σSY and δXI) and inside the populations (βSI and βXY). Finally, infected fish in the model, both wild and farmed, were removed depending on infection removal rates θ and γ.

The fundamental concept of basic reproduction ratio (R_o) (Anderson and May 1979a; Reno 1998) was adopted to measure the number of the secondary infections caused by single infected hosts in the course of the epizootics. Basically, when $R_o \leq 1$ an epizootic cannot be established, if $R_o > 1$ epizootic will take place. Subsequently this concept proved valuable in calculating the maximum stocking density of farmed fish under which epizootic cannot be established. As it was stated above, only densities of farmed fish were considered, assuming that real focal point for epidemics were farmed fish populations. For measure of the basic reproduction ratio (R_o) of microparasites the following equation adopted from Krkošek (2010) and based on model parameters described in Figure 1 was used.

$$R_o = \frac{\beta S}{\theta} \quad (7)$$

Macroparasite Model

Similar approach as in the compartmental model for microparasites was followed in construction of the model addressing epidemiology of macroparasites with direct life cycle between wild and farmed fish. However, due to the biology of macroparasites and dynamics of the metazoan parasitic epidemics, dividing populations simply to susceptible, infected and removed was not applied (Anderson and May 1979b). Instead division was made upon the existence of five basic populations, respectively two host populations (wild and farmed fish), two adult parasites populations (adult parasites on wild and farmed fish) and one population of infective stages, which was bring into life from the reproductive contribution of the adult parasites infesting both wild and farmed fish. The evidence for only one compartment of infective stages came from the study of Todd (2007), where was claimed that there was actually only one panmictic population of sea lice in North Atlantic. Thus, during the construction of the model it was logical to assume that there is only one compartment for the infective stages. Another assumption specific for the model addressing macroparasitic epidemiology was that the distribution of macroparasites followed Poisson form rather than negative binomial, macroparasites evenly or randomly distributed rather than clumped on their hosts (k→∞, parameter k approaching infinity) (Anderson and May 1979b; Krkošek 2010). The overall schematic representation of the model is provided in Figure 2 and the description of all model parameters along with their dimensional analysis are summarised in Table 2.

All compartments in the model were represented as densities. Again as in the SIR model, it was assumed that there was no recovery from the disease as in metazoan parasitic diseases fish do not build significant immune response to confer them with immune resistance to macroparasites (Guo and Woo 2009). The basic model parameters described in Table 2 were used to formulate the following

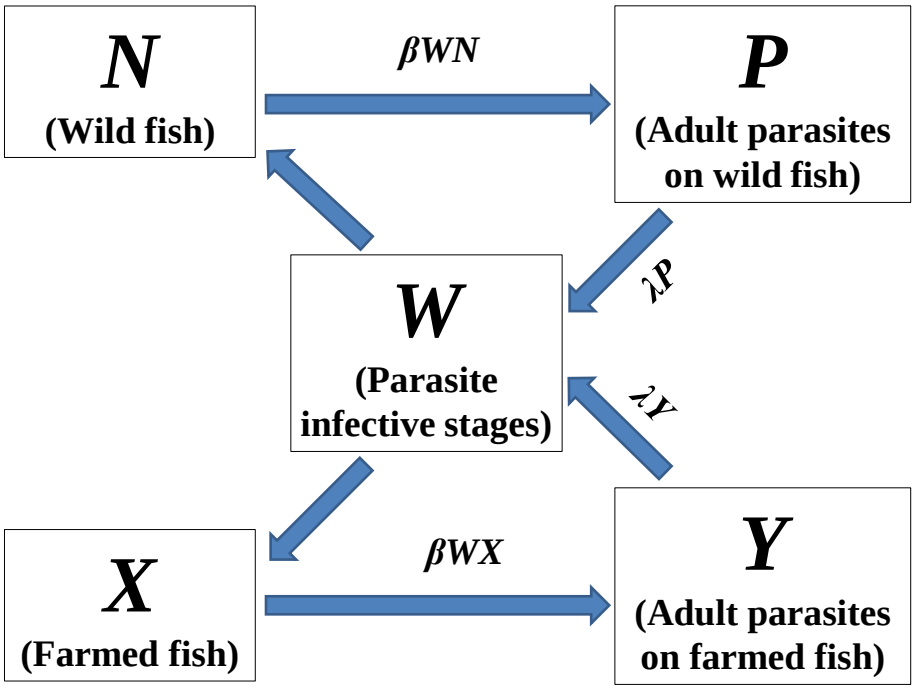

Figure 2. A schematic representation of the compartmental model of macroparasitic infestation between farmed and wild fish (all model parameters are described in Table 2)

Table 2. Description of the model parameters and variables used in the compartmental model of macroparasitic infestation between farmed and wild fish

Parameter symbol	Description	Dimension
N	Wild fish density	[M]
X	Farmed fish density	[M]
P	Density of adult parasites on wild fish	[M]
Y	Density of adult parasites on farmed fish	[M]
W	Density of infective stages	[M]
α	Parasite-induced host death rate	[M]
β	Transmission rate between host and parasite infective stages	$[M]^{-1}[T]^{-1}$
μ	Mortality rate of adult parasites on wild fish	$[T]^{-1}$
θ	Mortality rate of adult parasites on farmed fish	$[T]^{-1}$
c	Mortality rate of infective stages	$[M]^{-1}[T]^{-1}$
λ	The rate of production of infective stages by an adult parasite	$[M]^{-1}[T]^{-1}$

For parameter dimensions, T represents time and M represents host density

differential equations reflecting the dynamics of the epizootic:

$$\frac{dN}{dt} = -\alpha P \quad (8)$$

$$\frac{dX}{dt} = -\alpha Y \quad (9)$$

$$\frac{dP}{dt} = \beta WN - (\mu + \alpha)P - \frac{\alpha P^2}{N} \quad (10)$$

$$\frac{dY}{dt} = \beta WX - (\theta + \alpha)Y - \frac{\alpha Y^2}{X} \quad (11)$$

$$\frac{dW}{dt} = \lambda P + \lambda Y - cW - \beta WN - \beta WX \quad (12)$$

The differential equations above on which the model was based were subsequently used for writing the model code.

Macroparasite model, as it was schematically outlined in Figure 2 represented two distinct fish populations, wild and farmed denoted in the model as N (wild fish density) and X (farmed fish density). Infestations, depending on the transmission rate (β) started simultaneously both in farmed and wild fish

by contacts (βWN and βWX) with free swimming infective stages of the parasite (W) , which originated from adult parasites on wild fish (P). After successful infestations, infective stages of the macroparasites attached on wild and farmed fish produced adult parasite stages P and Y. Further production of infective stages (W) by adult parasites continued depending on parameter λ (the rate of production of infective stages by an adult parasite). Gradual accumulation of more infective stages (W) contributed for establishment of epizootics both on farmed and wild fish. Mortality of fish was dependent on parameter α, which in the model was denoted as parasite induced host death rate. Finally, adult parasites on farmed and wild fish were removed at mortality rates μ and θ.

As in the first model about microparasitic epizootics, the important concept of basic reproduction ratio (R_o) (Anderson and May 1979a; Reno 1998) was used to measure the number of the secondary infestations caused by a single macroparasite in the course of the epizootic. Again this concept proved valuable in calculating the maximum stocking density of farmed fish under which epizootic cannot be established. As it was stated above, only densities of farmed fish were considered, assuming that real focal point for epidemics were farmed fish populations. For measure of the basic reproduction ratio (R_o) of macroparasites the following equation adopted from Krkošek (2010) and based on model parameters described in Figure 2 was used:

$$R_o = \left(\frac{\lambda}{\theta+\alpha}\right)\left(\frac{\beta X}{c+\beta X}\right) \qquad (13)$$

Sensitivity Analysis

For the purpose of evaluating the relative importance of the model parameters in the dynamics of the modelled epizootics, the method of sensitivity analysis developed by Bode (1945) was applied. A one-at-a-time approach was followed where one basic input model parameter was changed by 1% keeping other on their default values in order to see what kind of effect it can produce in the model output. The output parameter in the first model addressing microparasitic epizootics was the maximum density of infected wild fish. The output in the second model, reflecting the macroparasitic epizootics, was the final density of wild fish. For measurement of the relative sensitivity of the model parameters following equation was used:

$$S_{x_i}^P = \frac{x_i}{P}\frac{\delta P}{\delta x_i} \qquad (14)$$

Where, $S_{x_i}^P$ was the relative sensitivity of the evaluated parameter, $\frac{\delta P}{\delta x_i}$ was the absolute change in the output in response to a 1% change in the parameter value and $\frac{x_i}{P}$ the ratio between initial output value to initial parameter value.

Writing the Model Codes and Analyses

Codes of the both models were coded in R (A Programming Environment for Data Analysis and Graphics Version 3.2.0 – The R Core Team 2015). Analyses were carried out both in R and Excel (Microsoft Excel, 2010).

Results

Microparasite Model

The simulation of the model started with initial model parameters with following values: $\beta = 0.4$, $\gamma = 0.18$, $\theta = 0.18$, $\delta = 0.02$ and $\sigma = 0.01$, densities of farmed $S = 10.0$ and wild fish $X = 2.0$ and with successful introduction of the microparasite in farmed fish with non-zero value of $I = 0.001$. The epizootic time series for the density-dependent SIR model are shown in Figure 3 where the epizootic follows typical SIR epidemic pattern. Initially, there was a sharp decrease of susceptible farmed fish density (S) as more effective contacts were made and sharp increase in infected farmed fish density (I), with gradual increase of removed farmed fish (R). Almost paralleled in time, with epizootics in farmed fish started the epizootic in wild fish, where microparasites from infected farmed fish were transmitted to wild susceptible fish depending on pathogen specific transmission rate δ. With increasing the infection level in wild fish the opposite trend also proceeded. In other words transmission of the pathogens, depending on pathogen specific transmission rate σ, took place between wild infected and farmed susceptible fish. Finally, the epizootics died off at which the lines in the graph were levelled off.

Sensitivity analysis of the model at default values of the basic parameters was performed (Figure 4). The analyses showed that the most sensitive parameter affecting the density of wild infected fish (denoted in the model as Y) was γ (infection removal rate of wild fish), followed by β (pathogen specific transmission rate), δ (pathogen specific transmission rate between infected farmed (I) and susceptible wild (X) fish) and θ (infection removal rate of farmed fish). Parameter σ (pathogen specific transmission rate between infected wild (Y) and susceptible farmed (S) fish) did not have significant effect on the model outputs. The only parameter in the model which allows direct human intervention such as fallowing, chemotherapeutic applications and

vaccination, with aim to eradicate the epizootic, respectively parameter θ, did not prove to be the most sensitive parameter of the model. In order to evaluate further the importance of this parameter, the model was simulated under three different scenarios (=different epizootic conditions): A) scenario 1: keeping the initial values of all parameters except β, where β was reduced to 0.2 pointing less acute epizootic and less force of infection; B) scenario 2 where the initial values of all parameters were kept except θ, respectively $\theta = 1.2$; C) scenario 3 where again the initial values of all parameters were kept except θ and β, respectively $\theta = 1.2$ and $\beta = 0.2$ (Figure 4).

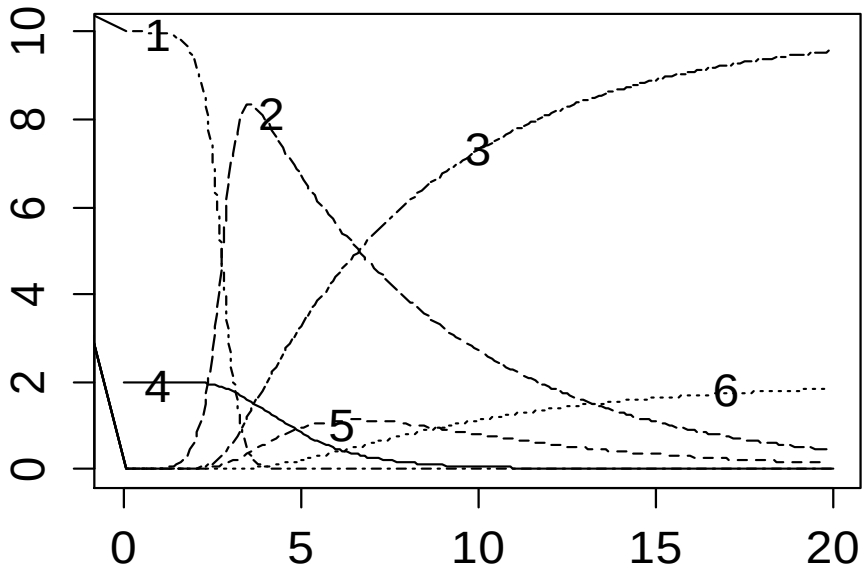

Figure 3. The epizootic time series for the Microparasite model. Line 1 - susceptible farmed fish density (S), line 2 - infected farmed fish density (I), line 3 - removed farmed fish density (R), line 4 - susceptible wild fish density (X), line 5 - infected wild fish density (Y), line 6 - removed wild fish density (Z)

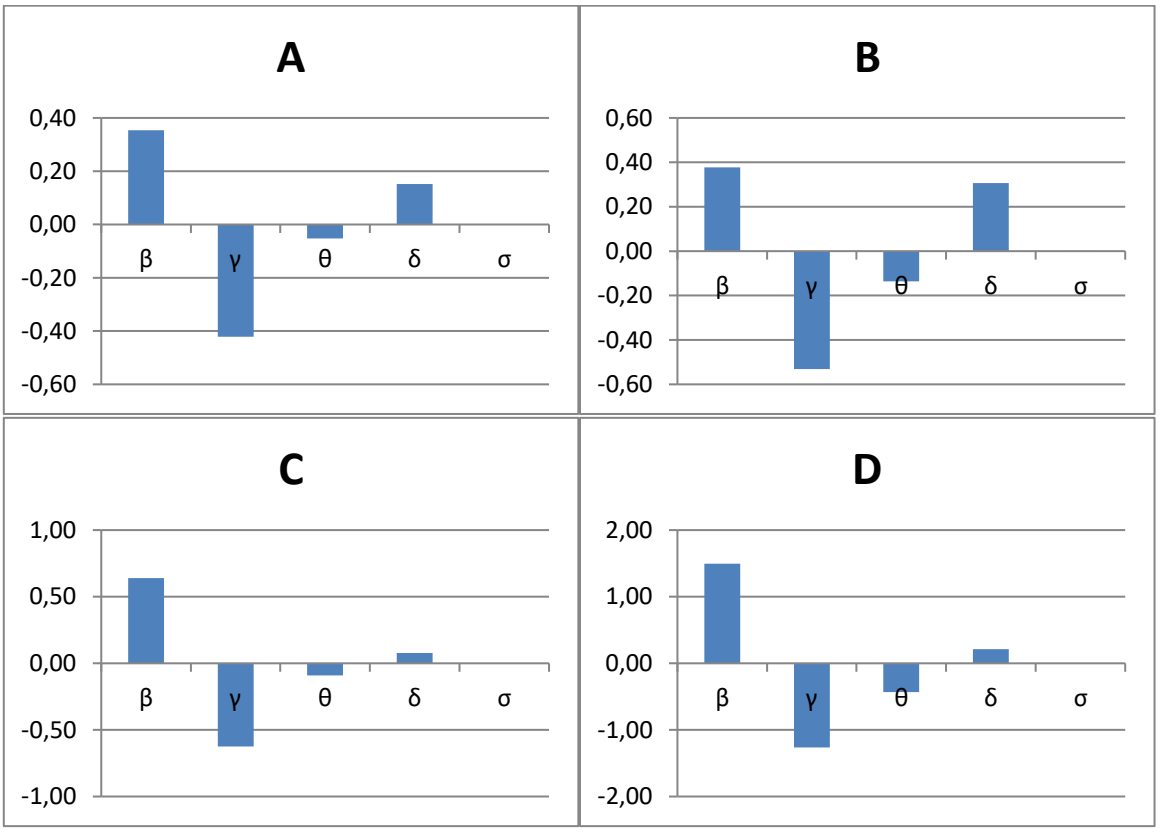

Figure 4. Sensitivity analysis results for the Microparasite model. The panels are: A) model simulated with the initial parameter values; B) scenario 1 - the initial values of all parameters kept except β, where β reduced to 0.2; C) scenario 2 where the initial values of all parameters kept except θ, respectively $\theta = 1.2$; D) scenario 3 where again the initial values of all parameters kept except θ and β, respectively $\theta = 1.2$ and $\beta = 0.2$

In scenario 2 and 3, θ (Infection removal rate of farmed fish) was increased near 6 times ($\theta = 1.2$) indicating more intense intervention procedures in order to eradicate the disease from farmed fish and shorter duration of the disease.

Sensitivity analysis for each scenario was carried out as shown in Figure 4. There was no change in the ranking of the sensitivity of each parameter in scenario 1. However, paralleled with increase in its value, the relative importance of θ (infection removal rate of farmed fish) increased in scenarios 2 and 3. Therefore, infection removal rate of farmed fish became the third most sensitive parameter.

Nevertheless, θ still proved to have relative impact on reducing the density of wild infective fish as shown in Figure 5, where the model was simulated with different θ values representing more effective removal rate of infective farmed fish.

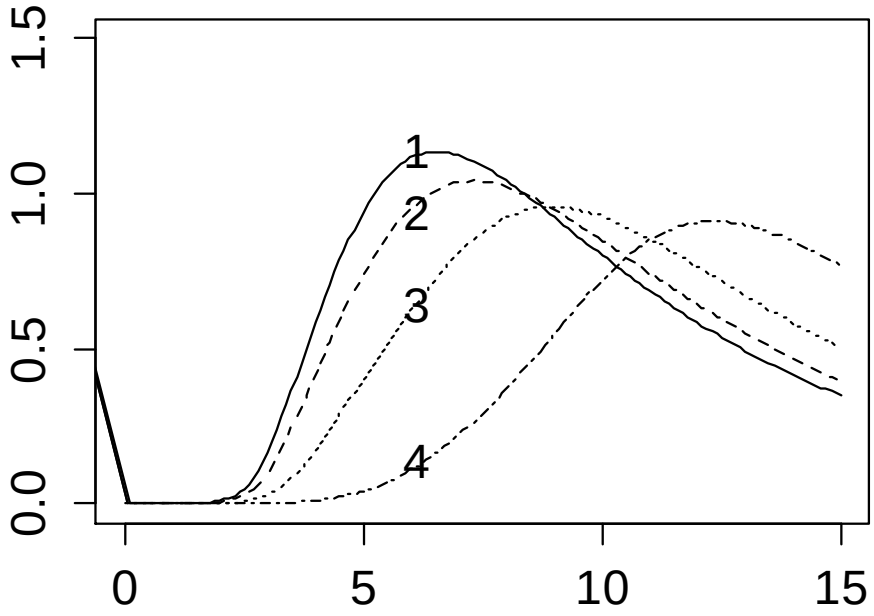

Figure 5. Simulation of the Microparasite model under different values of θ, while keeping other parameters in their default values. Line 1: $\theta = 0.18$; line 2: $\theta = 0.54$; line 3: $\theta = 1.26$; line 4: $\theta = 2.52$. Each line represents the time series of wild infected fish densities.

From the practical viewpoint, it was important to know the maximum density of farmed fish under which epizootic cannot be established. Therefore, in order to calculate the maximum density of stocked (S) fish under each scenario, the basic reproduction ratio (R_o) was set to 1 (the threshold value of R_o for establishment of infection) depending on the values of S, β and θ. The results are shown in Table 3. Although θ was not the most sensitive parameter of the model it was important in calculating the maximum density of stocked fish represented as density of susceptible farmed fish density (S) under different model scenarios.

Table 3. For each scenario in the Microparasitic model, the maximum stocking density of fish represented as density of susceptible farmed fish (S) when the basic reproduction ratio (R_o) is set to 1

Scenario	S	β	θ	R_o
Initial	0.45	0.40	0.18	1.00
1	0.90	0.20	0.18	1.00
2	3.00	0.40	1.20	1.00
3	6.00	0.20	1.20	1.00

Macroparasite Model

The simulation of the model started with initial model parameters with following values: $\alpha = 0.002$, $\beta = 0.4$, $\mu = 0.01$, $\theta = 0.01$, $c = 0.05$ and $\lambda = 0.3$, densities of farmed $X = 10.0$ and wild fish $N = 2.0$ and with preliminary established population of the

macroparasites in wild fish with non-zero value for $P = 0.2$ and $W = 0.5$. The epizootic time series for the compartmental model of macroparasitic infestation between farmed and wild fishes are shown in Figure 6. Macroparasites both on wild and farmed fish showed exponential growth, more profound in macroparasites on farmed fish. The latter also implied that infective stages mostly originated from farmed fish rather than from wild, which can also be deduced from the very similar slopes of the lines 3 and 5 rather than slopes of lines 4 and 5 (Figure 6). In contrast to the Microparasite model, the epizootics of macroparasites did not die off and the lines in the graph were not levelled off. When the number of the time steps in the simulation of the model was increased, the densities of both wild and farmed fish reached zero value. Therefore, host fish populations were driven by the parasites to extinction.

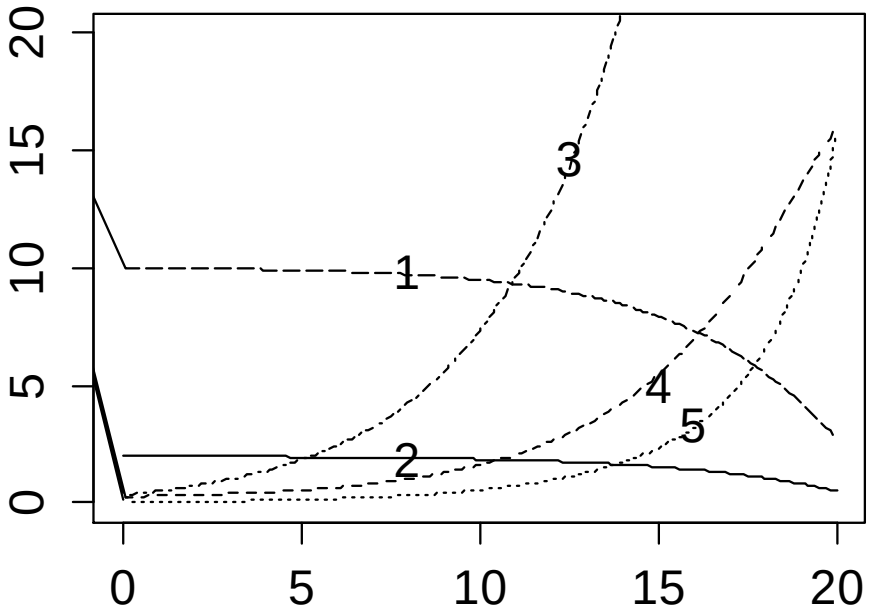

Figure 6. The epizootic time series for the Macroparasite model. Line 1 - Farmed fish density (X), line 2 - Wild fish density (N), line 3 - Density of adult parasites on farmed fish (Y), line 4 - Density of adult parasites on wild fish (P), line 5 - Density of infective stages (W)

Next step was performing the sensitivity analysis of the model with default values of the basic parameters (Figure 7). Analyses showed that the most sensitive parameter affecting the wild fish density was λ (the rate of production of infective stages by an adult parasite), followed by α (parasite-induced host death rate), β (transmission rate between host and parasite infective stages), θ (mortality rate of adult parasites on farmed fish), μ (mortality rate of adult parasites on wild fish) and c (mortality rate of infective stages). Parameter α, which can be influenced by management procedure such as vaccination, turned out to be the second sensitive model parameter in rank. However, the parameter θ in the model which with aim to eradicate the epizootic allows, as well as parameter α, direct human intervention such as fallowing, cleaning fish (wrasse) and chemotherapeutic applications did not prove to be the most sensitive parameter of the model. In order to evaluate further the importance of the parameter θ, the model was simulated under three different scenarios (=different epizootic conditions): A) scenario 1 - the initial values of all parameters were kept except α, where α was reduced to 0.001 B) scenario 2 where the initial values of all parameters were kept except θ, respectively $\theta = 0.1$ C) scenario 3 where again the initial values of all parameters were kept except θ and α, respectively $\theta = 0.1$ and $\alpha = 0.001$. (Figure 7).

In scenario 2 and 3, θ (mortality rate of adult parasites on farmed fish) was increased 10 times ($\theta = 0.1$) indicating more intense intervention procedures in order to eradicate the parasites from farmed fish.

There was no change in the ranking of the sensitivity of each parameter in scenario 1. However, in line with increase in its value, the relative sensitivity of θ (mortality rate of adult parasites on farmed fish), respectively its importance, increased in scenarios 2 and 3 and θ became the third most sensitive parameter.

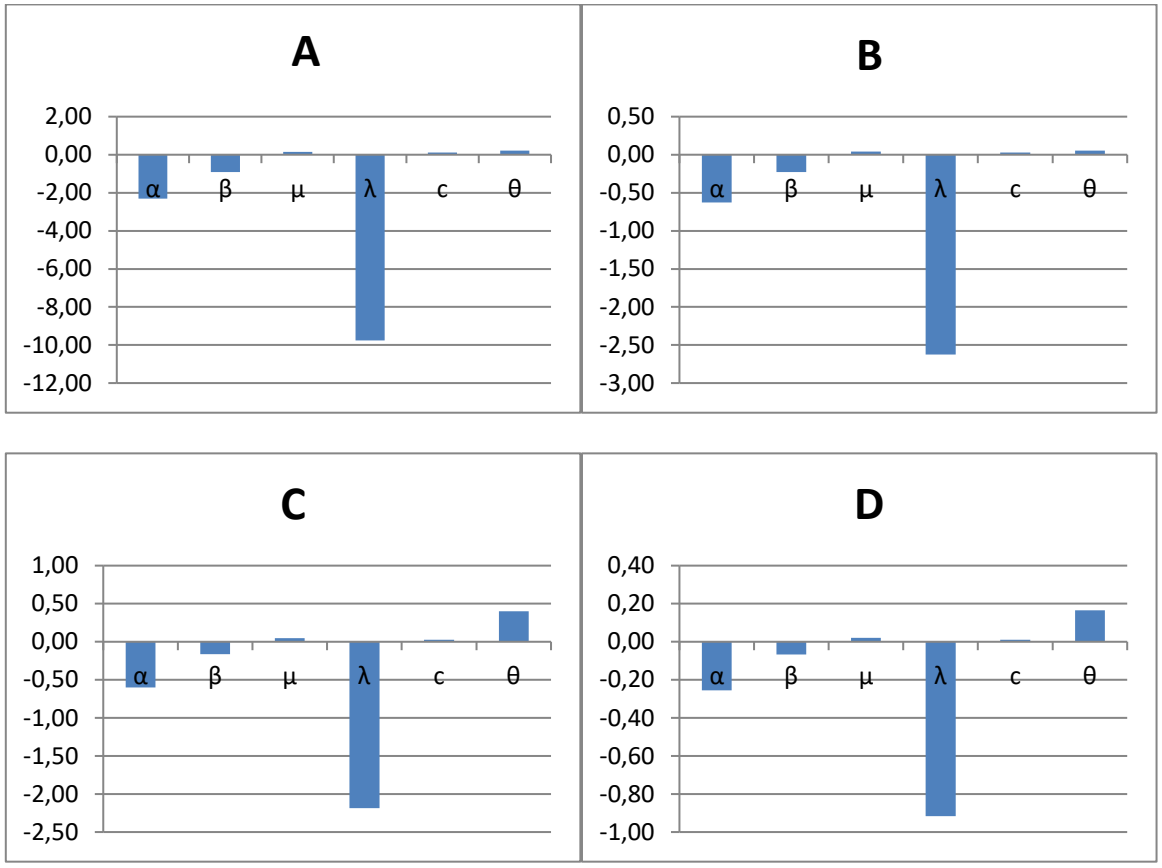

Figure 7. Sensitivity analysis results for the Macroparasite model. The panels are: A) model simulated with the initial parameter values; B) scenario 1 - the initial values of all parameters kept except α, where α reduced to 0.001 C) scenario 2 where the initial values of all parameters kept except θ, respectively $\theta = 0.1$ D) scenario 3 where again the initial values of all parameters kept except θ and α, respectively $\theta = 0.1$ and $\alpha = 0.001$

Furthermore, θ proved to have protective effect on the density of wild fish, which is shown in Figure 8, where the model was simulated with different θ values representing more effective removal rate of parasites on farmed fish (denoted with Y in the model).

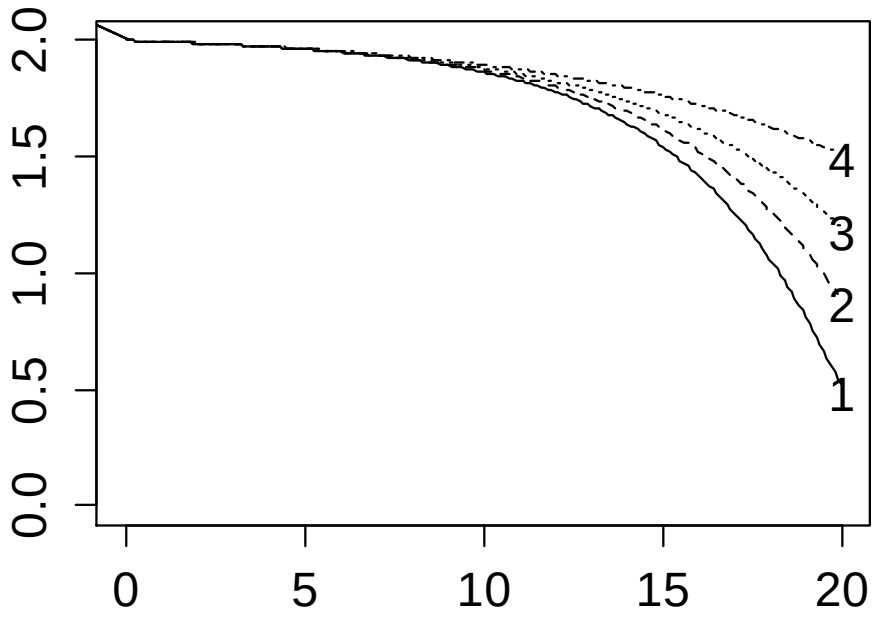

Figure 8. Simulation of the Macroparasite model under different values of θ, while keeping other parameters in their default values. Line 1: $\theta = 0.01$; line 2: $\theta = 0.05$; line 3: $\theta = 0.1$; line 4: $\theta = 0.2$. Each line represents the time series of the wild fish densities

In spite of θ not being the most sensitive parameter of the model, θ was important for calculation of the maximum density of stocked fish (X) under different model scenarios. For each scenario, depending on the values of X, α, β, λ, c and θ, the basic reproduction ratio (R_o) was set to 1 (the threshold value of R_o for establishment of infection) in order to calculate the maximum density of stocked fish (X). The results are shown in Table 4.

Table 4. For each scenario in the Macroparasitic model, the maximum stocking density of fish represented as density of farmed fish (X) when the basic reproduction ratio (R_o) is set to 1

Scenario	X	λ	θ	α	β	c	R_o
Initial	0.005	0.300	0.010	0.002	0.400	0.050	1.000
1	0.005	0.300	0.010	0.001	0.400	0.050	1.000
2	0.064	0.300	0.100	0.002	0.400	0.050	1.000
3	0.063	0.300	0.100	0.001	0.400	0.050	1.000

Discussion

The result of the present study indicate that parasitic pathogens first originated in wild fish populations after infecting and infesting farmed fish, subsequently causing epizootics under certain farming conditions in cultured fish, might have negative impact on wild fish populations. Similar views were expressed by Krkošek et al. (2005), Costello (2009) and Heuch et al. (2005) dealing with sea lice infestations on wild fish. Krkošek et al. (2005) suggested that fish farms were responsible for increasing sea lice infection pressure four times of magnitude than ambient levels and salmonid declines in Europe and North America may be attributed to salmon fish farming industry (Costello 2006, 2009). Heuch et al. (2005) claimed that there is a causal relation between salmon farming and sea lice epizootics on Norwegian South West coast due to high fish farming activity and low stocks of wild salmonids to generate these epizootics, but it was uncertain whether these epizootics effectively regulated the population size of Arctic char and sea trout.

Although in the present study, through computer models, testable hypothesis was generated, the results obtained *in silico* does not necessarily implied causation in reality (Turnbull et al. 2011). Indeed, this is in accord with findings of Green et al. (2012) who pointed out that paralleled to growing fish farming industry on the west coast of Scotland there was also decline in wild fisheries on the East coast, where fish farming is absent and answer might be simply lack of fishing effort instead of fish farming. Alleviating confounding factors in studying disease and observations supported by experimental data sufficiently repeated over time are needed to indicate that such correlation is based on cause and effect relationship (Costello 2009).

The present study models assumed homogenous dispersion of the wild fish populations similar to farmed fish. In reality, wild fish populations can have very patchy and chaotic distribution reducing contact rate, respectively protecting proportion of the wild fish populations from spreading of the diseases (Reno 1998; Green 2010). Thus, the results of the present models represented the worst-case scenarios, where wild fish populations were homogenously dispersed with enhanced disease transmission.

Another aspect of the present study was if under the developed models the hosts might be driven to extinction. According to Murray (2009), direct density-dependent transmission between hosts, as in the Microparasite model, cannot drive hosts to extinction, whereas macroparastic epizootics as in the Model II can. The results obtained indicated that after certain time microparasitic epizootics did not reach the state of equilibrium with their hosts and exterminated the host populations. Similarly, macroparasitic epizootics modelled in model II, did not reach the state of equilibrium and macroparasites continued their growth exponentially until reducing their host population density to zero. The latter was also consistent with the results obtained by Todd (2007) where the genetic distinction between wild and farmed populations was not discovered, dictating impossibility of eradicating sea lice as a pest from wild and farmed fish.

In the present models spreading of the microparasites as well as transmission of the macroparasite infective stages and their respective hosts was modelled under density dependency. Support for the latter and especially for microparasitic transmission came from the studies of Morrison et al. (2004) on amoebic gill disease (AGD), where infection was strictly amoeba density dependent and AGD function of amoeba cell density. In contrast, McCallum et al. (2001) proposed that simple mass action is not the proper model to explain effectively many terrestrial epizootics and does not account for the observed prevalence of many

diseases, with frequency dependence more accurately describing the disease patterns. However, due to the more contagious nature of the aquatic environment (Green 2010), assumptions of McCallum et al. (2001) were rejected while constructing the models and density dependency transmission finally adopted. With the correct models in place, it was easier to evaluate intervention steps and propose control procedures (Roberts and Heesterbeek 1993).

Results from the simulation of the Microparasite model indicated that the model parameter which is the least prone to management intervention, respectively infection removal rate of wild fish (γ) was the most sensitive parameter. It was evident that any attempt to apply chemotherapeutic agents for reduction of number wild infected fish is practically impossible and financially not feasible. Attempts to vaccinate wild fish from application viewpoint are simply futile when planning changes in (γ).

However, the second most sensitive parameter in model I respectively pathogen specific transmission rate (β) can be to certain degree manipulated. This parameter is influenced by the environmental factors such as population density, temperature, water flow and water chemistry (Reno 1998) so aquaculture can be geographically placed in areas (i.e. site selection) where the latter abiotic factors contribute to minimizing survivability of microparasites by reduction of basic reproduction ratio (R_o) (Krkošek 2010). Indeed, examples for reduction of β came from studies of Munday et al. (2001) who found that infections of salmonids with causative agent of amoebic gill disease (AGD) *Neoparamoeba pemaquidensis* occurred at high temperatures and high salinities and AGD can be easily managed and controlled at low temperatures and low salinities.

Equally important should be the measures applied to reduce the next sensitive parameter in the Microparasite model, pathogen specific transmission rate between infected farmed and susceptible wild fish (δ). This can be achieved by reducing the number of contacts between escaped farmed fish and wild (Heuch et al. 2005) and situating aquaculture production sites on places with greater residual current flow which leads to greater pathogen decay rate (Salama and Murray 2013 Green 2010).

Next sensitive parameter in the model evaluation, infection removal rate of farmed fish (θ), increased in importance paralleled to increase in its value and become the third most important model parameter. This had the most valuable implications for the model because the latter parameter encompassed all practically applicable procedures from aquaculture intervention viewpoint such as fallowing, application of chemotherapeutic agents and vaccination. All these procedures can inevitably affect infection removal rate of farmed fish (θ) and have protective impact on wild fish populations.

Beside the influence of aforementioned parameters susceptible farmed fish density had also important impact on onset of the parasitic epizootics via its influence on basic reproduction ratio (R_o). The lesser the density of susceptible farmed fish the lower R_o was and minimization of the microparasitic epizootics (Anderson and May 1979a; Roberts and Heesterbeek 1993; Reno 1998; Krkošek 2010). Reduction of farmed fish density at which R_o was still equal to 1 or below 1 dropped from 10.0 to 6.00. At density of 6.00 farmed fish can still be effectively and feasibly stocked (Table 3).

Macroparasite model results revealed that the most important, respectively the most sensitive parameter was the rate of production of infective stages by an adult parasite (λ), significantly surpassing the importance of all model parameters. Fortunately, compared to the most sensitive parameter of the Model I which was unaffected by human intervention, the rate of production of infective stages by an adult parasite (λ) can be to certain degree manipulated in order to protect wild fish. The higher the sea temperature the faster is the development and production of eggs by gravid female sea lice, which leads to higher infestation pressure on farmed and farmed fish stocks (Costello 2006; Guo and Woo 2009; Wagner et al. 2008). Support for the latter fact came from the modelling studies of Revie et al. (2005) in which they found that the second sensitive parameter in their model was the feedback rate at which gravid female louse produce viable eggs. Thus, in order to achieve effective eradication of the macroparasite epizootics, farm facilities must be located in areas where environmental factors, in that case temperature, hinder the development and production of viable eggs.

The next sensitive parameter in the Macroparasite model was parasite-induced host death rate (α), which can be influenced by management procedure such as vaccination. However, macroparasitic infestations do not confer their respective fish hosts with significant immune response and development of vaccines against macroparasites seems distant future (Anderson and May 1979b; Krkošek 2010). Consequently, if preventive intervention strategy are to be built over the model parameter (α), it should not be based on vaccination but on supporting the immune system of the host and reduction of environmentally caused stress in fish.

Transmission rate between host and parasite infective stages (β) was the third most sensitive parameter in the Macroparasite model reflecting

infestations between free infective stages of the macroparasites and their hosts. This parameter can be manipulated by relative physical isolation of captive fish populations in order to avoid contact with their wild counterparts (Reno 1998) by selecting farming areas which avoid spreading of infective stages by wind-driven circulation (Amundrud and Murray 2009; Salama and Murray 2013; Penston et al. 2008; Costello 2006). Similarly, stimulating migratory allopatry and avoiding migratory sympatry, in other words avoiding contact between migratory juvenile fish with adult farmed or escaped farmed fish (Costello 2009).

Special attention is deserved by the parameter θ (mortality rate of adult parasites on farmed fish) in the Macroparasite model which comprises the core of the integrated intervention strategy because this parameter can be modified by such control procedures as fallowing, use of cleaner fish (wrasse) and chemical control (use of parasiticides). Increase in the numerical value of the parameter led to increase in the sensitivity and relative importance of the parameter such as θ became the third most sensitive parameter in the second model. As Werkman et al. (2011) reported, synchronised fallowing is highly effective tool in disease control and eradication despite the common long distance contacts. However, there has been significant amount of accumulating data that even synchronised fallowing was not effective in reducing the abundance of sea lice *Caligus elongatus* (Nordmann,1832) on cultured Atlantic salmon in Scotland (Revie et al. 2002). Even when farms in Loch Torridon (Scotland) were synchronously allowed empty for 10 weeks before restocking greatest densities of sea lice nauplii were recovered around farms during the production season (Penston et al. 2008). McKenzie et al. (2004) reported that despite treatment applications against *Caligus elongatus* (Nordmann,1832) in Loch Sunart (Scotland) there was no significant impact on the parasitic infestations and there is substantial evidence that sea lice have been acquiring resistance against widely used and still effective chemotherapeutic agents such as Hydrogen peroxide and Emamectin benzoate (Guo and Woo 2009). Nevertheless, recent introduction of cleaner fish such as wrasse for biological control have had very promising results in reduction of sea lice infestations on salmonid fishes (Costello 2006). Wrasse can be used very efficiently against macroparasites such as sea lice.

In contrast to model I where farmed fish density had important impact on reducing basic reproduction ratio (R_o), in the Macroparasite model farmed fish density did not contribute significantly in reduction of R_o. Reduction of farmed fish density at which R_o was still equal to 1 or below 1 dropped from 10.0 to 6.00 in the Microparasite model, respectively these values in model II were 10.0 to 0.0634 (Table 4). The latter explicitly indicates that macroparasitic epizootics under farming conditions are very difficult to be eradicated.

Strategic models built in this study implicitly showed that parasites can be transmitted between farmed and wild fish populations in both directions and have potential to negatively impact both of them.

In conclusion, the strategic models presented in this study demonstrate the importance of epidemiological modelling in aquaculture disease management and prevention of epizootics caused by uncontrolled parasitic infestations in farmed fish, which can be transmitted to wild stocks with negative impacts on population dynamics. More refined biological data based on experimental and field data is needed for establishment of separate epidemiological models parameterised for specific parasitic disease. These disease specific models can reflect the dynamics of parasitic epizootics better and lead to better control and more effective environmental protection. New models, due to the impact on shortening of generation time of parasites, should mirror in their basic parameters the rise of global water temperatures predicted by climate change scenarios. Increase in the resistance of parasites against chemotherapeutic agents must also be a concern for future modelling efforts. This will be certainly advantageous to both wild fish defenders and aquaculturists.

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APPENDIX A. Code for the Microparasite Model

```
beta <- 0.4
gamma <- 0.18
teta <- 0.18
delta <-0.02
sigma<- 0.01
S <- 10.000
I <- 0.001
R <- 0
X <- 2.000
Y <- 0.000
Z <- 0
dt <- 0.1
t <- 0
max_time <- 20
time_steps <- max_time/dt
results<-c(t,S,I,R,X,Y,Z)
for (i in 1:time_steps) {
t <- t+dt
newI <- beta*S*I*dt+sigma*S*Y*dt
newR <- teta*I*dt
I <- I+newI-newR
S <- S-newI
R <- R+newR
newY <- beta*X*Y*dt+delta*X*I*dt
newZ <- gamma*Y*dt
Y <- Y+newY-newZ
X <- X-newY
Z <- Z+newZ
results<-rbind(results,c(t,S,I,R,X,Y,Z))}
colnames(results)<-
    c("t","S","I","R","X","Y","Z")
results<-as.data.frame(results)
plot(results$t,results$X,type="l",
    ylim=c(0,10))
lines(results$t,results$Y,lty=2)
lines(results$t,results$Z,lty=3)
lines(results$t,results$S,lty=4)
lines(results$t,results$I,lty=5)
lines(results$t,results$R,lty=6)
text (1,10,"1")
text (4.2,8.2,"2")
text (10,7.4,"3")
text (1,2,"4")
text (6.2,1.1,"5")
text (17,1.9,"6")
```

APPENDIX B. Code for the Macroparasite Model

```
alfa <- 0.002
beta <- 0.4
mu <- 0.01
lambda <- 0.3
si <- 0.05
teta <- 0.01
N <- 2.000
P <- 0.200
X <- 10.000
Y <- 0
W <- 0.500
dt <- 0.1
t <- 0
max_time <- 20
time_steps <- max_time/dt
results<-c(t,N,P,X,Y,W)
for (i in 1:time_steps) {
t <- t+dt
newP <- (beta*W*N-(mu+alfa)*P-
    alfa*P*P/N)*dt
newY <- (beta*W*X-(teta+alfa)*Y-
    alfa*Y*Y/X)*dt
newW <- (lambda*P+lambda*Y-si*W-
    beta*W*N-beta*W*X)*dt
P <- P+newP
N <- N-alfa*P
Y <- Y+newY
X <- X-alfa*Y
W <- W+newW
results<-rbind(results,c(t,N,P,X,Y,W))}
colnames(results)<-
    c("t","N","P","X","Y","W")
results<-as.data.frame(results)
plot(results$t,results$N,type="l",
    ylim=c(0,20))
lines(results$t,results$P,lty=2)
lines(results$t,results$W,lty=3)
lines(results$t,results$Y,lty=4)
lines(results$t,results$X,lty=5)
text (8,10,"1")
text (8,2,"2")
text (12.5,14.8,"3")
text (15,5.2,"4")
text (16,3.5,"5")
```

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Effect of Probiotic on Copper Nanoparticle Accumulation in *Dreissena polymorpha*

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ABSTRACT

Materials with dimensions between 0.1 and 100 nm are called nanoparticle (NP) materials. In recent years, the usage areas and quantities of NP materials have increased in parallel with the development of the industry. The need and usage areas of heavy metals such as Cu have also expanded in NP sizes. All these developments have led to problems on the ecosystem that are becoming more difficult to compensate. In this study, Zebra Mussel (*Dreissena polymorpha*) was chosen as a model to investigate the effect of probiotics on CuNP heavy metal accumulation. The model organism was exposed to three different concentrations of CuNP (5, 10, 50 mg/L) with probiotics and directly for 24 and 96 hours. CuNP accumulation amounts in *D. polymorpha* tissues treated directly and with probiotics were compared. The amount of accumulation in the test organism directly exposed to CuNP was higher compared to the groups administered with probiotics, but a statistically significant difference ($p<0.05$) was found only in the treatment group with the highest 24-hour concentration (50 mg/L). As a result, according to the findings obtained from the study, it has been determined that probiotics have positive developmental effects on aquatic organisms, as well as beneficial in the elimination of their accumulation in the organism.

Keywords: *Dreissena polymorpha*, Copper Nanoparticle, probiotic, bioaccumulation

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Probiyotiğin *Dreissena polymorpha*'da Bakır Nanopartikül Birikimi Üzerine Etkisi

Öz: Boyutları 0,1-100 nm arasında olan materyaller nanopartikül (NP) malzemeler olarak adlandırılmaktadır. Son yıllarda endüstrinin gelişmesine paralel olarak NP materyallerin kullanım alanları ve miktarları da artmıştır. Cu gibi ağır metallerin de NP boyutlarında gereksinimi ve kullanım alanları genişlemiştir. Tüm bu gelişmeler beraberinde ekosistem üzerinde telafisi zorlaşan sorunları doğurmuştur. Yapılan bu çalışmada probiyotiklerin CuNP ağır metal birikimi üzerine etkisini araştırmak için model canlı olarak Zebra Midye (*Dreissena polymorpha*) seçilmiştir. Model organizma probiyotikli ve doğrudan olmak üzere CuNP'ün üç farklı konsantrasyonuna (5, 10, 50 mg/L) 24 ve 96 saat süreyle maruz bırakılmıştır. Doğrudan ve probiyotik ile muamele edilen *D. polymorpha* dokularındaki CuNP birikim miktarları kıyaslanmıştır. CuNP'ye doğrudan maruz bırakılan test organizmasındaki birikim miktarı probiyotik uygulanan gruplara kıyasla daha fazla olduğu, ancak yalnızca 24 saatlik en yüksek konsantrasyon (50 mg/L) olan uygulama grubunda istatistiksel açıdan anlamlı ($p < 0,05$) fark bulunmuştur. Sonuç olarak çalışmadan elde edilen bulgulara göre, probiyotiklerin sucul canlılar üzerinde olumlu gelişimsel etkilerinin yanısıra organizmada birikim miktarlarının eliminasyonunda faydalı olduğu tespit edilmiştir.

Anahtar kelimeler: *Dreissena polymorpha*, bakır nanopartikül, probiyotik, biyobirikim

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Introduction

Copper (Cu), which is one of the basic nutrients for humans and other living things, also forms an important part of the oxidation-reducing enzyme system (Muralisankar et al. 2016). Cu has an important role in connective tissue, iron metabolism and the central nervous system (Turnlund 1994; Watanabe et al. 1997; Lall 2002). Some studies have revealed Cu requirements in various aquatic organisms (Lorentzen et al. 1998; Lee and Shiau 2002; Wang et al. 2013; Shao et al. 2012). However,

it has been reported that high Cu levels can be toxic to aquatic organisms as they increase reactive oxygen species (ROS) and oxidative stress (Clearwater et al. 2002). The effects of nano-based products may differ due to the nature of nanoparticles (NP), the characteristics of the environment, the way NPs are administered and the immune system of the living organism, and the production of aquatic organisms in different environments (for example, freshwater and saltwater or tropical and temperate regions) (Katuli et al., 2017). Considering the widespread use of NPs, attention should be paid to the environment and human health (Çimen et al. 2020). A product that is harmless on a larger scale can create a reactive and toxic state at the nanoscale. Problems with NPs have emerged with the use of NPs in human life (Dagani 2003; Dreher 2004; Hoet et al. 2004). The mixing of NPs in the aquatic environment leads to the accumulation of metals in organisms and produces long-lasting effects (Yu et al. 2020). It occurs when NPs enter the body through permeable membranes (such as gills) and interfere with different reactions by differentiating the natural metal ions of metabolic enzymes, causing disruption of protein structures and forming DNA cross-links that can disrupt the cell cycle (Garai et al. 2021). As a result of human consumption of NPs in aquatic organisms, similar effects such as neuronal, hepatic, renal and reproductive damage and cardiovascular and peripheral vascular diseases occur in humans (Azeh et al. 2019).

Feed additives such as prebiotics and probiotics are effective against NPs and pathogens by maintaining intestinal barriers and healthy intestinal bacterial count (Yukgehnaish et al. 2020; Arun et al. 2021; Yaqoob et al. 2021). Prebiotics and probiotics are used in fish, shrimp, etc. to improve growth performance, increase utilization of feed, strengthen the immune system, produce inhibitory compounds against pathogens, suppress virulence genes and reduce the expression of proinflammatory cytokines such as interleukin (IL). It is widely applied in the cultivation of aquatic organisms (Kakade et al. 2023).

The zebra mussel (*Dreissena polymorpha*) (Figure 1) is a reference species for ecotoxicological studies in aquatic ecosystems. These mussels are mainly distributed in lakes and reservoirs in Turkey. As a species that is not endangered and can be encountered continuously in nature, has stable behavior and sufficient body size, it is easier to sample than other species (Binelli 2015; Serdar 2021; Erguven et al. 2022) and has been preferred because it is not selective in food intake (Serdar 2021).

Figure 1. *Dreissena polymorpha*

In this study, it was aimed to investigate the effect of CuNP heavy metal on the accumulation amount with probiotic supplementation in the aquatic organism *D. polymorpha*.

Materials and Methods

Model Organism Supply and Adaptation

D. polymorpha individuals were collected from the Euphrates River (38º48'25" N, 38º43'51" E) (Figure 2) and quickly brought to the laboratory in plastic bottles. The temperature was set at 18 °C for 30days before using it in the experiments. Then, it was adjusted to laboratory conditions, which were similar to the natural living conditions, using 200 L oxygen-supplemented tanks. The aquatic environment was also refreshed by the addition of rested tap water. During the adaptation, a photoperiod of 12:12 light:dark cycle was applied and the organisms were fed with microalgae. Organisms at similar developmental stages were selected for the study and were not fed during the experimental study.

CuO Nanoparticles

The metal-based Cu-NP (60-80nm) chemical (Sigma-Aldrich) was purchased, and CuO-NP

(40nm) was purchased from SkySpring (Houston, TX, USA). No purification or analytical reagent classification has been done for Cu-CuO NP chemicals. In the bioassay studies, the shape and size data declared by the manufacturer were taken as a reference.

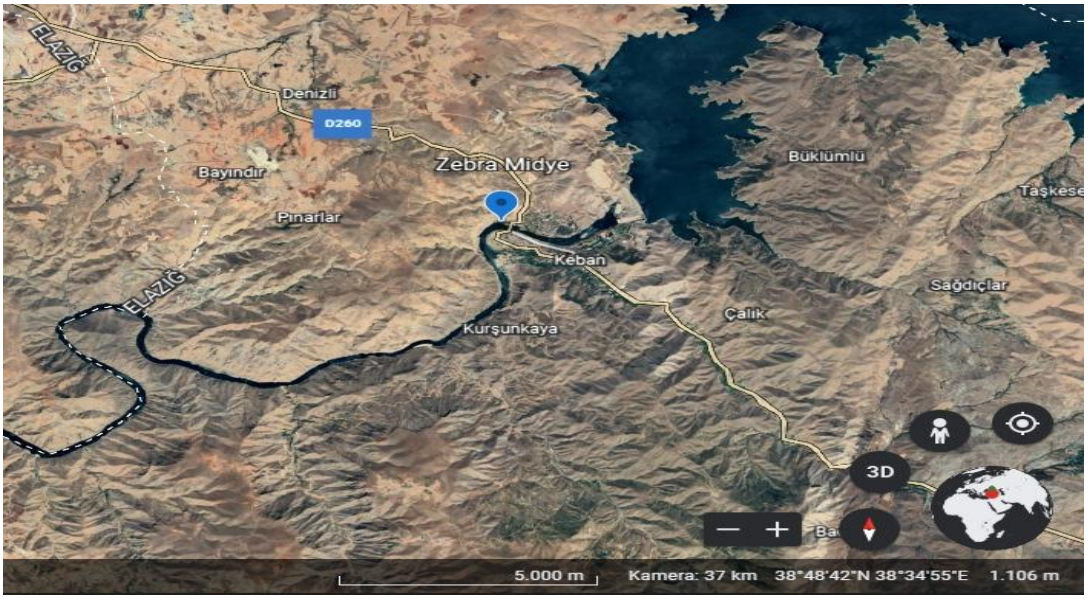

Figure 2. The area where living material is collected, Euphrates River (38º48'25" N, 38º43'51")

Probiotic Supply

The probiotic utilized in the study was acquired from Uğur Aqua Aquaculture Food Industry Trade. Bacteria species in probiotic content: *Lactobacillus plantarum, Lactobacillus casei, Enterococcus faecium, Bacillus subtilis, Pediococcus spp*. Bacterial colony count (CFU/ml): 2.2 x 10^{11}.

Acute Toxicity Tests (LC50)

Acute toxicity tests were not performed since the damage caused by pollutants to the environment was taken into account. Subacute values were determined by a literature review (Cimen and Serdar, 2022).

Experiment Design

After a 30-day adaptation period, 100 individuals with similar development and body size were selected from *D. polymorpha* individuals obtained from the Euphrates River and placed in 80-liter aquariums. Metric meristic data of the mussels used in the trial design (weight; 1.005+-0.29 g, length; 20.28+-2.09 mm, width; 10.04+-0.96 mm, height 9.74+-1 It was recorded as .07 mm). Dried microalgae was fed twice a day during the adaptation period. The stock aquarium/tanks were supplied with oxygen by aquarium air motors. Physico-chemical parameters of the ambient water during the experimental application; ambient water temperature 18 ± 1 °C; dissolved oxygen: 11.04 ± 0.15 mg/L; pH:8.12 ±0.45; electrical conductivity: 461 ± 43 µS cm-1; salinity: 0.11 ± 0.01 g/L were measured and recorded regularly daily.

Two different experimental design groups were created to investigate the effect of CuNp accumulation on probiotic use. In the first experimental group created, CuNp was applied at the determined concentrations without using probiotics (Figure 3).

CuNp was applied to *D. polymorpha* organisms in the 2nd experimental group at the determined concentrations of 3% of the water volume for 21 days. After the probiotic application, CuNp application and Probiotic + CuNp applications were applied simultaneously and in triplicate in both experimental groups.

As in all toxicological studies, application concentrations were determined by considering the application concentrations determined in CuNp application study, the rates of release to the environment and the values in this range. CuNP concentrations of 5, 10, 50 mg/L were established as sublethal concentrations.

After 21 days of probiotic application;

Groups of 8 organisms were formed in 1000 ml aquariums at 5, 10, 50 mg/L direct and CuNP and probiotic applied concentrations, and each concentration was repeated 3 times. 8 animals were used for each experimental group and hour (24 and 96). No changes were made in the water environment and the number of living things during the trial period (96 hours). A certain number of samples were taken from the application groups at 24 and 96 hours, labeled and stored at -86^{0}C until the accumulation amounts were measured.

C1: CuNP 5 mg/L
C2: CuNP 10 mg/L
C3: CuNP 50 mg/L
C4: Probiotic (30 ml) + CuNP 5 mg/L
C5: Probiotic (30 ml) + CuNP 10 mg/L
C6: Probiotic (30 ml) + CuNP 50 mg/L

Figure 3. Experiment design

Dissolving Process

For the dissolution process, a microwave dissolution treatment system (Berghof, Germany) was used to thaw *D. polymorpha* samples. Approximately 0.15 g of *D. polymorpha* sample was taken into the digestion vessel and 4 mL of concentrated nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) were added (Table 1). The mixture was shaken carefully and after waiting for at least 20 minutes, the container was closed and the disintegration program was applied (Serdar et al. 2019). After centrifugation, the clear solutions were diluted to 15 mL (Pala et al. 2019). It was mixed with ultrapure water and CuNP ion concentrations in solution were measured by electrothermal atomic absorption spectrophotometer (ETAAS).

The amount of *D. polymorpha* CuNP measurement values, which is a living material, was calculated with the calibration curve obtained from known standard solutions (Figure 4).

Table 1. *D. polymorpha* Sample Digestion Method

Biological Sample	Weight (gr)	Solvent	Volume(ml)	Temp.(°C)	Pressure (Atm)	Time(Min)
D.Polymorpha	0,15	HNO_3	4	150	15	4
		H_2O_2	1	180	25	5

Results

In treatment groups directly exposed to CuNP heavy metal (5, 10 and 50 mg/L), *D. polymorpha*. The amount of accumulation in *D. polymorha* tissues was measured at 24 and 96 hours. It was found that there was a linear increase between increasing concentration and application time, but the calculated increase was statistically insignificant ($P < 0.05$) (Figure 5).

CuNP accumulation amounts were measured in *D. polymorha* tissues treated with probiotics for 24 and 96 times. It was found that there was a linear increase between increasing concentration and application time, but the calculated increase was statistically insignificant ($P < 0.05$) (Figure 5).

When the amount of CuNP accumulation in *D. polymorha* tissues treated directly and with probioticswas compared 24 and 96 hours, it was found that there was a statistically significant ($P < 0.05$) difference only in the 24-hour highest concentration (50 mg/L) application group ($P < 0.05$) (Figure 5).

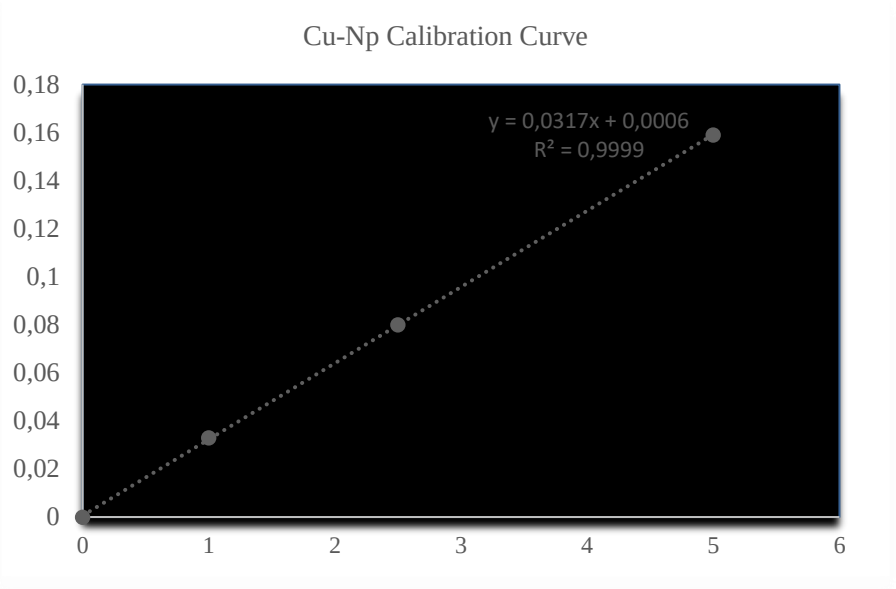

Figure 4. Calibration curve of Cu-Np

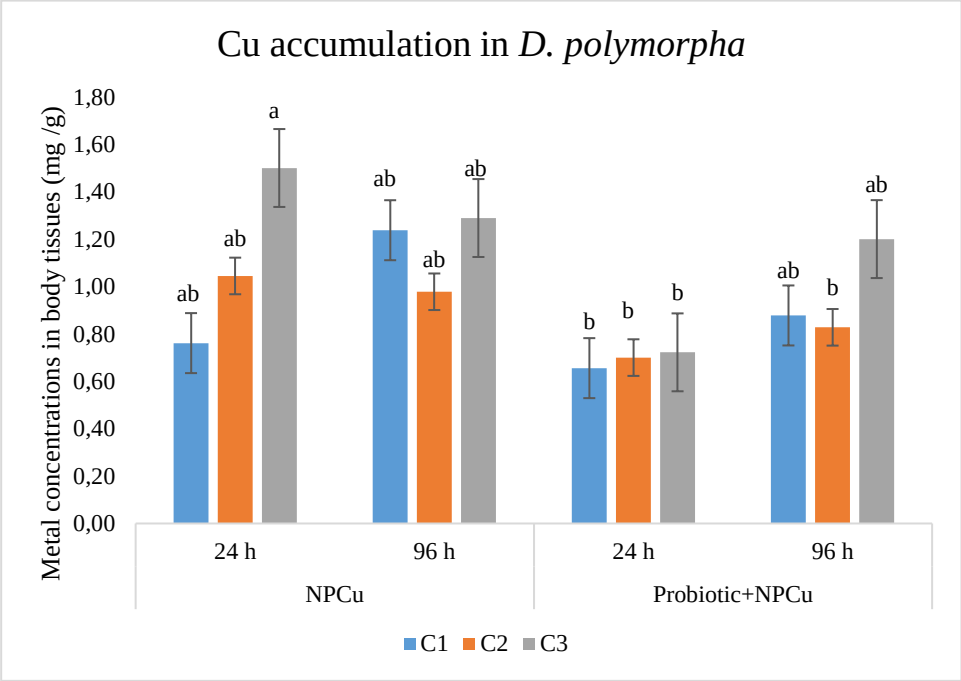

Figure 5. Cu accumulation in *D. Polymorpha*

Discussion

Çimen et al. (2020) investigated the accumulation rates of Cu (60–80 nm) and CuO (40 nm) nanoparticles in *Artemia salina*, and as a result, there was a difference in the accumulation and elimination rates of both NPs in parallel with the increase in concentration and at each application time they indicated. Dağlıoğlu and Öztürk (2016) evaluated single-celled freshwater algae (*Desmodesmus multivariabilis*) test organism for the bioaccumulation of boron nanoparticles. As a result, they observed that nano and micro boron particles accumulated in different amounts in algae. Le et al. 2021 investigated Cu accumulations under chronic exposure at various pH and sodium (Na) concentrations in *D. polymorpha* and stated that pH and Na have a significant effect on Cu uptake and accumulation rates in the body. According to Clayton et al. (2000), D. polymorpha showed a high tendency to accumulate copper and tributyltin (TBT) contaminants. When pooled, the replicated samples exhibited significant differences from the controls, despite the presence of relatively high inter-individual variability. Mersch et al. (1993) evaluated the potential of the freshwater organisms *D. polymorpha* and *Rhynchostegium riparioides* as

indicators of heavy metal contamination, consequently, exposure of both metals to Cu and Cd over a 27-day period resulted in the accumulation of both metals in *R. riparioides.* They stated that it was fast and it was not observed that it was slower for *D. polymorpha.* It has also been observed that *D. polymorha* individuals exposed to CuO NP accumulate in the model organism depending on time and concentration.

Sandeva et al. (2016) observed a slight improvement in some parameters (nitrates, nitrites, permanganate) by applying probiotics for 4 weeks in *Cyprinus carpio*. Sharifuzzaman et al. (2011) evaluated the efficacy of the cellular components of probiotics Kocuria SM1 and Rhodococcus SM2 to protect rainbow trout (*Oncorhynchus mykiss*) against vibriosis and stated that probiotics provide significant protection. Sami et al. (2020) evaluated the growth performance and survival rates of the examined species by adding probiotics to fresh algae, yeast and bacteria in *Ruditapes decussatus*. They reported that the highest specific growth rate and growth gain in total weight of test organisms were in mixed algae-probiotic feeding, and the highest in wet weight and dry weight was in the group fed with bacteria-probiotics. Giri et al. (2018) investigated the protective effects of the probiotic *Lactobacillus reuteri P16* against the toxicity caused by Pb exposure in *Cyprinus carpio* and stated that *L. reuteri P16* reduced the accumulation of Pb in tissues. Daisley et al. (2019) stated that *Lactobacillus rhamnosus GR-1* probiotic reduces the separation of Pb and Cd and their absorption through the intestinal epithelium. Tawwab et al. (2010) aimed to evaluate the growth response and resistance to water-borne copper toxicity of *Sarotherodon galilaeus* probiotic in *Galilee tilapia,* and as a result, it reduced Cu absorption; indicated that it had a positive effect on growth and feed utilization. Yu et al. (2020) examined the copper-induced accumulation and histopathological-biochemical parameters of dietary microbial flox in *Rhynchocypris lagowski dybowski*, and stated that their diet reduced Cu accumulation. Madreseh et al. (2018) investigated the accumulation and some parameters of some heavy metals (cadmium, lead, nickel, zinc) in *Oncorhynchus mykiss* tissues of feeding with encapsulated *Lactobacillus fermentum* and Lactulose; As a result of the examination, they stated that *L. fermentum* prevented the absorption and accumulation of heavy metals in the liver and gills of rainbow trout. Kargar and Shirazi (2020) examined the removal rates of Cu and Zn ions from the aquatic environment of *L. fermentum* and *Lactobacillus plantarum* probiotics for Cu and Zn in their study; indicating that both metals and both species showed rapid biosorption. Bhakta et al. (2012) examined water samples of Cd and Pb resistant lactic acid bacteria from some regions where aquaculture is used as probiotics, and as a result, these probiotics have the potential to remove heavy metals from the fish intestinal environment to control the progressive bioaccumulation of heavy metals in fish. They stated that they can be used as probiotic strains.

The data obtained from this study show parallelism with the information in the literature. It was determined that there was less Cu-Np accumulation in the groups treated with probiotics compared to the groups not given probiotics. In this respect, the study is similar to the information in the literature.

With this study, the effect of probiotics on heavy metal accumulation in the body was investigated and it was observed that they play an important role in positive excretion and elimination from the body, preventing or reducing the amount of accumulation. According to these results, the study is consistent with previous research in this field and is expected to lead the studies to be done in the future. It is believed that probiotics have an impact on the excretory system of the body and they are resistant to heavy metal accumulation in the body in this respect. *D. polymorha* is a suitable biomonitor to quantify the bioavailable of Cu-Np in freshwaters besides a continuous biomonitoring system as part of ecological risk assessment. Furthermore, this model can be used as a predictive tool to evaluate the quality of the environment.

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Effects of dietary soybean meal levels on reproduction parameters, the growth and gonad, gut, hepatopancreas histology of female African Cichlids *Pseudotropheus socolofi*

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ABSTRACT

This study was conducted to determine the effect of different soybean meal dietary supplementation levels on the growth, reproductive parameters, gonad, intestine and hepatopancreas histology of *Pseudotropheus socolofi*. Experimental groups were hand-fed to satiety with diets supplemented with soybean meal at levels of 3, 16, 27, 35, and 44% twice daily for 90 days. The results of this study showed that specific growth rate, weight gain, and survival rate were not affected by dietary soy proportions ($P > 0.05$). The worst FCR and final weight were found in those fed the diet containing 44% soy ($P < 0.05$). In female reproductive parameters, there was no significant difference between the groups in fertilization rate, egg production, hatching rate, egg diameter, gonadosomatic index and broodstock ovulation percentage ($P > 0.05$). However, due to pathological examinations in female individuals, a significant decrease was observed in the number of mature oocytes in the ovaries and goblet cells in the intestines with increasing soy levels in the diet ($P < 0.05$). As a result, using soybean meal up to 35% did not negatively affect growth. However, adding 44% soybean meal to diets caused histopathologically serious inflammatory reactions and decreased growth.

Keywords: *Pseudotropheus socolofi*, soybean meal, reproduction, histology, growth

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Diyetteki soya küspesi oranlarının Dişi Afrika Çiklitleri *Pseudotropheus socolofi'*nin üreme, büyüme parametreleri, ve gonad, bağırsak, hepatopankreas histolojisi üzerine etkileri

Öz: Bu çalışma, *Pseudotropheus socolofi*'nin büyüme, üreme parametreleri, gonad, bağırsak ve hepatopankreas histolojisi üzerine farklı düzeylerde soya küspesi diyet takviyesinin etkisini belirlemek amacıyla yapıldı. Deney grupları, 90 gün boyunca günde iki kez %3, 16, 27, 35 ve 44 seviyelerinde soya küspesi ile desteklenen diyetlerle doyana kadar elle beslendi. Bu çalışmanın sonuçları spesifik büyüme hızının, kilo alımının ve hayatta kalma oranının diyetteki soya oranlarından etkilenmediğini gösterdi ($P> 0,05$). En kötü FCR ve son ağırlık, %44 soya içeren diyetle beslenenlerde bulundu ($P < 0,05$). Dişi üreme parametrelerinde ise döllenme oranı, yumurta verimi, kuluçka oranı, yumurta çapı, gonadosomatik indeks ve anaç yumurtlama yüzdesinde gruplar arasında anlamlı bir fark bulunmamıştır ($P > 0,05$). Ancak dişi bireylerde yapılan patolojik incelemeler sonucunda diyetteki soya düzeylerinin artmasıyla birlikte yumurtalıklardaki olgun oosit sayısında ve bağırsaklardaki goblet hücrelerinde önemli bir azalma gözlendi ($P< 0,05$). Sonuç olarak soya küspesinin %35'e kadar kullanılması büyümeyi olumsuz etkilememiştir. Ancak diyetlere %44 oranında soya fasulyesi küspesinin eklenmesi histopatolojik olarak ciddi inflamatuar reaksiyonlara ve büyümede azalmaya neden olmuştur.

Anahtar kelimeler: *Pseudotropheus socolofi*, soya küspesi, üreme, histoloji, büyüme

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Introduction

Soybean meal (SBM) is used as plant-derived protein because of its abundance, relatively low cost, high protein levels and amino acid profiles in fish feeds (DiMaggio et al. 2016). SBM contains high levels of phenolic compounds, known as estrogenic compounds called isoflavonoid phytoestrogens (Bagheri et al., 2013). Therefore, the inclusion of SBM in fish feeds may introduce endocrine-disrupting compounds in the form of phytoestrogens (DiMaggio et al. 2016). SBMs phytoestrogens with estrogenic activity may affect reproductive development and sex differentiation in fish (El-Sayed et al. 2012).The pure forms of phytoestrogens such as genistein and daidzein in SBM were added to fish diets and their effects on growth and sex reversal were investigated by various researchers (Pelissero et al. 1991; Kaushik et al. 1995; Ko et al. 1999; Oca et al. 2005; Hernandez et al. 2007; El-Sayed et al. 2012; Bagheri et al. 2013; Brown et al. 2014; Jourdehi et al. 2014; Chakraborty et al. 2015; Ahmed et al. 2015; DiMaggio et al. 2016; Dong and Qiuyan 2016; Nezafatian et al. 2017). However, there are few studies about the effects of using high levels of SBM in fish feed on the reproductive systems of fish. Bagheri et al. (2013) reported that average egg number, sperm quality, fertilization and hatching decreased in *Carassius auratus*-fed diets added to high SBM. Pelissero et al. (1991) declared that the SBM (30%) based diet raised the plasma vitellogenin level of *Acipenser baeri*. In particular, no study has been detected on fish that reproduce more than one generation yearly. The objective of this study was to evaluate the effect of different levels SBM based diets on the growth, reproduction parameters, gonad, gut and hepatopancreas histology of *Pseudotropheus socolofi*.

Materials and Methods

The experiment conditions and design

In the present study, *Pseudotropheus socolofi* species was preferred, which gives offspring every two months, regarding the reliability of the hypothesis. *Pseudotropheus socolofi* broodstocks were obtained from the Faculty of Eğirdir Fisheries at the Isparta University of Applied Sciences. Each treatment was replicated three times. A total of 75 females with a mean weight of 5.43 g and 15 males with an average weight of 8.52 g ± were randomly distributed (5♀:1♂) in 15 aquariums (30 x 40 x 100 cm).

The experiment fish were fed diets containing SBM additions with three replicates for each treatment group for 90 days. The experimental groups were fed by hand ad libitum twice daily at 8:30 and 20:30. 100-watt thermostat heaters were used to keep the temperature in the aquariums at approximately 27 ± 1 °C. In addition, suitable shelters have been placed where fish can be easily stored. The aquariums cleaned 2 days a week, and the residual feed and feces were siphoned out. The dissolved oxygen ratio ranged from 6.15 to 6.35 mg L^{-1}. Fish were fed for 90 days under a natural lighting environment. Experimental fish were weighed at the beginning of the experiments and 90th day (at the end of the experiments). All fish were weighed individually a day before and at weighing days of fasting. Feed consumption was recorded daily.

Experimental Diets

Experimental diets were isonitrogenic (46% crude protein) and isoenergetic (9%). Five diets were prepared by adding different SBM levels (3%, 16%, 27%, 35% and 44%). The doses were determined based on the highest level of soybean in commercial feeds. The composition of experiment diets is shown in Table 1. Feed ingredients were obtained from a local fish feed producer. All ingredients were ground into small particles (0.5 mm) in a mill. Dietary ingredients were mixed in a mixer. Room temperature water was added to obtain a 30% moisture level. Diets were passed through a mincer with a 2 mm sieve. The pellets were fan-dried and stored frozen at –20 °C until used. YSI Pro Plus multi-measurement set and DAIHAN Wiseven model oven were used to prepare the diets.

Reproductive Performance

The females were observed as daily for spawning activity, and eggs were gently removed from the buccal cavity of females after ovulation. Photos of eggs were taken with a smartphone and the eggs were counted over photos. Fertilized eggs were separated based on the different coloring of unfertilized eggs (Ikhwanuddin et al. 2015). The diameters of eggs were measured with a micrometer using a microscope. The eggs were hatched in a special incubator (Biolife Turbojet Star X6) used for the first time in literature.

Histopathological examination

Histopathological examination was performed on 5 fish from each group. A complete necropsy was performed on each fish and visceral organ samples were collected during the necropsy. Tissue samples were fixed in 10% neutral formalin and processed by automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany). The samples were embedded in paraffin, and 5 μm sections were taken by a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch,

Germany). Then, sections were stained with hematoxylin and eosin (H&E) for histopathological examination, and periodic acid-Schiff (PAS) staining was used for histochemical analysis. After the coverslip, all slides were microscopically examined under a light microscope.

In the morphometric analysis of the small intestines, the villi length was measured in each fish at 40x with an Olympus CX41 light microscope. Five different villi were measured in each fish for statistical analysis. Morphometric evaluation was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan). The mucous cell counting was conducted in the anterior part of the intestine and the same region of the digestive tract for each fish. The mean number of mucous cells per 100 000 μm^2 of epithelial area in sections was compared and statistically evaluated. Histopathological and histochemical changes were graded in a blinded manner by a specialized pathologist from another university (Luna 1968).

Table 1. Formulation and proximate composition of experimental diets

Ingredients (%)	3%	16%	27%	35%	44%
Fish meal	30	30	30	30	30
Casein	22.5	16.6	11.34	6.85	3
Soybean meal	3	16	27	36	44
Corn starch	17.5	12.89	8.91	4.44	1.44
Corn gluten	5	5	5	5	5
Wheat meal	5.5	5.12	4.77	4.7	4.4
Sunflower meal	7.2	5	3.4	3.01	2.01
Fish oil	6.3	6.39	6.58	7	7.15
Vit[1]+Min[2]	2	2	2	2	2
Pellet binder	1	1	1	1	1
Crude protein (%)	46.24	46.28	46.18	46.29	46.254
Crude fiber (%)	2.44	2.32	2.28	2.48	2.48
Crude matter (%)	76.44	80.46	84.02	88.06	89.34
Crude lipid (%)	9.08	9.18	9.22	9.83	9.99
Crude ash (%)	12.49	11.67	10.80	11.31	11.03
GE:	3761.45	3761.91	3760.47	3761.61	3760.4

Vitamin premix.[1]; per kg, 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 mg niacin, 10,000 mg calcium D-pantothenate, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1,200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol. Mineral premix.[2]; per kg 23,750 mg Mn, 75,000 mg Zn, 2,000 mg Co, 2,750 mg I, 100 mg Se, 200,000 mg Mg.

NFE: Nitrogen Free Extract = 100-(% Moisture + % Crude protein + % Crude lipid + % ash + % Crude fiber) (Yeşilayar et al. 2020).

GE: Gross energy = (% crude protein×23.6) + (% crude lipids×39.5) + (% carbohydrates×17.3) (Koshio et al. 1993).

Statistical Analysis

The significance of differences among results of the intestinal data was analyzed by one-way analysis of variance (ANOVA). SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data.

The variables were assessed by the Bonferroni test, and ANOVA tests were used to compare groups. In comparing ovulation and egg quality, growth performance and gonodasomatic index data in among the groups were used ANOVA test (variance analysis). $P < 0.05$ was considered statistically significant.

Results

Growth Performance

The growth parameters of *P.socolofi* (female 93%; male 7%) fed diets added with different rates of SBM were given in Table 2. No statistical differences were found in terms of specific growth rate (SGR), weight gain (WG) and survival rate among groups ($P > 0.05$). Each other killings when many males in the aquarium

However, among groups, there were statistically significant differences in FCR and final weight ($P < 0.05$). Fish fed with a diet comprising 44 % SBM exhibited the highest feed conversion ratio (FCR) and the lowest final weight compared to other diets as statistical ($P < 0.05$).

Reproductive performance

The reproductive performance data of *P. socolofi*-fed diets added with different SBM levels are given in Table 3. According to the end of experiment results, no statistical difference was detected between the groups regarding fertilization rate, egg productivity, egg opening rate, egg diameter, gonodosomatic index and percentage of laying eggs ($P > 0.05$).

Table 2. Growth performance of *P. socolofi* fed different SBM levels for 90 days (mean ± SE)

	3%	16%	27%	35%	44%	df	F	P
Initial weight (g)	5.99 ± 0.06	6.00 ± 0.02	5.90 ± 0.11	5.95 ± 0.03	5.92 ± 0.11	4	0.34	0.85
Final weight (g)	7.95 ± 0.25^{a}	7.52 ± 0.30^{ab}	7.33 ± 0.21^{ab}	7.23 ± 0.11^{ab}	7.12 ± 0.28^{b}	4	1.83	0.02
WG	1.96 ± 0.30	1.52 ± 0.30	1.33 ± 0.12	1.38 ± 0.14	1.20 ± 0.36	4	1.27	0.35
FCR	3.69 ± 0.60^{a}	4.80 ± 1.42^{a}	5.26 ± 0.39^{a}	4.92 ± 0.58^{a}	7.48 ± 2.13^{b}	4	5.95	0.01
SGR	0.31 ± 0.04	0.25 ± 0.04	0.23 ± 0.02	0.23 ± 0.02	0.20 ± 0.06	4	1.09	0.41
Survival rate (%)	94.44 ± 5.56	88.89 ± 5.56	100 ± 0.00	83.33 ± 16.67	88.89 ± 5.56	4	0.54	0.71

Significant differences between treatments are indicated with different letter ($P < 0.05$).
Growth parameters were calculated using the following formulas
Weight gain (WG) g = (final body weight (g) - initial body weight (g))
Feed conversion ratio (FCR) = (total feed intake (g)) / (final body weight (g) - initial body weight (g))
Specific growth rate (SGR) % = [(ln final body weight - ln initial body weight)/experiment days] x 100
Survival (%) = 100*(final number fish−initial number fish) / initial number fish.

Table 3. Reproductive performance parameters of *Pseudotropheus socolofi* fed with experimental diets (mean ± SE)

	3 %	**16 %**	**27 %**	**35 %**	**44 %**	**df**	**F**	**P**
Fertility rate (%)	92.87 ± 1.41	94.68 ± 1.19	93.73 ± 1.46	95.31 ± 1.32	92.16 ± 1.57	4	0.65	0.60
Fecundity rate (%)	4.68 ± 0.20	4 ± 0.22	4.43 ± 0.26	4.37 ± 0.26	4.31 ± 0.30	4	1.17	0.33
Hatching rate (%)	89.24 ± 2.67	87.00 ± 3.09	91.50 ± 2.10	80.00 ± 5.00	88.71 ± 3.52	4	0.94	0.45
Egg diameter (mm)	3.01 ± 0.02	2.71 ± 0.18	2.90 ± 0.10	2.72 ± 0.19	2.83 ± 0.10	4	1.17	0.33
GSI	1.99 ± 0.40	1.82 ± 0.76	1.87 ± 0.53	0.79 ± 0.10	0.85 ± 0.35	4	1.16	0.39
Laying broodstock (%)	243.33 ± 54.87	235 ± 111.69	206.67 ± 35.28	196.67 ± 27.28	216.66 ± 20.28	4	0.11	0.98

Fertility rate (%) = (No. of fertilized eggs/No. of total eggs) *100
Fecundity rate (%) = No. of eggs/body weight of female(gr).
Hatching rate (%) = (No. of hatched eggs/No. of fertilized eggs) *100

Gonado somotic index (GSI) = (weight of gonad (g)/weight of fish) *100
Percentage of laying broodstock (%) = (Total number of laying broodstock / Number of female) *100
Significant differences between treatments are indicated with different letter in the same row (P < 0.05).

Histopathological examination of the female

No lesions were observed in any group at the gross examinations. Microscopical analysis revealed that guts were markedly affected by the increased ratio of the SBM. There was no inflammatory reaction, but numerous goblet cells were noticed in the anterior section of the gut in the 3% group. Meanwhile, there was a gradual decrease in goblet cells related to increased SBM additions. Severe inflammatory reaction was also observed in the 44% SBM added group (Figure 1). Statistical analysis results of villi length and goblet cell numbers are shown in Table 4. Our findings indicated that excessive SBM addition to a fish diet can cause harmful effects. No marked lesion in other organs in any group (Figure 2) except ovaries.

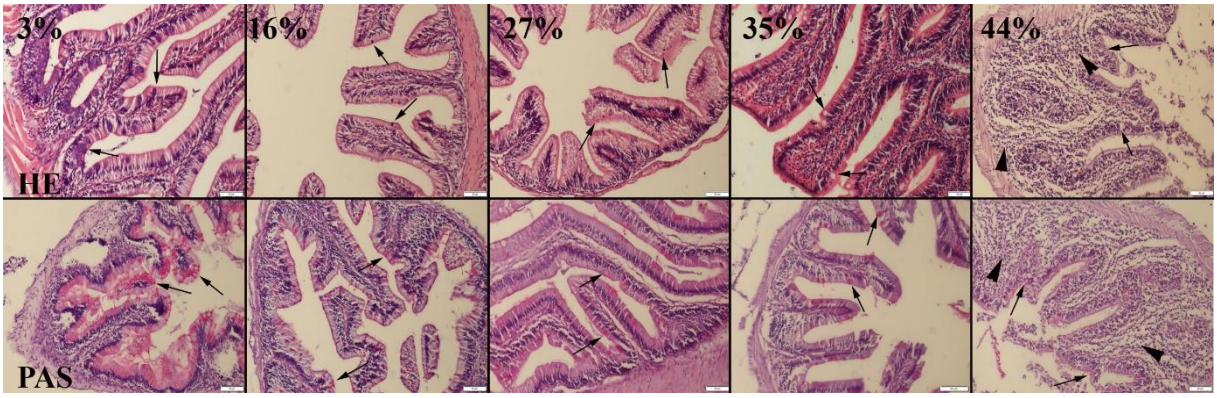

Figure 1. Representative histopathological figures between the group. Decreased number of goblet cells in 16%, 27%, 35% and 44% soy bean added groups compared to 3% group, severe inflammatory reaction 44% added group in gut (arrow head), Bars=50 µm.

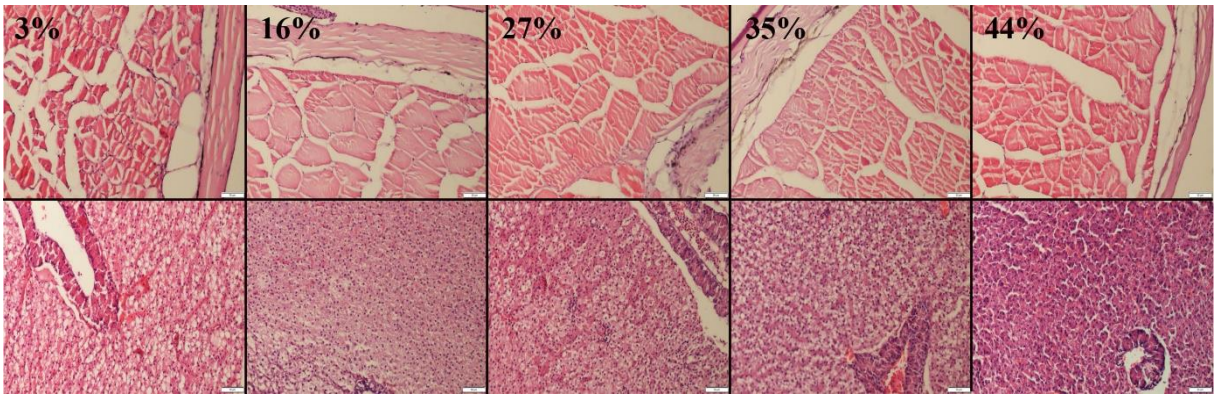

Figure 2. Histopathological appearance of muscle (upper row) and hepatopancreas (bottom row) between the group, HE, Bars=50 µm.

Table 4. Statistical analysis results of the intestinal data

Groups	Goblet cell numbers (mean ± SD)	Villi length (µm) (mean ± SD)
3%	53.80 ± 3.42^{a}	517.60 ± 10.73^{a}
16%	49.60 ± 1.67^{b}	517.40 ± 4.21^{a}
27%	34.40 ± 1.94^{c}	521.00 ± 6.78^{a}
35%	27.20 ± 1.30^{d}	521.40 ± 8.41^{a}
44%	16.40 ± 3.36^{e}	525.20 ± 11.54^{a}
P value	< 0.001	> 0.05

*: One-way ANOVA Bonferroni test
**: The differences between the means of groups carrying different letters in the same column are statistically significant (P < 0.001).

The ovarian follicles were microscopically evaluated at five different stages according to the appearance and diameters of the oocyte. At stage I (immature stage), oocyte diameter changed 55 to 60 µm without zona radiating and was irregular. At stage II, the diameter of the oocytes was between 163 to 175 µm throughout the long axis, and they had granular cytoplasm and ellipsoid in shape. At stage III, the oocyte's diameters were changing from 400 to 425 µm, and they had zona radiata with a 2 to 5 µm thickness. They had large cortical vacuoles with wavy margins in this stage. At stage IV, the diameters of the oocytes were between 758 to 825 µm; in addition, they had 8 to 10 µm thick zona radiate and were generally globular in shape. At stage V (maturation stage), oocyst diameter ranged between 843 to 889 µm and with 6 to 7 µm thick zona radiate, the oocytes without nucleus and granules in this stage. SBM caused decreased mature oocytes in ovaries related to ratio and a marked decrease was observed in the 44% added group (Figure 3).

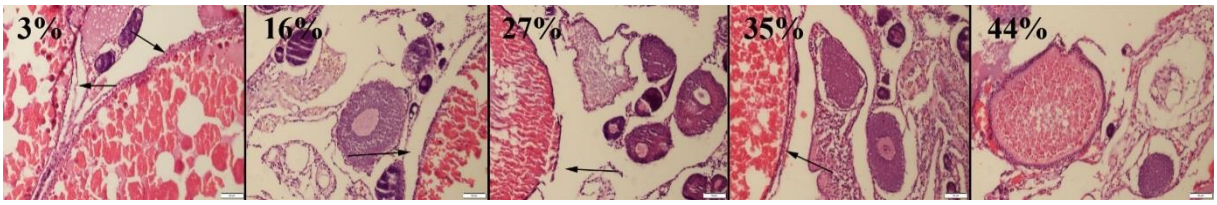

Figure 3. Microscopical appearance of the ovaries between the group, mature oocytes (stage V) (arrows) decreased according to increase of SBM ratio, HE, Bars=50 µm.

Statistical analysis of the percentage of the ovum developmental stages between the groups is shown in Table 5.

Table 5. Statistical analysis of ovum developmental stage percentages between the groups.

Groups	Stage I	Stage II	Stage III	Stage IV	Stage V
3%	9.00 ± 1.22^{a}	13.40 ± 2.51^{a}	21.40 ± 2.79^{ab}	39.20 ± 2.49^{a}	38.00 ± 3.53^{a}
16%	14.60 ± 2.70^{b}	20.80 ± 1.30^{b}	27.00 ± 4.12^{bd}	38.40 ± 8.61^{b}	40.20 ± 1.780^{ab}
27%	18.40 ± 1.51^{b}	25.40 ± 3.13^{c}	43.20 ± 1.92^{c}	24.00 ± 2.54^{c}	43.20 ± 3.76^{b}
35%	26.20 ± 3.89^{c}	41.60 ± 4.03^{d}	24.40 ± 3.64^{d}	18.40 ± 1.51^{d}	8.40 ± 1.14^{c}
44%	41.40 ± 6.10^{d}	42.00 ± 2.34^{d}	17.40 ± 2.07^{a}	10.60 ± 0.89^{e}	5.60 ± 2.60^{c}
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* The differences between the means of groups carrying different letters in the same column are statistically significant.
**: Values expressed as mean ± standard deviation.

Discussion

Effect on growth of soybean meal

In this study, the effects on the growth, reproduction parameters of supplementation of different levels of SBM (contains phytoestrogens) to diets were researched. Therefore, diet keeps fishmeal levels constant to avoid affecting reproduction parameters. Generally, there are studies on using SBM as a protein source instead of fish meal in the literature. In the present study, the growth and FCR were adversely affected by increasing SBM levels (especially 44%) in diets. However, a 35% SBM level may be used without adverse effects on the growth and reproduction performance of *Pseudotropheus socolofi*. The antinutrients contained in SBM may adversely affect the growth. Although not directly effective on growth, *Pseudotropheus socolofi*. is not a species fast growing for that is ornamental fish. The number of females in the trial groups was higher than that of males (5♀:1♂); females may have used for gonad development most of the nutrients obtained from

feed. Namely, the growth may be negatively affected by gonadal development. Similarly, Kaushik et al. (1995) indicated that replacing fishmeal with SBM (up to 50%) reduced the growth rate of the rainbow trout. Hernandez et al. (2007) indicated that high levels of SBM (60%) in their diets induced low final weight in *Diplodus puntazzo*. Gu et al. (2016) indicated that SBM caused dose-dependent decreases in growth performance and nutrient utilization of turbot *Scophthalmus maximus*. Krogdahl et al. (2003) reported that growth in Atlantic salmon decreased depending on the raising of the SBM ratio. Li et al. (2012) reported that high SBM levels (45-60%) negatively affected juvenile Japanese seabass growth. In the current study, pure phytoestrogen additions were not made to the diets. Nevertheless, researchers have reported that adding soy phytoestrogens such as genistein and daidzein to fish diets adversely affects growth. Ko et al. (1999) reported that the weight gain decreased in females of yellow perch, *Perca flavescens* fed with genistein diet. DiMaggio et al. (2016) indicated that the administration of diets of high-level genistein reduced the growth and survival of *Paralichthys lethostigma* fry. Dong and Qiuyan (2016) observed negative growth *Oreochromis niloticus* juvenile fed diet supplemented with genistein.

According to histopathological examinations, no remarkable lesions were identified in the muscle and hepatopancreas of all groups. However, a significant reduction of goblet cell numbers was determined in the gut with increasing SBM levels. A severe inflammatory reaction was also observed in the added 44% SBM group. Krogdahl et al. (2003) and Urán et al. (2009) reported that the inclusion of as low as 5–10% SBM level in the diet of Atlantic salmon could produce detrimental inflammatory effects in the intestinal tissues. Gu et al. (2016) indicated that enteritis developed in the distal intestine, and the severity of the inflammation increased depending on the dose of SBM (26–54%) in turbot. Zhang et al. (2018) pointed out that increasing the FM replacement level to 75% in Japanese seabass diets reduced villus height, but not at the 50% level. Similarly, villus height did not change even at the 44% SBM level in this study. This result pointed out that the addition of 44% SBM does not pose a digestive issue.

Effect of soybean meal on reproductive performance

In the present study, the fertilization rate, egg efficiency, egg opening rate, egg diameter, gonadosomatic index and spawning percentage paremeters of *Pseudotropheus socolofi* broodstock fed with diets supplemented with different SBM levels were no significant difference in among groups. However, SBM caused decreasing mature oocytes with increasing ratio and a marked decrease was observed in the 44% added group. There are very few studies investigating the effects of SBM used in fish diets on reproduction; Bagheri et al. (2013) reported that a long-term (5 months) feeding *Carassius auratus* with SBM (35, 65 and 100%) caused a reduction in maturation, fertilization, hatching rates average eggs number with increasing SBM inclusion. Unlike our study, they found a decrease in fertilization and hatching rates. This result may depend on the application of a longer feeding time than our study. Ko et al. (1999) indicated that estrogenic effects on reproductive function were not observed in *Perca flavescens* fed diet supplemented with genistein. Oca et al. (2005) indicated that the supplementation of genistein and daidzein to the diets of Oreochromis niloticus was no effect towards the masculinization of gonads in females. Brown et al. (2014) reported that *β*-sitosterol or genistein did not have significant effects on steroids or gonads of Betta splendens females.

In conclusion, 44% SBM addition to diets of *Pseudotropheus socolofi* negatively affected growth. In pathological examinations, negative effects were observed in gonads while the high SBM levels no affected reproductive parameters of females. The results of this study showed that long-term and high-dose SBM can cause harmful effects in fish, so it should take into account this subject when using SBM.

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Akuakültürde Balık Refahı

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ÖZ

Balıkların korunmasına ilişkin mevzuat diğer çiftlik hayvanlarını kapsayan mevzuat ile aynı zamanda yürürlüğe girmiştir. Ancak balık refahına ilişkin gelişmeler nispeten daha yavaş ilerlemektedir. Bunun en önemli nedeni çok sayıdaki balık türü ve çeşitli yetiştirme sistemlerinin karmaşık doğasıdır. Mevcut araştırmalar balıklarda bilinç ve duyarlılık kapasitesinin varlığını tanımlamış ve balıkların ağrı, acı ve ızdırap hissedebildiklerini ortaya koymuştur. Ayrıca araştırmalar balıklar ile yetiştirme çevresi arasındaki etkileşimlerin balıklarda stres yanıtları oluşturduğunu ve refah kayıplarına neden olduğunu göstermektedir. Bu makalede akuakültürde yetiştirilen balıkların refahına ilişkin kavram, mevzuat ve refah değerlendirme yöntemleri ile balık yetiştirme, taşıma, kesim ve öldürme uygulamalarının balık refahına etkilerine ilişkin güncel araştırma bulgularının derlenmesi amaçlanmıştır. Ayrıca su ürünleri alanı çalışanları ile tüketicilerin balık refahına ilişkin farkındalığının arttırılması amaçlanmıştır. Yüksek stok yoğunluğu, su kalitesi, hastalıklar ile balık nakilleri ve kesim ve öldürme uygulamaları balık refahı için en önemli risklerdir. Balıklarda türe özel olmak üzere refah ihtiyaçlarının tanımlanması ile etkili ve pratik refah değerlendirme metotlarının geliştirilmesine ihtiyaç vardır. Ayrıca su içinde veya dışında iken stres faktörlerine karşı uyum kapasitesi oldukça sınırlı olan balıkların korunması için yetiştirici, bakıcı ve tüketicilerin iyi hayvan refahı konusunda eğitiminde fayda olduğu kanaatine varılmıştır.

Anahtar kelimeler: Akuakültür, Balık refahı, Çevre faktörleri, Refahın değerlendirilmesi

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Fish Welfare in Aquaculture

Abstract: Legislation for fish welfare was implemented concurrently with that for other animals. Nonetheless, advancements in fish welfare have been comparatively slow. This is mainly due to the complexity of fish species and aquaculture systems. Recent studies have shown that fish are conscious and sentient. Furthermore, research indicates that fish interactions with their environment can induce stress responses in fish leading to welfare losses. This article aims to summarise the concepts, regulations and assessment methods related to the welfare of farmed fish in aquaculture, and to summarise current research on the effects of fish farming, transport, slaughter and killing practices on fish welfare. In addition, the aim is to raise awareness of fish welfare issues among professionals in the fisheries sector and among consumers. High stocking density, water quality, diseases, and fish transfers, as well as slaughter and killing practices, are the most significant risks to fish welfare. There is a need to develop species-specific welfare requirements and effective and practical welfare assessment methods for fish. It was also concluded that educating producers, caretakers and consumers about good animal welfare practices can beneficial to fish welfare, given the limited adaptability of fish to stressors both in and out of the water.

Keywords: Aquaculture, Environmental factors, Fish welfare, Welfare assessment

Alıntılama

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1. Giriş

Bilinç hissedebilme, zekâ ile içsel ve dışsal uyarıların farkında olma ve dünyadaki yerinin bir anlayışına sahip olmayı ifade etmektedir (Dawkins 2004; Sneddon 2020). Hissetme ise rahatlık gibi olumlu duygular ile acı, kaygı veya ızdırap gibi olumsuz duyguları deneyimleyebilme yeteneğini kapamaktadır (Mercogliano ve Dongo 2023). Balıkların pozitif veya negatif duyguları hissetmesini açıklamak kolay değildir (Broom 2007; Brown ve Dorey 2019). Ancak balıklarda bilişsel kapasite ve hissedebilme yetisinin zayıf olduğu, balıkların basit bir beyine ve temel davranış biçimlerini kontrol eden birkaç sinir devresine sahip olduğuna ilişkin ilk görüşlerin aksine (Rose vd. 2014) balıklar tarafından sergilenen bilişsel karmaşıklık seviyesinin karasal omurgalılarla çoğunlukla aynı seviyede olduğu ve onlarda bulunanlarla benzer nöroseptif işleme sistemlerine sahip oldukları belirlenmiştir. Eğer herhangi bir hayvan düşünülebiliyorsa, o zaman balıkların da düşünülebileceğini ileri süren kanıtlar giderek artmaktadır (Sneddon 2003; Chandroo vd. 2004).

Rekabetçi ve sürdürülebilir bir akuakültür için balık refahının arttırılması stratejik bir hedeftir. Ancak akuakültürde balık refahı yaklaşımlarının hayvan refahı etiği ve ekonomisinin tüm yönlerini dengeli bir şekilde kapsaması çok önemlidir. Etik değerleri ve bilimsel gerçekleri dikkate alarak, insan ve balık ilişkisinde mümkün olan en iyi uzlaşma sağlanmalıdır (Seibel vd. 2020). Nitekim hayvan refahı için aktivist kuruluşlar ile eleştirel tüketicilerin duygu ve doğa temelli bir anlayışı benimsediği, çevre, etik ve şefkate dayalı normatif tepkiler gösterdiği buna karşın araştırmacıların ve yetiştiricilerin fonksiyon temelli bir yaklaşımı daha fazla önemsediği bildirilmektedir (Vanhonacker vd. 2011). Balık refahı, sadece etik bir sorun olmayıp, çiftlikten sofraya gıda güvenliği stratejisinin hedeflerinden birisidir. Ayrıca Tek Sağlık ve Tek Refah yaklaşımlarına göre de balık refahı halk sağlığı ve ürün kalitesinin önemli bir yönünü oluşturmaktadır (Mercogliano ve Dongo 2023). Sevgi ve şefkat gibi etik ilkeler ile çevre ve gıda güvenliği endişelerine bağlı olarak yakın dönemde insanların balık refahı konusuna daha fazla ilgi göstereceği görülmektedir (Röcklinsberg 2015)

Bu derleme makalede balıklarda refah kavramı ve ilgili mevzuat, akuakültürde balık yetiştirme, taşıma, sedasyon ve kesim uygulamalarının balık refahına etkileri ile balıklarda hayvan refahı değerlendirmesine ilişkin güncel araştırma bulgularının derlenmesi amaçlanmıştır. Ayrıca akuakültür yetiştiricileri ve çalışanları ile tüketicilerde balık refahına ilişkin farkındalığın arttırılması hedeflenmiştir.

2. Hayvan Refahı Kavramı ve Mevzuat

Bir hayvanın çevresiyle başa çıkma çabalarına ilişkin durumu olarak tanımlanan hayvan refahı kavramına (Broom 1986) sağlık, zindelik ve hayvanların ne istediklerine ilişkin tamamlayıcı işlevsel kriterler eklenmiştir (Dawkins 2004). Daha sonra hayvan refahının kapsamı acı ve ızdırap yokluğundan öteye geçerek yaşamaya değer iyi bir hayatı ifade edecek şekilde genişlemiştir (Mellor 2016). Zaman içinde gelişen bu hayvan refahı kavramı balık refahının sadece sağlıklı olmanın ötesinde değerli bulunan kaynaklara ulaşma, doğal davranışlarını sergileyebilme, kontrol ve seçim yapabilme ve poziyif duyguları da deneyimleyebilme ile ilişkili olduğunu ortaya koymaktadır (Sanchez-Suarez vd. 2020). Bu nedenle akuakültürde kısıtlanmış sakinler olan balıkların yüksek hayvan refahı standartlarında yetiştirilmesi için bilim insanları (Mustapha 2014; Kurtoğlu vd. 2021; Köse vd. 2023), tüketiciler (Pieniak vd. 2013), sivil toplum örgütleri (Petersson 2022) ve yasa yapıcıların (EFSA 2008) çabaları giderek artmaktadır (Broom 2007).

Balık refahına ilişkin huhuki yaptırımlar diğer çiftlik hayvanları ile aynı zamanda başlamış ise de akuakültür endüstrisinin gelişimi henüz yakın zamanda ivme kazanmıştır (Röcklinsberg 2015; Sanchez-Suarez vd. 2020). Çiftlik Amaçlı Yetiştirilen Hayvanların Korunmasına ilişkin Avrupa Sözleşmesi (ETS No.087) Daimi Komitesinin çiftlik balıkları ile ilgili tavsiye kararı balıkların korunmasına ilişkin standartları tanımlamıştır (Council of Europe 2005). Lizbon Antlaşması hükümlerine dayanılarak, 2006/88/EC sayılı AB Konsey Direktifi Birlik içindeki tüm su ürünleri yetiştiriciliği faaliyetlerinde yer alan sucul canlıların sağlık kontrollerini tek bir çatı altında birleştirmiştir. Ayrıca, gıda üretimi amacıyla yetiştirilen balıklar ve diğer çiftlik hayvanlarının çiftlik ve yetiştirme ortamlarında (1998/58/EC), nakilleri sırasında (EC1/2005) ile kesim ve öldürülmeleri sırasında (EC/1099/2009) ve deneylerde kullanımlarında (2010/63/EU) sağlanacak minimum hayvan refahı standartlarına ilişkin bir dizi AB mevzuatı yürürlüğe girmiştir. Organik su ürünleri yetiştiriciliği üzerine ayrıntılı kuralları belirleyen (EC) No 889/2008 ve (EC) No 710/2009 sayılı yönetmelikler bazı balıklar için stok yoğunluğu kriterlerini hükme bağlamış, (EC) No 2065/2001 sayılı yönetmelik ise balıkçılık ve su ürünleri ürünleri hakkında tüketicilere bilgi verilmesini hükme bağlamıştır. İlgili mevzuat, yetiştiriciler ve hayvan bakıcılarını sorumlulukları altındaki hayvanların refahını sağlamak için makul adımları atmaları ve bu hayvanların gereksiz acı ve ızdırap yaşamaması için tedbir almaları konusunda

yükümlülük altına almaktadır. Ancak bu mevzuat yetiştiriciliği yapılan yüzlerce balık türünün bireysel farklılıklarını dikkate alan türe özgü refah düzenlemelerini içermemektedir (Röcklinsberg 2015). Bunun en önemli nedenlerinden birisi akuakültür balıklarının biyolojik ve etolojik özellikleri ile refah gereksinimleri konusundaki bilgilerin henüz yeterli olmamasıdır. Bununla birlikte, balıkların acı ve ağrı hissedebilme kapasiteleri ile hayvan refahının verimler, ürün kalitesi ve halk sağlığıyla olan ilişkilerini aydınlatan yeni bilgiler elde edilmiştir ve akuakültürde sürdürülebilir gelişme ve hayvan refahının arttırılması birlikte ele alınmaya başlanmıştır (Hastein vd. 2005; Kamali vd. 2022).

3. Akuakültürde Balık Refahını Etkileyen Faktörler

3.1. Balık Yetiştirme Uygulamaları

Stok Yoğunluğu

Akuakültürde balıklar minimum ağrı, korku ve stres yaşamalı ve mümkün olduğunca çok türüne özgü doğal davranışını sergileyebilmelidir (Council of Europe 2005; OIE 2019). Akuakültür üretiminde stok yoğunluğunun balık refahına etkileri Tablo 1'de özetlenmiştir. Stok yoğunluğu su kalitesini sürdürebilme kapasitesi, yetiştirme sistemi ve balık besleme yöntemi (OIE 2019) gibi yetiştirme ortamı koşulları ile balıkların biyolojik ve davranışsal ihtiyaçları, sağlık ve refah durumları dikkate alınarak planlanmalıdır (OIE 2019). Herhangi bir yetiştirme ortamında stok yoğunluğu artışları kademeli yapılmalı ancak maksimum stok yoğunluğu belirlenirken mümkün olan en düşük seyide tutulmalıdır (FAWC 2014) ve bu amaçla su ortamının taşıma kapasitesi aşılmamalıdır (Ellis vd. 2002; Saraiva vd. 2022). Stok içinde stres, kanibalizm ve saldırganlığın en aza indirilebilmesi için balıklar büyüklüklerine göre sınıflandırılmalı, balık grubu sağlık durumu ve ölüm oranı yönünden düzenli olarak takip edilmeli ve günde en az bir kez grup içindeki balıklar suyun altından gözlemlenerek davranış anormalliği yönünden değerlendirilmelidir. Bu amaçla mümkün ise video tabanlı davranış izleme yöntemleri ve telemetri sistemleri gibi teknolojiler kullanılmalı ancak suyun içinden alınan görüntülerin net olmasına özen gösterilmelidir (Council of Europe 2005). Balık çiftliklerinde otomatik sistemlerin takibi için alarm bulundurulmalı, güç kaynağı ve ekipman arızaları durumunda balık sağlığı ve refahını güvence altına almak için yedek sistemler hazır bulundurulmalıdır. Kafes, havuz ve göletler gibi akuakültür sistemleri daha yapım aşamasında iken planlanan yetiştirme yönü ve balık tipine göre tasarlanmalıdır. Balıklara zarar verebilecek keskin köşe ve çıkıntılardan ve malzemelerden kaçınılmalıdır. Balıkların bireysel kontrolü gerektiği durumlarda balığın izole edilmesi için yapılacak girişimin diğer balıkları rahatsız etmemesi için gerekli dikkat gösterilmeli, gerekirse izole balığın yemliğe ulaşımını sağlamak için gerekli donanımlar tasarlanmalıdır. Balık sınıflandırma işlemi aynı zamanda balıkların bireysel kontrollerinin yapılması için uygun bir fırsat olarak değerlendilmelidir (FAWC 2014).

Yüksek stok yoğunluğu O_2 miktarının azalmasına neden olur ve amonyak (NH_3), nitrit (NO_2), karbondioksit (CO_2) ve pH düzeylerindeki artışlar ile balıkların daha fazla aktivite sergilemesi sonucu oluşan sudaki parçacık miktarındaki artış gibi nedenlerle su kalitesi düşer (Council of Europe 2005; OIE 2019; Carbonara vd. 2020). Amonyak ve NO_2, balıklar için çok toksiktir ve bunların su ortamlarında birikmesi engellenmelidir. Amonyak ve NO_2'in birikmesinin önlenmesi için su akış hızının arttırılması, biyofiltrasyon uygulanması ile stok yoğunluğu, balık besleme veya su sıcaklığının azaltılması gibi çeşitli yöntemler kullanılmalıdır (Council of Europe 2005; FAWC 2014). Balıklar boşaltılmadan önce suyun kalitesi kontrol edilmelidir (FAWC 2014). Balıkların solunumu ile üretilen CO_2 suda birikir. Bu nedenle suyun kalitesini izlemek için CO_2 ve pH seviyeleri izlenmelidir. Stok yoğunluğu yüksek tutulduğunda yetersiz su akış hızı veya uygun olmayan besleme ekipmanları gibi diğer çevresel koşulların su kalitesini desteklemediği durumlarda stok yoğunluğunun olumsuz etkileri katlanarak artmaktadır (Ellis vd. 2002; Gauy vd. 2023). Yüksek stok yoğunluğuna bağlı akut ve kronik stres balıklarda yem tüketimi ve yemden yararlanma oranından başka immunite ve büyüme hızını da olumsuz etkilemektedir (Andrew vd. 2002; Boujard vd. 2002). Ayrıca yüksek stok yoğunluğunda yetiştirilen balıklarda parazit ve hastalık insidansında artış (Stevenson 2007; Broom 2007; Cascarano vd. 2021) yanı sıra oksijen tüketimi, saldırganlık, anormal davranışlar ve yaralanmalarda artış olduğu (Ellis vd. 2002, Anras ve Lagardere 2004) ve balıklarda kötü vücut kondüsyonu (Turnbull vd. 2005) görüldüğü bildirilmiştir. Stok yoğunluğunun balık refahına etkisi balık türü ve yaşı gibi bazı faktörlerden etkilenmektedir. Yüksek stok yoğunluğu gökkuşağı alabalığı (*Oncorhynchus mykiss*), levrek (*Dicentrarchus labrax*) ve çipura (*Sparus aurata)* gibi akuakültür balıklarının türe özgü gece-gündüz ritmi, doğal davranış deseni, aktivite düzeyi, saldırgan davranış ve fiziksel hasar düzeyleri ile su kalitesini olumsuz etkilemektedir (Anras ve Lagardere 2004; Turnbull vd. 2005; Ashley 2007; Calabrese vd. 2017).

Stok yoğunluğunun balık refahı üzerindeki etkileri karmaşık olup bazen çelişkili sonuçlar da bildirilmektedir (Carbonara vd. 2020). FAWC (2014)'e göre stok yoğunluğu maksimum 15 kg/m^3 olmalıdır. Ancak yapılan araştırmalarda stok

yoğunluğu 22 kg/m^3 veya 30 kg/m^3'ün üzerinde olduğunda balıkların refahının olumsuz etkilenmediği bildirilmiştir (Greaves ve Tuene 2001; Turnbull vd. 2005). Ancak 50 kg/m^3 ve daha yüksek yoğunluklarda tutulan kalkan balıklarının (*Scophthalmus maximus*) büyüme performansı, biyokimyasal profili, ozmolarite düzeyi, gen ekspresyonunu ve refahının olumsuz etkilendiği bildirilmiştir (Liu vd. 2019). Buna karşın Atlantik somonu (*Salmo salar*) yavru balıkları hem düşük (15 kg/m^3) hem de yüksek (35 ve 75 kg/m^3) stok yoğunluklarında olumsuz etkilenmiştir (Adams vd. 2007; Calabrese vd. 2017). Yüksek stok yoğunluğunun gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) büyüme hızı ve yüzme performansını düşürdüğü ve yemden yararlanma oranını arttırdığı bildirilmiştir (McKenzie vd. 2012). Düşük stok yoğunluğunda (3 kg/m^3) yavru ve yetişkin çupra balıklarının *(Sparus aurata)* yem için rekabet ettiği (Sara vd. 2010) ve daha yüksek stok yoğunluğunda (40 kg/m^3) ise balıklarda fizyolojik değişiklikler meydana geldiği görülmüştür (Montero vd. 1999). Greaves ve Tuene (2001) Atlantik halibutu (*Hippoglossus hippoglossus* L.) balıklarının yüksek stok yoğunluklarından fazla etkilenmediğini bildirmiştir ancak Kristiansen vd. (2004) yüksek yoğunlukta yetiştirilen bu türe ait balıklarda yüzeyde yüzme davranışında artış, yem tüketimi ve büyüme hızında düşüş belirlemişlerdir. Yüksek stok yoğunluğunda Atlantik halibutu (*H. hippoglossus* L.) balıklarının dikey yüzme davranışı olası bir refah problemi ile ilişkilendirilebilirken Rayler balıklarında (*Raja sp*.) görülen yüzeyi kırma davranışı hem bir stereotipik davranışla (Scott vd. 1999; Ashley 2007) hem de bir besleme tekniği ile ilişkili normal bir davranış (Kristiansen vd. 2004) olarak değerlendirilmiştir. Bu bilimsel kanıtlar stok yoğunluğu için bir "altın oran" önermenin oldukça güç olacağını ortaya koymaktadır (Saraiva vd. 2022). Bu durum balık, stok yoğunluğu ve diğer çevresel faktörler arasındaki ilişkilerin daha net anlaşılması için yeni araştırmalara ihtiyaç olduğunu göstermektedir.

Su Kalitesi

Balıkların yetiştirildikleri su ortamında sıcaklık, tuz oranı, pH ile O_2, CO_2, NH_3 seviyeleri ve su akış hızı gibi çevresel faktörler balık türü ve stok yoğunluğuna göre uygun olmalıdır (OIE 2019). Balık yetiştiriciliğinde su kalitesinin balık refahına etkileri Tablo 1'de sunulmuştur. Su kalitesini etkileyen parametreler aynı zamanda birbiri ile ilişkilidir ve her birisindeki değişim diğerlerini ve dolayısıyla balık refahını etkilemektedir (Council of Europe 2005). Bu nedenle su akış hızı, solüt konsantrasyonu ve su sıcaklığı gibi kritik değerler yönünden su kalitesi sürekli olarak izlenmelidir (FAWC 2014). Muhtemel sorunlara karşı ek O_2 sağlama, CO_2 giderme, su akışının arttırılması veya stok yoğunluğunun azaltılması gibi yönetimsel tedbirler her zaman kullanılabilir olmalıdır (Mustapha 2014). Su kirliliği için acil durum planları hazır bulunmalıdır (FAWC 2014). Ayrıca yosun ve su bitkilerinin üremesine ilişkin tedbirler alınmalıdır (Kamali vd. 2022; Saraiva vd. 2022). Su kalitesi her zaman belirli bir balık türünün normal aktivite ve fizyolojisini sürdürebileceği bir aralıkta olmalıdır ancak balık türlerinin gereksinimlerinin farklı yaşam aşamaları arasında değişebileceği dikkate alınmalı veya yetiştirme uygulamasına ilişkin istisnai durumlar söz konusu olduğunda bu parametrelere ilişkin uygun yönetim sağlanmalıdır. Su kalitesini etkileyen parametrelerde meydana gelen ani değişiklikleri en aza indirmek için uygun önlemler alınmalıdır. Geri dönüşüm sistemlerinde su kalitesinin izlenmesine ve yönetimine özel bir dikkat gösterilmelidir (Council of Europe 2005).

Balıklar, su kalitesindeki değişikliklere farklı derecelerde adapte olabilirler ise de bu durum strese neden olur ve değişikliğin şiddetine bağlı olarak balıkların ölümüne kadar varan refah kayıpları meydana gelebilir (Conte 2004). Su kalitesi balıklarda sağlık, üreme ve besi performansı ile davranışları etkilemektedir (Ellis vd. 2002; Relic vd. 2010) ve bu nedenle balık refahının önemli bir göstergesidir (Saraiva vd. 2022). Çünkü su kalitesinin kötüleşmesi ile akut ve kronik stres, homeostazı kontrol güçlüğü, büyüme geriliği, solungaçlarda ve yüzgeçlerde yaralanmalar, hastalıklara karşı artmış duyarlılık ve ölümler görülebilir (Ellis vd. 2002; Mustapha 2014). Su sıcaklığı önemli bir su kalitesi parametresidir ve su sıcaklı ile stok yoğunlu, pH ve amonyak düzeyi arasında çeşitli etkileşimler bildirilmiştir (Ellis vd. 2002; Shabani vd. 2016).

Balık Bakım ve İdaresi

Balık Besleme

Balıklara sağlık ve verimliliğin sürdürebilmesi için fizyolojik ihtiyaçlarını karşılayabilecek uygun içerik ve miktarda yem verilmelidir (EC/98/58 ve Çiftlik Hayvanlarının Refahına İlişkin Genel Hükümler Hakkında Yönetmelik, 22 Kasım 2014, R.G.No:29183). Besleme ekipmanı suda en az kirliliğe neden olacak ve tüm balıkların yeterli miktarda yeme ulaşmasını sağlayacak şekilde tasarlanmalı, yerleştirilmeli ve verilen yem miktarının izlenmesine olanak sağlayacak ve tüm iklimsel koşullarda çalışacak şekilde yönetilmelidir (Council of Europe 2005). Kullanılan veya depolanan yemlerden düzenli örnekleme yapılarak pelet büyüklüğü, yemde toz, nem ve yabancı maddeler yönünden fiziksel kontroller ile bakteri ve

küf gibi mikroorganizmalar yönünden kontroller düzenli olarak yapılmalıdır (Hastein vd. 2005; FAWC 2014; Mustapha 2014). Yemin bileşimi beslenecek balıkların türü ve yaşı ile üretim programının özelliğine göre hazırlanmalıdır. Yemde ve besleme programında ani değişikliklerden kaçınılmalı, balıkların refahını veya su kalitesini olumsuz etkileyebilecek yöntemler kullanılmamalıdır (OIE 2019; FAWC 2014). Yemleme programı ve yemin dağıtılma şekli balıklar arasında zararlı sonuçlar oluşturabilecek rekabete neden olmamalıdır (Andrew vd. 2002; Council of Europe 2005). Özellikle yavru ve genç balıkların beslenmesi düzenli olarak izlenmelidir (Council of Europe 2005; FAWC 2014).

Balık türünün doğal yapısına ve davranış özelliklerine uygun olmayan besleme uygulamaları balıklarda stres tepkilerine neden olmakta, stres toleransı ile balık sağlığını olumsuz etkilemekte ve saldırgan davranışlarda artışa yol açmaktadır. Bu tip hatalı beslemeyle balıklarda düşük büyüme hızı, baskılanmış immun yanıt, morfolojik anormallikler ve normalden faklı bir şekilde yüzme gibi anormal davranışlar ilişkilendirilmektedir (Conte 2004; Damsgard vd. 2004; Hastein vd. 2005; Mustapha vd. 2014). Yem dağıtılan alanın oransal olarak dar olması yem alımı için rekabeti arttırarak saldırgan davranışlarda ve büyüme yönünden grup içi varyasyonda artışlara neden olabilir ve sosyal hiyerarşinin güçlenmesine yol açabilir (Stevenson 2007). Uygun şekilde yapılmayan yemlemenin Atlantik somonunda hastalıkların artmasına neden olduğu, levrekler arasında saldırgan etkileşimleri arttırdığı bildirilmiştir (Andrew vd. 2002; Damsgard vd. 2004). Kafeste yemleme yapıldığı sırada iri yapılı somonların fazla miktarda yem aldığı belirlenmiş ancak rekabet gücü zayıf olan küçük yapılı somonların az yem aldığı ve kafes çevresine yakın bölgelerde daha yoğun şekilde bulundukları bildirilmiştir (Broom 2007). Yemde besin madde miktarlarının dengesiz veya yetersiz olmasının balık sağlığını olumsuz etkilediği bildirilmiştir (Poli 2009). Gece beslenen Afrika kedi balığının (*Clarias gariepinus*) akuakültürde genellikle gündüz beslenmesinin bu türün yemden yararlanma ve büyüme hızını olumsuz etkilediği bildirilmiştir (Ashley 2007).

Doğada birçok balık türü, mevsimsel gıda kıtlığı, göç veya üremeyle ilgili faktörlere bağlı olarak zaman zaman kısa veya uzun süre beslenmeden kalabilir (Miller vd. 2009). Balıklar ektotermik olup vücut sıcaklıkları ortam sıcaklığından etkilenir ve açlık döneminin etkileri endotermik olan canlılara göre daha düşük olmaktadır. Bu nedenle balıkların sağlıklı kalabilmek için sık sık beslenmeye ihtiyaçları olmadığı ve açlık dönemlerinde enerji depolarının daha yavaş tükendiği bildirilmiştir (Wang vd. 2006; Bar 2014; Hvas vd. 2020). Ayrıca Salmon alfavirusun neden olduğu pankreas hastalığı ile su sıcaklığında meydana gelen mevsimsel sıcaklık dalgalanmaları ve hipoksinin de gönüllü açlığa neden olabileceği kaydedilmiştir (McLoughlin ve Graham 2007; Röcklinsberg 2015; Hvas vd. 2020).

Su ürünleri yetiştiriciliğinde tuzlu suya geçiş, hasat, nakil ve kesim gibi işlemler sırasında balıklarda oluşacak stres ile metabolizma hızı, oksijen tüketimi ve atık üretim seviyelerini azaltılmak ve ayrıca sindirim sistemini boşaltmak (Hvas vd. 2020), et kalitesini arttırmak (Mustapha 2014) ve hastalıkların tedavisi gibi amaçlarla balıklar geçici olarak aç bırakılabilmektedir (Ashley 2007). Uygun koşullar altında balıkları kısa süreyle aç bırakmak hayvan refahı kayıplarına neden olmayabilir (Hastein vd. 2005; Relic vd. 2010). Ancak daha önce düzenli olarak beslenen balıklarda açlığın etkileri veya göç ve hastalıklar gibi durumların gönüllü açlıkla ilişkisine dair bilgiler çok sınırlıdır. Bununla birlikte açlık ile metabolik aktivite ve saldırgan rekabet davranışı arasındaki ilişkilere dair önemli kanıtlar bulunmaktadır (Hastein vd. 2005; Ashley 2007; Stevenson 2007). Bu durum, balıklar için gönüllü veya gönülsüz açlığın balık refahını nasıl etkilediğini belirleyebilmek için daha fazla araştırma yapılmasına ihtiyaç olduğunu göstermektedir. Uzun süreli açlığın balık refahı için risk oluşturup oluşturmadığını değerlendirebilmek için dinlenme metabolik hızı, enerji tasarrufuna başlama zamanı ve vissera içindeki depoların azalma durumu gibi parametrelerin yararlı olabileceği bildirilmiştir (Hvas vd. 2020).

Balık Hastalıkları

Akuakültürdeki balıklar hastalanma ve yaralanma risklerinden korunmalı, yaralanma ve hastalık hallerinde uzman veteriner hekimlerce hızlı tanı konulmalı ve en uygun tedavi yapılmalıdır (EC/98/58 ve Çiftlik Hayvanlarının Refahına İlişkin Genel Hükümler Hakkında Yönetmelik, 22 Kasım 2014, R.G.No:2918). Balıklarda anormal davranış, yaralanma, hastalık ve ölümlerde artış görüldüğünde balık bakım ve idaresinden sorumlu kişiler nedenlerin belirlenmesi ve gerekli ise tedavinin başlatılması için derhal harekete geçmeli ve veteriner hekime bilgi vermelidir (FAWC 2014). Balık hastalıkları ile hayvan refahı etkileşimleri Tablo 1'de gösterilmiştir. Balıklarda vücudun hassas bir bölümünün kaybedilmesine veya zarar görmesine, yüzgeç, solungaç veya kemik yapısının değiştirilmesini içeren herhangi bir cerrahi müdahaleye izin verilmez. Yapılacak olan sağlık müdahalelerine karar verirken akuakültür koşulları, balıkların fizyolojik ve davranış özellikleri, stres, iştah ve büyüme düzeyleri ile yaralanma, hastalık ve ölüm oranları gibi sağlık ve refah göstergeleri dikkate alınmalıdır (Council of Europe 2005).

Enfeksiyon hastalıkları ve paraziter invazyonlar balıklarda en önemli refah sorunlarındandır (Damsgard vd. 2004; FAWC 2014). Etkin ve hızlı tedavi, hastalık tehditlerinin azaltılması ve iyi sağlık koruma uygulamaları için veteriner ilaçların kullanımındaki sınırlandırmaların azaltılmasına ihtiyaç bulunmaktadır (FAWC 2014). Ayrıca veteriner ilaçların kullanım şekli, yan etkileri ve çevresel etkileri bakımından balık türleri arasındaki geniş farklılıklar tedavi uygulamalarını güçleştirmektedir (OIE 2019; FAWC 2014). Akuakültürde yüksek balık stok yoğunlu uygulamalarıyla ve özellike açık su balıkçılığında hastalıkların bulaşma riski artmaktadır. Kısa sürede yüksek ölüm gerçekleşen bulaşıcı hastalıklar su ürünleri yetiştiriciliğinin gelişmini tehdit etmektedir ve kültür balık türlerinin hastalık kontrolü ve sağlık yönetimi endüstride ön öncelikli konulardan birisidir (Rajee ve Alicia 2019).

Akuakültürde görülen balık hastalıklarından korunma ve yapılan tedavilerin başarısı için biyogüvenlik ve biyoteknoloji olanaklarının kullanımı dahil proaktif çalışmalara ihtiyaç vardır (Broom 2007; Mustapha 2014). Hastalıkların önlenmesi ve etkin tedavi için temizlik, dezenfeksiyon ve uygun koşullarda bekletme gibi tedbirler alınmalıdır (Council of Europe 2005). Balıkların ihtiyaçlarını karşılayacak kalitede temiz su ve yemin sürekli temin edilmesi çok önemlidir. Aşılamalar ve diğer sağlık koruma uygulamalarının balık türü, yetiştirme sistemi ve tesislere uygun şekilde planlanması ve yönetilebilmesi için yetiştirici, bakıcı personel ve veteriner hekim arasında uzlaşılmış bir hayvan sağlığı ve refahı koruma planı yürütülmelidir (Council of Europe 2005). Bu plan yazılı olarak bulundurulmalı ve ayrıca balık idaresi, balıkların fiziksel yaralarının izlenmesi, balık ölümlerinin nedenlerinin kaydedilmesi, biyogüvenlik, salgın hastalık ve doğal afetlerle mücadele stratejileri ile personel eğitimi konularını kapsamalıdır (FAWC 2014). Üreticiler ve bakıcı personel balıklarda kötü refah ve hastalık belirtilerini tanıyabilmeli, aşılama ve ilaç uygulama ile bunlara ilişkin kayıtları tutma, balık sağlığı ve refahı sorunlarını izleme ve araştırma konularında eğitilmelidir. Bununla birlikte balık sağlığı ve refahı konusunda deneyimli veteriner hekim sayısının fazla olmaması da sektördeki diğer bir önemli sorundur (FAWC 2014). Ayrıca, hastalık riskini ve veteriner ilaçların kullanımını azaltmak için temizlik, dezenfeksiyon, hasta balık izolasyonu ve karantina işlemlerine özen gösterilmelidir (Council of Europe 2005, OIE 2019).

Sağlık koruma veya tedavi amaçlarıyla ele alınma, çözeltiye batırılma, aşılama ve enjeksiyon gibi uygulamalar balıklar üzerinde strese neden olabilmektedir (McLoughlin ve Graham 2007; Mustapha 2014). Damsgard vd. (2004) anestezik uygulamasının ve aşılamanın Atlantik somonunda (*Salmo salar*) yem alımını ve büyümeyi düşürdüğünü belirlemiştir. Kültür balıkçılığı uygulamaları balıklarda bazı hastalıklar (*vibrio, Lepeophtheirus salmonis, Ceratothoa oestroides*, vs.) ile dolaşım, kas ve iskelet problemlerinin yaygınlaşmasıyla ilişkilendirilmiştir (Poppe vd. 2002; Mordue ve Pike 2002; Cascarano vd. 2021). Yüksek stok yoğunluğu, düşük su kalitesi, balıklar arasında saldırgan sosyal etkileşimler ile yüksek verimler yönünde yapılan seleksiyonların balıklarda fizyolojik sınırların aşılmasına neden olmuş olabileceği ve refah standartlarındaki düşüşlerin balıkları geniş bir hastalık yelpazesine duyarlı hale getirebileceği endişeleri dile getirilmiştir (Hastein 2004; Mustapha 2014). EFSA (2008), kültüre alınan balıklarda hastalıkların genellikle birincil patojenler tarafından değil, yetiştirme çevresiyle ilişkili olduğunu kaydetmiştir. Son dönemde küresel ısınma ve su ortamlarındaki değişiklikler balık sağlığı ve refahını etkileyebilecek potansiyel riskler oluşturabilir (Mustapha 2014; Cascarano vd. 2021).

Seleksiyon ve Biyoteknoloji Uygulamaları

Mikroyosunlar, biyosensörler, DNA aşıları ve genom maniplasyonları gibi biyoteknolojik uygulamalar, su ürünleri yetiştiriciliğinde verimleri ve üretimi arttırmak için kullanılmaktadır (Forabosco vd. 2013; Zhou ve Gui 2018; Rajee ve Alicia 2019). Farklı su ürünleri türleri arasında yapılan hibridasyonlar, genom tabanlı seleksiyon ve gen transferi ile elde edilen ticari melez balık türleri akuakültür koşullarına daha iyi adapte olabilmekte ve daha yüksek üterim potansiyeli sunmaktadırlar (Rajee ve Alicia 2019). Ayrıca balıklarda eksojen steroid hormon veya endokrin bozucu kimyasalların uygulamasıyla cinsiyet dönüşümü yapılmaktadır. Tek cinsiyet veya steril stoklar yemle alınan besinleri üreme faaliyetleri yerine büyümede kullanarak yüksek büyüme hızı ve hasat yeteneği göstermektedir (Forabosco vd. 2013; Rajee ve Alicia 2019).

Faydalarına rağmen, su ürünleri yetiştiriciliğindeki biyoteknoloji uygulamalarının insan sağlığı ve çevre üzerinde çeşitli antropojenik etkiler yapabileceği kaydedilmiştir (Mustapha 2014; Rajee ve Alicia 2019). Genetiği değiştirilmiş bir balık türünün doğal ortama kaçmasının bu balıkların habitattaki yerli türlerle yiyecek, barınak ve çiftleşme için rekabeti arttırabileceği, çevreye daha iyi uyum yeteneği olan bu melez balıkların zamanla yerli türlere üstünlük sağlayabileceği ve yerli türlerle çiftleşerek genetik kirlenmeye ve hatta yerli türlere ait popülasyonun yok olmasına neden olabileceği endişeleri bulunmaktadır (Mustapha 2014, Rajee ve Alicia 2019). Bu nedenle transgenik su ürünlerinin

kullanımının sıkı bir protokol ile denetlenmesi ve DNA aşılarıyla aşılanmış balıklara veya hibrid balık ürünlerine ilişkin tüketicinin bilgilendirilmesinin önemi vurgulanmıştır (Rajee ve Alicia 2019). Balıklarda indüklenmiş üreme için doğal ve sentetik hormonların kullanılması, alıcı ve bağışçı balıklar için birçok refah sorununa neden olabilir. Bir bağışçı balığın hipofiz bezinin çıkarılması için kurban edilmesinin önemli bir refah problemi olduğu açıktır ve ayrıca biyoteknoloji uygulamalarının stres, ağrı ve doku hasarına yol açabileceği bildirilmiştir (Zhou ve Gui 2018). Erken büyüme için yapılan seleksiyonun balıklar arasında saldırganlığın artmasına ve yaralanma, ağrı, rahatsızlık, korku ve sıkıntıya neden olabileceği kaydedilmiştir. Ayrıca balıklarda hızlı büyüme ile anormal kalp şekli, kalp fonksiyon bozukluğu, omurga deformasyonu, katarakt ve düşük yaşama gücü arasında ilişki olduğunu gösteren bulgular artmaktadır (Wall ve Richards 1992; Poppe vd. 2002; Stevenson 2007; Mustapha 2014).

Normal Davranışların Sergilenmesi ve Anormal Davranışlar

Gıda amaçlı yetiştirilen hayvanlarda minimum refah ihtiyaçları "Beş Özgürlük" şeklinde gruplanmıştır (FAWC 2014). Yetişitiricilik koşullarında balıkların da dâhil olduğu çiftlik hayvanlarının refahını sağlamak için asgari standartları belirleyen mevzuat bu refah ihtiyaçlarına dayanmaktadır (EC/98/58 ve Çiftlik Hayvanlarının Refahına İlişkin Genel Hükümler Hakkında Yönetmelik, 22 Kasım 2014, R.G.No:29183). Bu mevzuata göre balıklar ağrı, korku ve kaygı hissetmeden, yem alma ve sosyal etkileşim gibi normal davranışlarını sergileyebilmelidir (OIE 2019). Duyguları da yansıtan davranışlar balıklarda stresi ve refahı değerlendirme ve izleme için en önemli hayvan tabanlı refah göstergeleridir (OIE 2019). Dolayısıyla fizyolojik ve sağlık durumu ile kötü refahı yansıtan davranış modelleri iyi izlenmeli ve kaydedilmelidir (FAWC 2014; Council of Europe 2005). Bu amaçla su ürünleri yetiştirme sistemlerinde balık davranışları, biyolojisi ve refah gereksinimleri konusunda türe özgü bilgilere sahip eğitimli ve deneyimli yeterli sayıda personel bulunmalıdır. Bu personelin sürekli eğitimi sağlanırken, verilen eğitim sertifikalandırılmalıdır (OIE 2019).

Üretim miktarının artışına odaklanılan yoğun üretim sistemlerinde anormal balık davranışlarındaki artış akuakültürde balık refahı kayıplarının arttığını göstermektedir (Adams vd. 2007; Sneddon 2020). Anormal davranış, belirgin bir işlevi olmayan ve amaçsızca tekrarlanan davranışlardır (Dawkins 2004). Balıklarda anormal davranışlar ve yaralanmalara ilişkin etkileşimler Tablo 2'de gösterilmiştir. Su ürünleri yetiştiriciliğinde pek çok balık davranışının pozitif deneyimler (oyun, keşif, vs.) veya stres faktörlerine uyum veya kontrol çabasıyla (kaçma, sığınak arama, vs.) ilişkili olduğu bilinmektedir (Dawkins 2004; Kristiansen vd. 2004; Ashley 2007). Ancak stres faktörünün etkisinin uzun süreli ve şiddetli olması durumunda değişen davranışlar maladaptif bir hal alabilir. Eğer durum daha da kötüleşir ve uyum çabaları başarısız olur ise balıklarda yılgınlık meydana gelebilir (Clement vd. 2005; Madaro vd. 2020). Akuakültür barındırma koşullarının balıklar için yeterince duyuşsal ve bilişsel uyarıları içermediği durumlarda can sıkıntısı ve yılgınlık daha da . artmaktadır (Kleiber vd. 2023). Bu nedenle anormal davranış frekansı balıklarda psikolojik stres faktörlerine bağlı refah kayıplarının ölçülmesi ve değerlendirilmesi için geçerli bir hayvan tabanlı refah göstergesidir (Alfonso vd. 2020).

Stok yoğunluğu arttıkça balıklar için azalan yaşam alanı davranışların daha da kısıtlanmasına neden olurken artan sosyal stres baskısı da duyulan kaygıyı derinleştirmektedir (Ashley 2007; Carbonara vd. 2020). Sosyal üstünlük sıralamasında baskın balıklar tarafından yem alması engellenen pasif balıklarda sindirim fonksiyonlarında ve immun kapasitede kayıplar ve büyüme geriliği görülebilmektedir. Bu durum stok içinde beden iriliği yönünde heterojeniteye neden olur ve gelişme geriliği olan küçük balıklar yetiştirme uygulamaları için bir sorun haline gelebilir (DiBattista vd. 2005; Ashley 2007). Refah kayıplarının önlenebilmesi için taşıma kapasitesinin aşılmaması ve ayrıca işlevsel veya bilişsel uyarıcıları içerecek şekilde su ortamlarının zenginleştirilmesi önerilmektedir (Ashley 2007; Kleiber vd. 2023). Çevresel zenginleştirme refahı arttırarak balıkların davranışsal, fizyolojik ve psikolojik ihtiyaçlarının karşılamasına yardımcı olacak yeni duyusal ve motor uyarıcılar sağlayabilmektedir. Ancak çevresel zenginleştirme stratejileri (gürültü ve müzik gibi işitsel, koku ve tat gibi kimyasal, aydınlatma ve renkler gibi görsel uyarıcılar ile akıntılar ve egzersiz, vs.) her türün ve yaşam evresinin ihtiyaçları, tercihleri ve doğal geçmişi ile ilişkilidir ve balık yetiştirme sisteminin özellikleri ile de etkilenebilmektedir (Arechavala-Lopez vd. 2022; Kleiber vd. 2023). Balıklar için beslenme veya olumsuz olayların tahmin edilebilirliğini sağlamak için otomatik yemliklerden balıkların kendisinin yem alması gibi işlevsel koşullanma ve öğrenme deneyimlerinden de yararlanılabilir (Galhardo ve Oliveira 2009; Kleiber vd. 2023).

3.2. Nakil

Omurgalı hayvanların taşınması onlara zarar veya aşırı acı verme olasılığı olan bir şekilde yapılamaz ve bu hayvanlara balıklar da dâhildir (EC/1/2005). Ancak ilgili yönetmelik nakilleri sırasında korunmaları için balıklara özel detaylı

kuralları içermemektedir (Gimenez-Candela vd. 2020). Bu Avrupa Birliği yönetmeliği ulusal mevzuata aktarılmıştır ancak canlı balıklar kapsam dışı bırakılmıştır (Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliği, R.G. No:28152, 24 Aralık 2011). Akuakültürde nakil işlemlerinin balık refahına etkileri Tablo 2'de özetlenmiştir.

Nakilden önce balıklar yolculuk için yeterince formda ve sağlıklı olup olmadığı yönünde kontrol edilmeli ve tedavi amaçlı olanlar hariç yolculuk için yeterince durumu uygun olmayan veya hasta olan balıklar taşınmamalıdır. Taşıma işlemleri balık türene özgü davranış ve özel ihtiyaçlar dikkate alınarak planlanmalıdır (Council of Europe 2005). Somon ve alabalık gibi büyük balıkların uzak mesafelere taşınması için yapılan uzun yolculuk sırasında balıkların kalabalık şekilde tutulmamasına dikkat edilmelidir. Pompalama hızı ve basıncı ile boru çapı balık büyüklüğüne göre ve taşıma tanklarında ulaşılacak son stok yoğunluğuna göre ayarlanmalıdır (FAWC 2014). Balıkların yüklenip boşaltılacağı tesislerde yürütülen işlemleri denetleyecek personel bulunmalı ve balık yükleme ve boşaltma işlemlerinin minimum stres veya yaralanmaya yol açacak şekilde gerçekleştirilmesi sağlamalıdır (OIE 2019). Bazı durumlarda balıklar kısa mesafeler için kafesleriyle taşınabilir. Daha küçük olan alabalıklar genellikle çiftlikte kesilebilir ancak bazen çiftlik dışındaki kesim tesislerine de taşınabilirler. Balıkların kesim tesislerine kadar taşınacakları süre varılan tesiste balıkların sedasyon ve kesimi için planlanan işlemlerle uyumlu olarak gerçekleştirilmelidir. Bu nedenle öldürme veya kesim noktası ile nakil tekneleri arasında sağlıklı ve uyumlu bir iş birliği sağlanmalıdır. Ağ ile balık taşıma, titreşim, gürültü, su kalitesi, basınç ve sıcaklık değişikliği ile fiziksel hasarlar balıklar için stres yapıcı diğer faktörlerdir. Nakil sürecinde su kalitesi izlenmeli, kaydedilmeli ve oksijen tankları ile gerekli takviyeler yapılmalıdır (FAWC 2014).

Nakilde görevli tüm personel taşımanın balıklar üzerindeki olumsuz etkilerinden sorumludur. Yükleme, balıkların davranışı ve özellikleri konusunda bilgi ve deneyime sahip olan operatörler tarafından yapılmalıdır (OIE 2019). Taşınacak tür için uygun olan ve arızalı olmayan bir araç ile taşıma yapılmalıdır. Balıkları ele alma ve taşıma sırasında stresi en aza indirmek için acil durum planları oluşturulmalıdır. Nakil personeli balık sağlığı ve refahı konusunda eğitilmelidir. Eğitim, her balık türüne özel olmak üzere, balık davranışı ve fizyolojisi, hastalık ve kötü refahın genel belirtileri, yükleme, boşaltma ve yolculuk sırasında canlı balıkların ele alınması ve yönetimi, ilgili ekipmanın kullanımı ve bakımı, taşıma sırasında sıkça karşılaşılan durumların yönetimi, balıkların muayenesi ile su kalitesini izleme, olumsuz hava koşulları ve acil durum planındaki uygulamalara ilişkin detayları içermelidir (OIE 2019). Balıkların taşınması için kullanılan araçlar ve konteynerler, taşınacak balıkların türüne, boyutuna, ağırlığına ve sayısına uygun olmalı ve bu amaçla kullanılan tüm ekipman ve cihazlar balıklarda fiziksel yaralanmaları en aza indirecek şekilde tasarlanmalı ve üretilmelidir (OIE 2019). Çapraz bulaşmaları önlemek için kullanılan ekipmam temizlenmeli ve dezenfekte edilmelidir (Council of Europe 2005).

Balıklarda nakil stresi yolculuğun süresi, su kalitesi ve nakil sırasındaki balık idaresiyle önemli ölçüde etkilenmektedir (Iversen vd. 2005; Ashley 2007; Relic ve Markovic 2021). Ancak bu faktörlerin etkileri veya birbirleri arasındaki etkileşimlere ilişkin araştırmalar yetersizdir. Kurtoğlu vd. (2021) Sibirya mersin (*Acipenser baeri*) balıklarının 15°C su sıcaklığında 50 kg/m^3 stok yoğunluğunda 20 saat boyunca güvenli bir şekilde taşınabileceğini, ancak, 100 kg/m^3 ve üzeri stok yoğunluğunda yapılacak nakillerde 16 saat yolculuk süresinin balık refahı ve sağlığını tehdit edebileceğini belirlemiştir.

Nakil uygulamaları ve sınıflandırma işlemi balıklar üzerinde oluşturduğu yüksek stres nedeniyle hastalıklara duyarlılığı arttırmaktadır. Iversen vd. (2005) Atlantik somonu (*S. salar* L.) yavru balıklarının denize transferinin ardından görülen enfeksiyonların arttığını ve özellikle yükleme sonrası kortizol düzeylerinde artış görüldüğünü bildirmiştir. Chandroo vd. (2005) nakil sırasında ve sonraki 48 saat içinde gökkuşağı alabalıkların (*Oncorhynchus mykiss*) aktivitesinde artış olduğunu, dinlenme sonrası bile balıkların normal aktiviteye yavaş döndüklerini tespit etmiştir. Shabani vd. (2016) ise bu tür balıkların nakil sonrasında dinlendirilmesinin nakil stresini azalttığını belirlemiştir. Akdemir vd. (2022) canlı gökkuşağı alabalığı (*Oncorhynchus mykiss*) taşımasında kullanılan seramik topların sudaki pH değeri ve amonyak seviyesi ile solungaç doku hasarlarının azaltılmasında fayda sağladığını belirlemiştir. Ayrıca başka bir tanka transfer sırasında balıkların ani ışık yoğunluğu değişiminden de strese girdikleri bildirilmiştir (Relic ve Markovic 2021).

Canlı balıklar için nakil işlemi korku, acı ve kaygı eşliğinde yeni bir çevre ile çeşitli temaslar içerdiğinden strese neden olmakta ve balık refahını düşürmektedir. Sedasyon oluşturacak bir anestezik madde uygulanmasının taşınacak balıklarda stresi azaltabildiğini gösteren çalışmalar bulunmaktadır (Neiffer ve Stamper 2009). Lopez-Canovas vd. (2020) de β-CD içinde nanoenkapsüle edilmiş karanfil yağını kesim öncesinde anestezik amaçlı olarak Atlantik somonu, Nil tilapyası, levrek ve gökkuşağı alabalığı türlerinde

Tablo 1. Akuakültürde stok yoğunluğu, su kalitesi ve hastalıkların balık refahına etkileri
Table 1. The impact of stocking density, water quality and diseases on fish welfare in aquaculture

Çevre Faktörleri	İlgili refah problemleri	Balık refahını arttırmak için yaklaşımlar
Yüksek stok yoğunluğu Üretim artışı için yoğun üretim tekniği kullanılırken stok yoğunluğu arttırılmaktadır (Carbonara vd. 2020; Saraiva vd. 2022)	• Rekabette artış (Sara vd. 2010; Calabrese vd. 2017; Saraiva vd. 2022), heterojen büyüme (Ellis vd. 2002), normal davranışların sergilenememesi (Adams vd. 2007) saldırganlık ve anormal davranışlarda artış (Greaves ve Tuene 2001; Ellis vd. 2002) fiziki hasarlarda artış (Galhardo ve Oliveira 2009; Kurtoğlu vd. 2021) • Balık sınıflandırmaya duyulan ihtiyacın artması (Ellis vd. 2002; Dawkins 2004) • Akut ve kronik stres, düşük yemden yararlanma ve büyüme (Ellis vd. 2002, Boujard vd. 2002; Andrew vd. 2002), immun despresyon (Liu vd. 2019), parazit ve hastalıklar (Stevenson 2007; Cascarano vd. 2021), kötü vücut kondisyonu (Turnbull vd. 2005), düşük su kalitesi (Calabrese vd. 2017)	• Maksimum stok yoğunluğu belirlemede balık türünün ihtiyaçlarını dikkate alma (McKenzie vd. 2012; Calabrese vd. 2017; Saraiva vd. 2022), uygun ekipman ve besleme uygulamaları ile heterojen büyümenin engellenmesi (Boujard vd. 2002), sık sık sınıflandırmadan kaçınma (Andrew vd. 2002; Iversen vd. 2005; FAWC 2014) • Çevresel zenginleştirme (Carbonara vd. 2020; Kleiber vd. 2023) • Otomatik yemlikler ve su kalitesi için izleme (Galhardo ve Oliveira 2009; Liu vd. 2019; Alfonso vd. 2020) • Balık boyuna uyumlu parti besleme gibi yenilikçi besleme politikaları (Council of Europe 2005; OIE 2019; Mustapha 2014) • Balıklara zarar verebilecek keskin köşe ve çıkıntıları giderme (Council of Europe 2005)
Düşük su kalitesi Yüksek stok yoğunluğu, hatalı besleme ve balık idaresi nedeniyle su kalitesi bozulabilir (Alfonso vd. 2020)	• Suda oksijen yetersizliği, amanyok ve CO_2 artışı (Kamali vd. 2022) • Suda asılı partikülllerin solungaçlara zarar vermesi ve kirlilik (Ellis vd. 2002) • Hastalıklarda artış ve verimlerde düşüş (Calabrese vd. 2017)	• Su akış hızının arttırılması (Mustapha 2014) • Uygun yemleme tekniği (Hastein vd. 2005; FAWC 2014) ve aşırı yemlemeden kaçınma (Council of Europe 2005) • Stok yoğunluğunun azaltılması (Kamali vd. 2022; Saraiva vd. 2022) • Balık aktivitesinde artışkan kaçınma (Turnbull vd. 2005; Calabrese vd. 2017)
Hastalıklar Paraziter, bakteriyel ve viral bulaşıcı hastalıklar balık refahını düşürmektedir. Ayrıca ürün kalitesi ve halk sağlığı yönünden de riskler meydana gelmektedir (McLoughlin ve Graham 2007; Cascarano vd. 2021)	• Etkinliği kanıtlanmış ticari aşı ve veteriner ilaçlardaki kısıtlar (OIE 2019; FAWC 2014) • Aşı adjuvanların ve veterimer ilaçların yan etkileri (Damsgard vd. 2004; Broom 2007; McLoughlin ve Graham 2007) • Bit ve bakterilerde ilaçlara genetik direnç artışı (Mordue ve Pike 2002; Zhou ve Gui 2018), tedavi ve dezenfeksiyon ile çevre kirliliği, enjeksiyon ve ele alma stresi (Montero vd. 1999; OIE 2019), farklı su sıcaklıkları, immunsupresyon (Montero vd.1999; Liu vd. 2019) • Yüzgeç kesme ile numaralama (Hastein vd. 2005, Mustapha 2014) • Genetik seleksiyon, rekombinat gen teknolojisi ve diğer biyoteknoloji uygulamalarına bağlı sağlık ve davranış problemleri (yüzgeç ve omurga deformiteleri, kardiyovasküler fonksiyon bozukluğu, yüksek ölüm oranları (Forabosco vd. 2013; Stien vd. 2013; Zhou ve Gui 2018; Rajee ve Alicia 2019)	• DNA aşıları gibi alternatif antiviral tedavilerin geliştirilmesi (Broom 2007; FACW 2014) • Hastalık direncine yönelik seçilim (Zhou ve Gui 2018; Liu vd. 2019), parazit direncine yönelik seçici üreme (Stevenson 2007; Zhou ve Gui 2018) • Sürdürülebilir yetiştirme uygulamaları ile düşük stres (Forabosco vd. 2013) • Biyolojik kontrol yöntemleri (FAWC 2014), invazif olmayan yeni tedavilerin geliştirilmesi (Damsgard vd. 2004), hasta balıklar için kafes tasarımları (Gauy vd. 2023) • Bağışıklık sistemini destekleyici takviyeler ve türe özel diyet (Saraiva vd. 2022; Köse vd. 2023), yemlerde mikrobiyolojik ve içerik kontrolleri (Mustapha 2014) • Düşük stok yoğunluğu (Turnbull vd. 2005; Adams vd. 2007), balıklarda rekabetin önlenmesi ve etolojik ihtiyaçların karşılanması (Kamali vd. 2022; Kleiber vd. 2023), bilişsel ve işlevsel zenginleştirme (Ashley 2007; Galhardo ve Oliveira 2009, Kleiber vd. 2023) • Yüksek su kalitesi ve pratik su kalitesi izlenme yöntemleri (Ellis vd. 2002) • İlgili sağlık verilerin düzenli kaydı, ulusal veri tabanına veri girişi (FAWC 2014)

Tablo 2. Akuakültürde anormal balık davranışları ile nakil ve kesimin balık refahına etkileri

Table 2. The effects of abnormal fish behaviors, and handling and slaughtering on fish welfare in aquaculture

Çevre Faktörleri	İlgili refah problemleri	Balık refahını arttırmak için yaklaşımlar
Anormal davranışlar ve yaralanmalar Yüksek stok yoğunluğu, kötü su kalitesi ve idare gibi nedenler saldırganlık gibi anormal davranışlara neden olabilir. Fiziksel çevre ve balıklarla sık temasla yaralanmalar artmaktadır (Anras ve Lagardere 2004)	• Sosyal streste artış (Montero vd. 1999; Cascarano vd. 2021; Saraiva vd. 2022), artan rekabet ve saldırganlık ile küçük balıkların zarar görmesi (Greaves ve Tuene 2001; Clement vd. 2005; Sara vd. 2010), yaralanma ve sekonder enfeksiyonlarda artış (Turnbull vd. 2005; Ellis vd. 2002). • Yüksek aktivite ve O_2 tüketiminde artış (Anras ve Lagardere 2004), su kalitesinde hızlı bozulma (Turnbull vd. 2005; Ashley 2007; Calabrese vd. 2017) • Sosyal hijerarjisinde ve grup içi varyasyonda artış (Greaves ve Tuene 2001; Neiffer ve Stamper 2009) • Stres toleransı, immundepresyon ve büyüme kayıpları (Conte 2004; Stevenson 2007; Mustapha 2014)	• Düşük stok yoğunluğu (Gauy vd. 2023) ve sürdürülebilir su kalitesi (Alfonso vd. 2020), duyuşsal ve bilişsel uyarıları içeren barındırma (Kleiber vd. 2023) ve çevresel zenginleştirme (Arechavala-Lopez vd. 2022; Kleiber vd.2023), rekabeti arttırıcı yemlemeden kaçınma (Council of Europe 2005), aynı yaş, cinsiyet ve tür balıkların birlikte tutulması, heterojenitenin engellenmesi, aktivitenin arttırılmasından kaçınma (FAWC 2014) • Pratik refah değerlendirme yöntemlerinin geliştirilmesi (Hastein vd. 2005; FAWC 2014), personel eğitimi (OIE 2019), Acil Durum ve Afet Planı (Mustapha 2014; FAWC 2014),Anormal aktiviteyi arttırıcı proaktif uygulamalarından kaçınma(Mustapha 2014)
Nakil Nakil nakil öncesi ve sırasında yakalama, yükleme, taşıma, boşaltma ve stoklama sırasında balıklarda stres ve refah kayıpları görülmektedir (Relic ve Markovic 2021; Kurtoğlu vd. 2021; Akdemir vd. 2022)	• Yabancı çevre ve nakil uygulamalına bağlı korku, kaygı ve stres artışı (Wang vd. 2006; Hvas vd. 2020) • Yükleme ve boşaltma sırasında kötü balık idaresi ve hatalı ele alma (Chandroo vd. 2004) • Balık türüne uygun olmayan yakalama, zaptı-rapt ve nakil araç ve ekipmanları (Broom 2007; Shabani vd. 2016), yetersiz su kalitesi ve kalabalık taşıma (Relic ve Markovic 2021;Akdemir vd. 2022) • Nakil sırasında yaralanmaların ve sonrasında hastalıkların artması (Iversen vd. 2005; Shabani vd. 2016), nakil öncesi uzayan toplulaştırma ve uzun yolculuk süresi (Iversen vd. 2005; Ashley 2007; Relic ve Markovic 2021)	• Taşıma kapasitenin aşılması (Kurtoğlu vd.2021), nakil öncesi sedasyon (Neiffer ve Stamper 2009), türe göre ihtiyaçların karşılanması, yeni nesil nakil araç ve ekiman tasarımlarımı (Akdemir vd. 2022;FAWC 2014) • Hayvan dostu balık nakli için standartlar, sertifikasyonlar ve etiketlemenin geliştirilmesi (Lopez-Canovas vd. 2020), nakil sırasında balık türene göre mevzuat ve rehberlerin geliştirlmesi, personel eğitimi (OIE 2019; Gimenez-Candela vd. 2020), hayvan refahı görevlilerinin bulunması (OIE 2019), nakilde balık refahı standartları için etkin izleme, denetleme ve kontroller (Council of Europe 2005; FAWC 2014). Balık hareketlerinin izlenmesi için ulusal veri tabanı (Council of Europe 2005)
Sedasyon, Kesim ve Öldürme Sedasyon ve öldürme yöntemleri balık türlerini farklı etkilemektedir.. Bu uygulamalar balıklarda dramatik refah kayıplarına neden olmaktadır (Sara vd. 2010; Van De Vis vd. 2003; Clemente vd. 2023)	• Canlı kesim ve ölüme kadar bilincin açık olmasına bağlı ağrı ve ızdırap (Van De Vis vd. 2003; Clemente vd. 2023), sedasyon ve bilinç kaybının izlenmemesi, kesime bağlı ağrı (ekipman, uzman, vs) (Relic ve Markovic 2021; Clemente vd. 2023), insancıl olmayan sedasyon ve kesim yöntemleri (hava veya buzlu su içinde boğma, CO_2 ile doyurulmuş su içinde bekletme, bilinç açık iken solungaçların kesilmesi (Kestin vd. 1991; Van De Vis vd. 2003) • Sedasyon ve kesim için hatalı hayvan idaresi, balıkların yere düşmesi, yaralanması veya ezilmesi (Poli 2009; Sara vd. 2010; Relic ve Markovic 2021), deri, yüzgeç, kas ve kemik hasarları (EFSA 2009). • Stresli muameleler ile kas aktivitesinin artması, ölüm sonrası vücuttaki biyokimyasal süreçlerin zarar görmesi ve etin kalitesinde kayıplar (Morzel vd. 2003; Relic vd. 2010)	• Uygun sedasyon (Neiffer ve Stamper 2009), bilinç kaybının ve ölümün izlenmesi (Anras and Lagardere 2004), türe özel insancıl sedasyon ve kesim teknikleri (Rob ve Kestin 2002) ile otomatik sedasyon sistemlerinin geliştirilmesi (Sara vd. 2010) • Kesim operatörlerinin eğitimi (Rob ve Kestin 2002; Mustafa 2014; OIE 2019), Standart İşletme Prosedürlerinin kullanımı (SOP'lar)(Lopez-Canovas vd. 2020; Mercogliano ve Dongo 2023) • Hayvan dostu balık kesimi için standartlar, sertifikasyonlar ve etiketlemenin geliştirilmesi (FAWC 2014; Lopez-Canovas vd. 2020) • Kesim sırasında hayvan refahı görevlilerinin bulunması (OIE 2019) • Kesimde balık refahı standartları için etkin izleme, denetleme ve kontroller (Council of Europe 2005; FAWC 2014)

denediklerinde stres tepkilerinin ve anestezi indükleme süresinin azaldığını ve taze balık raf ömrünün uzadığını gözlemlemişlerdir. Iso-eugenol tabanlı anesteziklerin aynı amaç için Yeni Zelanda, Avustralya, Şili ve Kore'de de kullanıldığı görülmektedir (Iversen vd. 2003). En yaygın sedatif uygulama yöntemi solüsyon içinde bekletmedir ancak anastezik madde kas içi veya intravenöz de uygulanabilir. Genel olarak balıklarda anestezi ve sedasyon uygulamaları diğer omurgalılarda yapıldığı gibidir. Ancak balıklarda analjezi uygulamalarına ilişkin yapılan araştırmalar çok azdır (Neiffer ve Stamper, 2009). Diğer yandan, sedatif kullanımı ile ilişkilendirilen potansiyel riskler bakımından sedasyondaki balıklara dikkatli bir izleme yapılması çok önemlidir (Lopez-Canovas vd. 2020).

3.3. Sedasyon, Kesim ve Öldürme

Balıkların kesim sırasında acı, sıkıntı veya ızdıraptan korunması Avrupa Birliği'nin (EC)1099/2009 sayılı yönetmeliği ile hükme bağlanmıştır. Ancak diğer çiftlik hayvanlarından farklı olarak balıklar için izin verilen kesim ve öldürme yöntemleri belirtilmemiştir. Buna karşın Avrupa Komisyonu Yeşil Anlaşmasının Tarladan Sofraya stratejisi ve hayvan refahı stratejisine göre ilgili mevzuatın gözden geçirilmesi ve güncellenmesine karar verilmiştir (Clemente vd. 2023). Ayrıca sedasyon, acil kesim ve öldürme sırasında balık refahının korunmasına yönelik tavsiye ve yönergeler bulunmaktadır (Council of Europe 2005; FAWC 2014; OIE 2019). Su ürünleri yetiştiriciliğinde sedasyon ve kesim teknikleri çok çeşitlidir ve bu tekniklerin her balık türünde oluşturduğu tepkisel yanıtlar da farklıdır (Morzel vd. 2003). Bu nedenle balıkların kesim ve öldürülmesi amacıyla kullanılan yöntemler sırasında korunması için Dünya Hayvan Sağlığı Örgütü (WOAH, OIE olarak kuruldu) ve Avrupa Gıda Güvenliği Otoritesi (EFSA) tarafından tavsiye ve görüşler yayınlaşmıştır (Gimenez-Candela vd. 2020; Relic ve Markovic 2021).

Su ürünleri yetiştiriciliğinde sedasyon ve öldürmenin balık refahına etkileri Tablo 2 verilmiştir. Kesimden önce metabolik aktiviteyi azaltmak, yükleme ve nakil sırasında oksijen talebini azaltmak için 72 saatte kadar değişen sürelerle yem çekme ve balıkları aç bırakma işlemi uygulanabilmektedir ancak bunun balık refahına etkilerine ilişkin bilgiler oldukça azdır. Yakalamak için yapılan toplulaştırma balıklar arasında ve ağ ile olan teması ve fiziki hasarları arttırılabilir ve bu sürenin en fazla 2 saat olması önerilmektedir. Genellikle hasada kadar gelişemeyecek yavru balıklar için ve hastalık kontrolleri için hasta balıkların öldürülmesi gereken acil durumlarda öldürme işlemi yapılmaktadır (FAWC 2014).

Sedasyon yapılmadan kesim ve öldürme sırasında balıklar güçlü bir şekilde mücadele edebilir. Balıklar büyük kan damarları kesildiğinde tam bilinç kaybına kadar 15 dakika veya daha fazla bilinçli kalabilirler, acı ve ızdırap hissedebilirler (Van De Vis vd. 2003). İyi bir kesim yöntemi balık için daha az acı ve ızdıraba neden olmalıdır (Mustapha 2014). Nakillerde olduğu gibi, kesimden önce sedasyon işlemi balıklarda kesim ve öldürmeye bağlı oluşan stresi ve ızdırabı azaltabilir. Ancak sedasyon etkinliği iyi izlenmelidir. Uygulayıcılar balıklarda operkulumun ritmik hareketi, vestibül-oküler refleksi, yüzme, dik durmaya veya dengeyi yeniden kazanmaya çalışma gibi belirtileri ayırt edebilecek şekilde eğitilmelidir ve bu belirtiler en az 10 dakika süresince görülmediğinde balığın ölümü doğrulanmalıdır. Bu işlemi büyük gruplar halindeki balıklar için saha koşullarında gerçekleştirmek ise çok zordur (Clemente vd. 2023).

Somon ve daha büyük alabalıklar için insancıl sedasyon ve öldürme yöntemi mekanik perfüzyon bayıltmadır. Bu amaçla otomatik perfüzyon bayıltma giderek yaygınlaşmaktadır. Perfüzyon bayıltma ve öldürme için darbe, başın üstüne, gözlerin hemen arkasına uygulanmalıdır (FAWC 2014). Mekanik sedasyon yönteminde serbest bir mermi veya beyine penetre olan bir iğne kullanılarak balık beyninde geri dönüşümsüz bir tahribat sağlanmakta ve balıkta şiddetli bir beyin sarsıntısı ve beyin disfonksiyonu oluşturulmaktadır (Relic ve Markovic 2021). Mekanik sedasyon yöntemleri sazan, alabalık ve ton balığı için önerilmiştir (OIE 2022). Manuel perfüzyon bayıltma işleminin özellikle çok sayıda balıkta uygulandığı durumlarda uygulayıcının işlemin doğruluğunu sürdürebilmesi çok önemlidir. Personel eğitimi de dahil olmak üzere, otomatik perkusif sedasyon cihazları balık refahının artmasına katkı yapabilir. El değmeden, balıkların kanal girişine yönlendirildiği sistemlerde uygun olmayan boyutlardaki balıkların ayıklanması ve manuel olarak bayıltılması çok önemlidir (Ashley 2007). İskandinav alabalıkları için genellikle özel olarak tasarlanmış mekanik bir sedasyon cihazı balığın hızla bilincini kaybetmesi ve ölüm anına kadar öyle kalmasına yetecek güçte tek bir vuruş sağlamaktadır (Van De Vis vd. 2003). Diğer bir sedasyon yöntemi küçük balıklara uygulanan elektrikli sedasyondur. Elektrikli sedasyon balıkların içinde bulunduğu suya elektrik akımı verilerek (elektronarkoz) veya elektriğin doğrudan balığın başına uygulanmasıyla (electrocution) yapılmaktadır (EFSA 2009). Doğrudan başa uygulanan elektrikli sedasyon yöntemi sazan, yılan balığı ve somon balığı için önerilmiştir (EFSA 2009; Relic ve Markovic 2021; OIE 2022; Clemente vd. 2023). Doğru uygulandığında hızlı bir sedasyon sağlayan bu yöntemde düşük voltaj uygulamaları balıklarda felç

durumuna neden olurken yüksek voltaj uygulamaları ise kanama, kas ve kemik hasarları oluşturabilmektedir (Van De Vis vd. 2003; Clemente vd. 2023). Balıkların vücudunun tamamının elektirik akımı verilen suyun içinde olması, sedasyon tankının tümüne homojen bir elektirik akımının gitmesi veya yarı kuru uygulamada elektriğin balığın başının her iki tarafına uygulanması çok önemlidir. Ashley (2007) doğru uygulanan perfüzyon veya elektrikli bayıltma yöntemlerinin Atlantik somonu (*S. salar* L.), kalkan (*S. maximus* L.) ve gökkuşağı alabalığı (*O. mykiss*) gibi balıklarda insancıl kesim gereksinimlerini karşılayabildiğini bildirilmiştir.

Sudan çıkarılarak hava veya buzlu su içinde boğma (asfiksasyon) balıklar için çok streslidir (FAWC 2014) çünkü sudan çıkarılmış ve havaya maruz kalmış balıklar solungaçlarının çökmesi nedeniyle oksijen alamaz (Relic ve Markovic 2021). Balıklar buzlu su içine bırakıldıklarında ise hızlı ve yoğun bir soğuma gerçekleşeceğinden kas felçleri meydana gelmektedir (Roth vd. 2006) ve çok rahatsız edici bu uygulamayla balıklar maksimum stres yanıtlarının eşliğinde şiddetli kaçış davranışları sergilemektedirler (Robb ve Kestin 2002; Ashley 2007). Gökkuşağı alabalığı (*O. mykiss*) kesiminde ise ölüme kadar süren 14 dakikalık sürede stres yanıtlarının devam ettiği bildirilmiştir (Kestin vd. 1991; Van De Vis vd. 2003). Sazan balıklarında özellikle düşük çevre sıcaklığı ve yüksek nem oranı koşullarında aynı yöntemlerin kullanılması durumunda ölüme kadar geçen sürenin daha da uzadığı belirlenmiştir (EFSA 2009; Relic ve Markovic 2021). Somon ve alabalıklarda CO_2 doymuş suya batırılarak yapılan öldürme işleminin sedasyon ve beyin fonksiyonlarında kayba neden olduğu ancak bilinç kaybının birkaç dakika daha sürdüğü ve balıklarda ciddi strese ve et kalitesinde kayıplara neden olduğu görülmüştür (Van De Vis vd. 2003; Clemente vd. 2023). Bu nedenle acil durum öldürmeleri dışında bu yöntemin kullanımı giderek azalmaktadır. Sedasyon yapılmadan solungaçların kesilmesi yöntemiyle yapılan kesim işlemi ölüme kadar bilincin açık olması nedeniyle ızdıraplıdır (Clemente vd. 2023).

Balık türü ve yetiştirme koşullarına göre en uygun sedasyon ve kesim yöntemi seçilmeli ve eğitimli personelin takip edeceği Standart Operasyonel Prosedürler (SOP'lar) kullanılmalıdır (Kestin vd. 1991; Robb ve Kestin vd. 2002; Broom 2007). Tüm sedasyon ve öldürme yöntemleri tek seferde ve hatasız uygulanmalı, tam bilinç kaybı oluşmuş balıklar için kesim veya öldürme işlemi hızla yapılmalıdır. Kesim için keskin bıçaklar kullanılmalıdır (OIE 2022).

Balıkların yakalanması sırasında personel muameleleri veya kullanılan ağ balıklara zarar vermemelidir. Ayrıca balıkların ağ ve bekleme tanklarından düşmesi, balıkların boş konteynerlere konulması veya çok sayıda balığın üst üste konulması gibi hatalı uygulamalar deri abrazyonu, yüzgeç, kas ve kemik hasarları, yüzgeç içindeki gaz basıncında ani değişiklikler ile ezilme ve ölümlere neden olur ve balıklar acı ve ızdırap hissedeler (EFSA 2009). Kesimden önce balıkların iki saatten fazla süreyle sıkıştırılmamasına ve kalabalık şekilde tutulmamasına dikkat edilmelidir. Ayrıca sedasyon ve kesim sırasında ekipmanların kullanımından kaynaklanan ani gürültü bekletme tankındaki balıkları ciddi şekilde rahatsız edebilir (Clemente vd. 2023). İnsan tüketimi için yetiştirilen balıkların kesim ve öldürülmeleri sırasında maruz kaldıkları stresli muameleler kas aktivitesinin artmasına, ölüm sonrası vücuttaki biyokimyasal süreçlerin zarar görmesine ve etin kalitesinde kayıplara neden olur (Relic vd. 2010). Anestezi ile sedasyon altında yapılan kesim et kalitesini de arttırabilir (dinlenmiş hasat) (Van De Vis vd. 2003).

4. Balıklarda Refahın Değerlendirmesi

Balıklarda refahın değerlendirilmesi için kullanılan parametrelere refah göstergeleri denir ve çeşitli refah göstergeleri hem balık refahının değerlendirilmesindeki isabet düzeyi hem de ölçüm ve değerlendirme kolaylığı bakımından farklılık göstermektedir. Hayvan tabanlı refah göstergeleri tek bir balığın davranışı, deri ve yüzgeçlerinin durumu ile sağlık durumu gibi gözlemlere dayanabilir veya bir grup balığın grup davranışı, ölüm oranı veya suda kan ve pulların varlığı gibi grup gözlemlerine dayanabilir (Relic vd. 2010). Grup halindeki balıkların grup eğilimi ve davranışları, grup iştahı ve ölüm oranı gibi grup seviyesinde yapılan gözlemlere dayanan sonuç tabanlı refah göstergelerine grup tabanlı refah göstergeler denir (Poppe vd. 2002). Balık stok yoğunluğunun yüksek olduğu bir yetiştirme sistemi içindeki tüm balıkları sonuç tabanlı göstergelerle değerlendirmek güçtür. Ancak çiftlik seviyesinde balık refahının pratik bir şekilde ve kısa sürede değerlendirilmesi için operasyonel refah göstergeleri tercih edilmelidir. Ayrıca refah değerlendirmesi için bir laboratuvar veya diğer analitik tesislere erişim gerektiren refah göstergelerine ise laboratuvar tabanlı refah göstergeleri denmektedir (Dawkins 2004; Hastein vd. 2005).

Balıklarda refahın değerlendirilmesine ilişkin şema veya protokollerin geliştirilmesinin henüz başında bulunulmaktadır. İlk olarak RSPCA tarafından 2002 yılında Atlantik somonu (*Salmo salar*) için ve 2014 yılında gökkuşağı alabalığı için refah standartları tanımlanmıştır (Noble vd. 2018). Hayvan dostu balık üretimi için balık refahını garanti eden standartlar bireysel girişimciler (Carrefour

sertifikası) veya özel sektör işletmelerinin oluşturduğu ittifaklar tarafından geliştirilebilmektedir (Global G.A.P akuakültür sertifikasyonu). Ayrıca kar amacı bulunmayan hayvan aktivisti kuruluşlar tarafından belirlenen üçüncü taraf standartlar da bulunmaktadır (Dünya Hayvan Koruma ve İnsancıl Kesim Derneği yönergeleri; WWF su ürünleri yetiştiriciliği diyalogları ve standartları; Dolphin Dostu, vs) (Reis vd. 2021). Küresel eko etiketleme alanında diğer bir önemli girişim vahşi av balıkçılığı alanında Marine Stewardship Council (MSC) tarafından gerçekleştirilmiştir (Hønneland 2020). Yine küresel ölçekte sekiz büyük su ürünleri işletmesi tarafından kurulan Deniz Ürünleri İşletmeleri için Okyanus Sorumluluğu (SeaBOS) standartları da bulunmaktadır (Reis vd. 2021). Sadece türe özgü balık refahı standartları geliştiren girişimler de bulunmaktadır. Bunlara örnek olarak Label Rouge, RSPCA Assured, Red Tractor, Soil Association (Reis vd. 2021), Aquaculture Stewardship Council (ASC) ve Best Aquaculture Practices (BAP) sayılabilir. Ayrıca Avrupa Gıda Güvenliği Otoritesi (EFSA) ve Hayvan Sağlığı ve Refahı Bilimsel Paneli (AHAW) balık sağlığı ve refahı konusunda bağımsız bilimsel tavsiyeler yayınlamıştır (Reis vd. 2021).

Atlantik Somonlarında kafes yetiştirme sistemini değerlendirmek üzere "welfare meter" değerlendirme protokolü geliştirilmiştir (Turnbull vd. 2005; Mustapha 2014). Gökkuşağı alabalıkları (*Oncorhynchus mykiss*) için dış morfolojik hasarın değerlendirilmesine dayalı bir balık refahı değerlendirme endeksi (fWEI) akışkan sistemlerde büyüme sırasında kullanılan güvenilir ve uygulanabilir çiftlik göstergelerinin değerlendirilmesini amaçlamaktadır. Buna göre, potansiyel çiftlik refah göstergeleri, çevresel (suyun temini, değişimi ve kalitesi) ve yönetim parametrelerini (stok yoğunluğu, besleme sıklığı), davranış ve sağlık gözlemlerini (hastalık ve kötü refahı gösteren davranışlar, sosyal davranış, aktivite düzeyi) ve dış morfolojik hasarı (deri, yüzgeç, göz, solungaç hasarı, iskelet deformiteleri, zayıflık) içermektedir. Farklı şiddet derecelerinde bozulma içeren fotoğraflı görüntüler kullanılarak değerlendirilen dış morfolojik hasar, hızlı ve maliyeti olmayan bir yöntemdir. Bu indeks (fWEI) balık yetiştiricileri, veteriner hekimler, sertifikasyon ve izleme programları tarafından kullanılabilir (Weirup vd. 2022).Somon refahı endeksi modeli (SWIM 1.0) mevcut en uzun modeldir ve somon çiftliklerinde su sıcaklığı ve tuzluluk düzeyi, oksijen doygunluğu, su akış hızı, stok yoğunluğu ve aydınlatma gibi çevresel parametrelerden başka balıklarda rahatsızlık, ölüm oranı, iştah, deniz biti enfestasyon oranı, kondisyon skoru, zayıflama durumu, omurga deformitesi, olgunlaşma ve smoltifikasyon durumu, yüzgeç durumu ve deri durumu gibi bir dizi refah göstergelerinin kullanılmasına dayanmaktadır (Stien vd. 2013). Balık sağlığı profesyonellerince geliştirilen bir set kullanarak resmi ve standart bir balık refahı değerlendirme yöntemi olarak tasarlanan SWIM 2.0 ise SWIM 1.0'ı tamamlamaktadır. SWIM 2.0' de kullanılan göstergeler gözler, kalp, karın organları, solungaçlar, operküler, iskelet kasların durumu ile aşıyla ilgili patolojiler, balık anormallikleri, ötenazi ve ölü balıkların otopsi bulgularını kapsamaktadır (Stien vd. 2013).

5. Sonuç

Son yıllarda hızla büyüyen su ürünleri yetiştiriciliğinde çok sayıda balık türü farklı sistemlerde yetiştirilmektedir. Balıkların evcilleştirilme sürecinin henüz başında olduğu, akuakültür ve balık arasındaki etkileşimlere dair mevcut bilgilerin de yetersiz olduğu dikkate alındığında diğer çiftlik hayvanlarına göre balıklarda refah ihtiyaçlarının daha fazla olduğu görülmektedir. Balık yetiştiriciliğinde su kalitesi, yüksek stok yoğunluğu ve hastalıkların en önemli refah problemleri olduğu anlaşılmaktadır. Özellikle büyük balıkların kesimi için yapılan nakiller ile sedasyon yapılmaksızın yapılan öldürme ve kesim uygulamalarının balık refahı bakımından önemli diğer riskler olduğu anlaşılmaktadır. Balıkların önlenebilir acı ve ızdıraptan korunmasına ilişkin yönetmelikler bulunmakla birlikte türe özgü ihtiyaçlar temelinde kapsamlı ve detaylı balık refahı mevzuatı veya rehberleri bulunmamaktadır. Benzer şekilde, bazı yeni girişimlere rağmen, balık refahının değerlendirilmesi ve izlenmesi için çiftlik düzeyinde ve hayvan tabanlı parametreleri içeren etkin protokoller de yoktur. Ancak akuakültürde düşük balık refahı hem üretim kapasitesini ve ürün kalitesini olumsuz etkilemekte hem de tüketicilerin konuya ilişkin ilgi ve endişeleri artmaktadır. Sürdürülebilir su ürünleri yetiştiriciliğinin gelişimi için bir sonraki aşama, yüksek balık refahı kalite standartlarının küresel pazarlarda avantaj oluşturabilmesi için hayvan ve çevre dostu etiketlemenin bir standardizasyona doğru evrilmesi olacaktır. Türkiye'nin su ürünleri üretimi alt yapısı ve doğal su kaynaklarıyla sahip olduğu üretim potansiyelini yüksek balık refahı ile destekleyerek küresel rekabet gücünü arttırılabileceği düşünülmektedir.

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