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Review

The Effects of Climate Change on Aquatic Ecosystems in Relation to Human Health

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ABSTRACT

This review paper aimed to summarize the climate change impacts on water sources and their relation with human and ecosystem health and evaluate better management strategies. In aquatic environments, climate change causes alteration of biodiversity and species distribution, changes in the duration of biological functions, decreasing productivities, alteration in food web structures, as well as triggering the invasion of various species, and variation in the presence, abundance, and concentrations of various co-stressors. Since the beginning of the 20th century, the surface water temperature in the oceans has risen by about 1°C. Consequently, human well-being is directly and indirectly affected by these alterations. The World Health Organization (WHO) estimates 3.5 million people die from water-related diseases each year. It is projected that the number of water-related diseases will increase due to the effects of climate change. To cope with these problems, alternative water management strategies should be developed to have resilient water systems in terms of both ecological and technological perspectives. Thus, water management requires the cooperation of many sectors including citizens, institutions, public and private sectors, etc. within a multi-stakeholder approach.

Keywords: Climate change, ecosystem services, public health

INTRODUCTION

"Climate change" defines any alterations in climatic conditions including frequency of extreme weather events (i.e., heat waves, floods, storms), rising atmospheric temperatures and sea levels, melting of ice sheets, etc. caused by both natural events and anthropogenic activities (NASA, 2020). Rising surface temperature is one of the main drivers of climate change and global warming was noted particularly in the late '70s as being attributed mostly to greenhouse gas emissions resulting from industrial and domestic sources (Lipczynska-Kochany, 2018; NASA, 2020; Sun et al., 2020).

Any changes in climate have impacts on the ecosystem, the economy, as well as social welfare including clean air, safe and adequate drinking water, sufficient food, etc. (WHO, 2018a). Climate change is one of the most important drivers for environmental alterations severely affecting biodiversity and ecosystem capacity and functions (Bai et al., 2019). It can result in water temperature rises, acidification, and deoxygenation in aquatic ecosystems. The water temperature rose two-fold more than that in 1993 (Yadav & Gjerde, 2020). The ocean acidity has increased by 30% since preindustrial times and the dissolved oxygen levels have decreased almost 1-2% since the mid-20th century. Furthermore, due to the rising water temperature, the intervals and durations of heatwaves have increased. These changes cause alteration of species and biodiversity, as well as productivities and food web structures in the aquatic environments (Yadav & Gjerde, 2020). The changing environmental conditions will also trigger the invasion of various species and have direct impacts on ecosystem well-being (Figure 1).

Particularly, it is projected that the Mediterranean region will suffer from climate change most which will affect available water quantity and assimilation capacities of the water sources and cause poor water quality (Rocha et al., 2020). Decreasing the water levels may cause poor water quality revealing problems for the ecosystem and public health. The World Health Organisation (WHO) has projected that climate change will cause approximately 250,000 deaths per year in the period 2030-2050 mainly from malnutrition, malaria, diarrhea, and heat stress (WHO, 2018a).

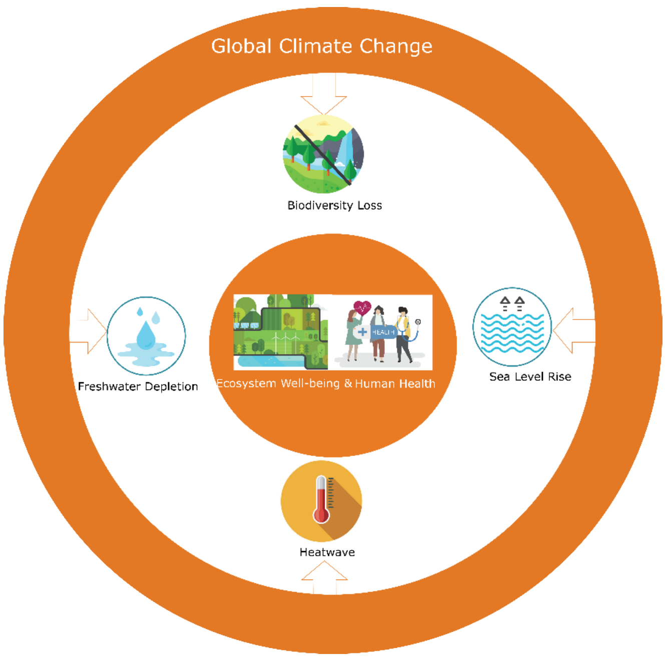

Figure 1. Climate change effects.

Water management requires the cooperation of many sectors including citizens, institutions, public and private sectors, etc. within a multi-stakeholder approach. This paper aimed to address the climate change impacts on water resources and their relation to human and ecosystem health.

The relation between ecosystem services loss and climate change

The ecosystem, which is under threat by the acceleration of the demand for resources and environmental changes, provides many services and products for human well-being directly and/or indirectly. The Millennium Ecosystem Assessment revealed that many of the ecosystem services such as water purification and natural hazard regulation have been under deterioration and the level of disturbance will be in an upward trend in the following years (Bai et al., 2019). Ecosystem functions are directly correlated with biodiversity, and the physicochemical properties of the environment and higher biodiversity are usually attributed to higher rates of ecosystem functions (Heinrichs et al., 2016). There are dynamic interactions between various species and their environments in which specific ecosystem functions are attributed to a specific group of species (Heinrichs et al., 2016). Since global warming is a fact in this century, there is a growing body of research evaluating its effects on biodiversity and its relation to the capacity and function of ecosystems. To understand these interactions, phylogenetic databases should be improved. One of the main drivers for shifts in the functioning of the ecosystem and biodiversity loss is climate change. Any kind of difference in climatic conditions (i.e., temperature, precipitation) results in the transformation of hydrological regimes of freshwater ecosystems (Tsang et al., 2021) and aquatic ecosystems are considered more susceptible to the effects of climate changes compared to terrestrial ecosystems in which the climate change scenarios showed that the biodiversity loss rates are greater in the aquatic environments (Huang et al., 2021). Increasing water temperatures, deoxygenation, and acidification are by far the most significant impacts of climate change. Rising temperature leads to changes in basal metabolic functions, species distribution, and the duration of biological functions (Griffith & Gobler, 2020). Moreover, climate change reveals alterations in the presence, abundance, and concentrations of various co-stressors. The stressors resulting from climate change will also have critical interactions with other factors in water and may have complex synergistic effects on the aquatic ecosystem (Griffith & Gobler, 2020). A greater focus on revealing the link between climate change and the food web can produce critical findings that account for the sustainability of aquatic environments. It affects the whole food web in the ecosystem and the responses vary depending on species, level of the effect, location, etc. Climate change also causes the spreading of new species in uncolonized areas and shifts in their distribution across the world (D'Amen & Azzurro, 2020). The invasion may cause decisive environmental and ecological problems triggering the alterations of ecosystem dynamics and functions, extinction of native and endemic species, reducing populations, etc. (D'Amen & Azzurro, 2020).

The climate change forecasts revealed that the Mediterranean region will be one of the most vulnerable areas to global warming and other extreme environmental conditions caused by climate change. With the other stressors in the area, the effects have already been recognized (Lejeusne et al., 2010). The Mediterranean Sea has the highest number of invasive species worldwide (D'Amen & Azzurro, 2020), and these species mostly originate from the Red Sea (e.g. Lessepsian species) (Lejeusne et al., 2010). The thermo-tolerant exotic organisms have become dominant by the warming in which cold stenothermal local species have rarified, conversely.

Rising temperatures promote the proliferation of harmful algae in excessive amounts in inland water bodies. Examples are provided in Figure 2. It also causes acceleration of both the growth rates and bloom frequency and durations (Gobler et al., 2017). Cyanobacteria blooms and their toxins have emerged as a global concern due to the acceleration of the eutrophication process in freshwater habitats (i.e., lakes and drinking water reservoirs) which then reduce the water quality and finally the use of water (Albay et al., 2005; Cai et al., 2014; Koker et al., 2017). It has been reported that some cyanobacteria species are dominant in the aquatic ecosystems due to their adaptation abilities to various environmental conditions thanks to their functional properties such as phosphorus storage, buoyancy regulation, nitrogen fixation, and akinete formation (resting spores) (Mantzouki et al., 2018). For instance, despite the density barrier of water, potentially toxic species, e.g., *Microcystis aeruginosa* and *Anabaena spiroides*, can migrate 12 m to access light and nutrients (Ganf and Oliver 1982). Thus, the fast migratory ability can provide a competitive advantage to these species over other non-migratory or slow migrating phytoplankton. The temperature was found as one of the main parameters responsible for the continental-scale distribution of different cyanotoxins. Thomas and Litchman (2016) stated that optimum growth temperatures of 12 strains of *Microcystis, Raphidiopsis*, and *Dolichopsermum* are in the range of 27–37°C. It was found that an increase in the water temperature stimulates the dominance of well-adapted toxic strains (Mantzouki et al., 2018).

It is thought that climate change will trigger the increase of toxic cyanobacteria in the coming years and therefore pose a serious threat to human and ecosystem health. Chorus et al.(2021) argued that trophic and climatic changes will affect health risks from cyanobacteria. Cyanotoxins are considered one of the most lethal toxin groups with high risks of health disorders and mortality (Albay et al., 2003). Health risks depend on the quantity and time span by which cyanotoxin concentrations exceed WHO guideline values (1 µg/L Microcystin LR in drinking water) for the respective exposure pathway and time span (Chorus et al., 2021). They also cause taste and odor problems, hypoxia, and fish kills. Furthermore, increasing water temperatures stimulate stratification and thus limit nutrient flows (Griffith & Gobler, 2020).

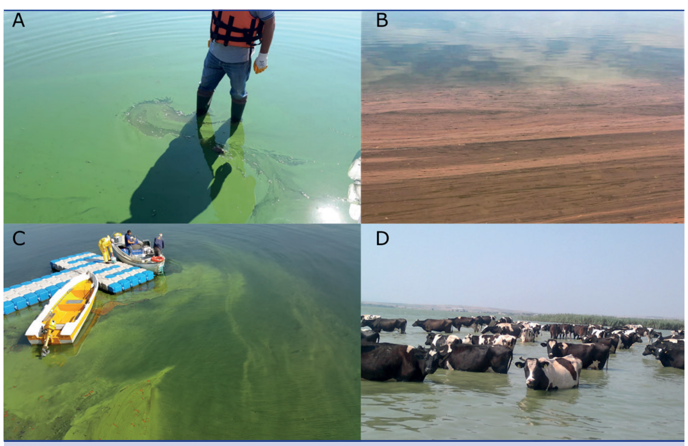

Figure 2. Cyanobacterial bloom in **A)** Kurtboğazi Reservoir (June 2019), **B)** Sapanca Lake (April 2019), **C)** Ömerli Reservoir (October 2015), **D)** Manyas Lake (July 2011).

Fishes are essential for the maintenance of the biogeochemical process and ecosystem functions and climate change causes widespread disturbances to the fish population. They pose different adaptation strategies to combat changing environmental conditions including growth alterations, migration, and mass mortality. Warming of the seawater may also cause an invasion of fish species. An example of invasion within the Mediterranean Sea can be found in the work of Sara et al. (2005) where the authors detected the thermophilic fish species, *Thalassoma pavo*, in the northwest of the Mediterranean Sea. The stresses caused by climate change include rising temperature and the lowering of dissolved oxygen concentrations which mostly result in body size and growth reduction (Huang et al., 2021; Weiskopf et al., 2020). The average body size of fish is projected to fall 14-24% by 2050. Besides, climate change affects the ecological organization in the aquatic ecosystem (Huang et al., 2021). It will detrimentally affect the fish industry in which the quality, quantity, and distribution of the commercial species would decrease (Weiskopf et al., 2020).

Climate changes also affect aquatic plants, especially the invasion of species including formation, distribution, and their impact which then cause an alteration of species interactions, the ecosystem, and human health (Sun et al., 2020). Another group of organisms that also suffer from warming temperatures is corals. The increasing number of heat waves leads to coral bleaching as well as coral cover loss, which also threatens fish communities (Weiskopf et al., 2020; Yadav & Gjerde, 2020). Increasing water temperature also causes bacterial biofilm formation and accelerated decomposition (Lejeusne et al., 2010).

The jellyfish's abundance is dependent on water temperature and salinity. Besides the human-based effects, the higher abundance of jellyfish is attributed to higher salinity levels related to climate change factors. Moreover, some species became abundant due to the higher salinity and temperature conditions (Purcell, 2012). Rising water temperatures have also caused higher asexual production rates in jellyfish (Purcell, 2012).

The relation between public health, water, and climate change

It is now a fact that climate change negatively affects marine and terrestrial biodiversity, and damages ecosystems. As a consequence, human well-being is directly and indirectly affected by these changes (i.e., access to safe water sources, clean air, and sufficient food) (WHO, 2018a).

One of the most important health problems is related to not being able to access safe water sources. The quality and quantity of drinking water are directly and indirectly impacted by climate change, and the use of poor quality water in daily life (i.e., drinking, washing, food preparation, etc.) may cause waterborne diseases (Harper et al., 2020). It is projected that the number of water-related diseases, which are categorized into four groups: water-borne diseases (such as typhoid fever, cholera, etc.), water-based diseases (Schistosomiasis, etc.), water-related vector-borne diseases (malaria, dengue fever, etc.), and water-scarce diseases (scabies, trachoma, etc.), will increase due to the effects of climate change (Hinrichsen, Robey, & Upadhyay, 1997). Diarrheal disease, which is among the water-borne diseases, is a notable public health problem, especially in undeveloped and developing countries. Increasing air and water temperatures, changing rainfall patterns and extreme rainfalls, and seasonal changes affect the transmission of these diseases. Diarrheal diseases peak depending on weather conditions both with droughts and heavy storms (CDC, 2020a). It is estimated that there are 485,000 deaths each year due to diarrheal diseases caused by contaminated drinking water (WHO, 2019). Furthermore, changes in the climatic conditions can result in alteration of the transmission seasons or the geographical area of some infectious diseases (WHO, 2018a). For instance, whereas the water-based disease Schistosomiasis is an endemic disease in some regions of China, it is estimated to expand its impact region and reach 8.1% of China's surface area by 2050 (Zhou et al., 2008).

Malaria and dengue fever, which are among the water-related vector-borne diseases, are highly affected by climatic conditions. Transmitted by Anopheles mosquitoes, malaria kills over 400,000 people every year. These deaths are generally seen in children under 5 years of age in African countries. Studies show that Dengue fever cases will increase as a result of climate change (WHO, 2008). The warming of seawater due to climate change causes the proliferation of some pathogenic bacteria such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*. These bacteria can cause illness through ingestion of raw or undercooked seafood or contact while swimming. On the other hand, the warming in freshwater resources can increase the abundance of *Naegleria fowleri,* which causes primary amebic meningoencephalitis (PAM) disease in humans (CDC, 2020c; USGCRP, 2016).

Temperature increases due to climate change increase drought on a regional and global scale (IPCC, 2014a). It is estimated that by 2030, half of the world's population will be affected by water scarcity and 700 million people will have to migrate for this reason (WHO, 2021a). Water scarcity and drought will adversely affect agricultural, livestock, and fish production, especially in poor regions. It is known that 3.1 million deaths occur each year due to malnutrition and the prevalence of malnutrition will increase due to both decreased food production and poverty in the future (CDC, 2020b; WHO, 2018a, 2021a). On the other hand, studies showed that psychosocial stress and related mental health disorders will be seen in regions affected by water scarcity (WHO, 2021a).

Due to global climate change, glaciers are melting, sea levels are rising, precipitation patterns are changing, and extreme weather events are more frequent and intense. The number of natural disasters caused by weather events has more than tripled since the 1960s (WHO, 2018a). Flood events cause different health consequences. After heavy rains and floods, the contamination of food, drinking water, and recreational water causes water-borne disease outbreaks. Moreover, respiratory system diseases are observed due to mold growth in buildings. Lower respiratory tract infections such as upper respiratory tract symptoms, asthma, pneumonia, and respiratory syncytial virus (RSV) pneumonia occur in those who live in damp residences, schools, and workplaces. Mental health is adversely affected due to the disaster experienced (CDC, 2020a, 2020c, 2020d, 2020e; WHO, 2018a, 2021b). After a flood, vector (such as mosquitoes) breeding areas are formed, and the number of vector-borne diseases increases. Damage to health facilities during floods also negatively affects the provision of healthcare services (WHO, 2018a, 2021b). Additionally, harmful chemicals may be released into the environment during floods, and contact with them may cause toxic effects (CDC, 2018). Considering more than half of the world's population lives 60 km from the sea, rising sea levels will force people to migrate, and this will have various health consequences, not only from infectious diseases but also mental health effects (WHO, 2018a).

Shifting rainfall patterns, floods, and drought will also affect water supply and water management, and sanitation. Sanitation is one of the determinants of health status (WHO, 2018b). Flood, drought, and rising sea levels threaten sanitation systems (e.g., toilets, septic tanks, treatment facilities) (UN, 2020) in which 4.2 billion people today do not have access to safe sanitation. Climate change is expected to worsen health problems related to inadequate sanitation due to the increase in environmental contamination and damage to sanitation systems.

However, within the scope of Sustainable Development Goal No.6, everyone should have sanitation that can withstand climate change by 2030. This forces nations to take precautions and build resilient environments (UN, 2020).

Building Resilient Ecosystems

The influences of climate change vary spatially and temporally due to the variation in susceptibility and adaptive capacity of the affected area (Weiskopf et al., 2020). To determine the level of impact in the short and long term, modeling studies should be conducted to make projections for diversity changes, ecosystem function, and ecosystem services loss, accordingly. With the help of modeling studies, a trend towards ecological responses can be determined. Thus, the necessary precautions and adaptation strategies can be prepared and implemented in the specific region (Morid et al., 2020). It should be valued that a great number of parameters attributed to hydrological and hydraulic characteristics need to be evaluated for these studies. The modeling projects revealed that, besides warming temperatures, heavy storms will occur in some regions and result in poor water qualities due to increasing depositions, particulate materials, and nutrient concentrations. On the other hand, intensification of wind events will directly impact lake thermal stability as well as decrease dissolved oxygen concentrations (Messina et al., 2020).

Resilience can be defined as the ability to overcome and adapt to problems to maintain services for human use considering the natural environment for today and the future (Ofwat, 2015). It is vitally important to maintain diversity, ecosystem functions, and services mainly in relation to sustainability and the constitution of resilience in natural environments (Boltz et al., 2019) as well as human well-being. To make the residential areas resilient to climate change, three main factors namely, temporal variations, spatial heterogeneity, and hydrological connectivity should be sustained. Moreover, the actions should be implemented in the watershed considering stress factors including pollutants, invasive species, etc. (Grantham et al., 2019).

Healthy ecosystems and the continuity of ecosystem services are strongly related to resilience in the watersheds (Dorendahl & Aich, 2021). Resilience contributes to ensuring the maintenance of the functionality of the ecosystem and helps to recover itself in case of any system failures. Building climate resilience includes the cooperation of all stakeholders including governments, private sectors, universities, and civil organizations. It starts with the development of national policies, defining vulnerable locations and problems, involvement of stakeholders, and implementing environment-oriented solutions. Within this concept, ecosystem-based adaptation strategies have a great concern about using nature-based solutions (Dorendahl & Aich, 2021). Reconnection of rivers to the floodplains is one of the green infrastructures that support the re-establishment of its natural course, reducing the risk of erosion and flooding (Brears, 2018). In Singapore, the drainage network was revised by the government and there is a regulation for the private sector which requires the building of detention tanks for stormwater collection to reduce the stress on the public stormwater infrastructures. As another example of green infrastructure, a rooftop park was built in Rotterdam to contribute to flood prevention (Centre for Liveable Cities and Urban Land Institute, 2020). In Tuscon (Arizona, USA), rainwater, which is used for irrigation purposes, is harvested commercially (EPA, 2014).

CONCLUSION

After the 2000s, many countries especially the United Nations and the European Union initiated very detailed research and published reports to minimize the impact of climate change on water resources. Undoubtedly, the measures taken and the studies carried out are quite inadequate. If more effective and sustainable measures are not put into practice, the Mediterranean ecosystem, which is the most affected area by climate change, and the inland water resources of the countries in the Mediterranean belt will experience greater problems.

Since climate change has serious pressures on aquatic ecosystems, alternative solutions should be developed to meet human and ecosystem needs. For this purpose, engineers and ecologists have been working on more efficient, flexible, modular infrastructures and strategies. Thus, green infrastructures come into prominence and are supported by the national governments (Grantham et al., 2019) in which nature serves to have more resilient water systems in terms of both ecological and technological perspectives. An integrated approach including all stakeholders from policymakers to the citizens should be applied for the better management of water resources.

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Research Article

Cyanobacterial Diversity and the Presence of Microcystins in the Küçük Menderes River Basin, Turkiye

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ABSTRACT

Although cyanobacteria are commonly associated with eutrophic lakes, they are the basic components of phytoplankton communities in lakes that have different trophic statuses. In inland waters, both nutrient loading from watersheds and warmer conditions promote phytoplankton growth and cause extensive cyanobacterial blooms. Certain bloom-forming cyanobacterial species can pose a health risk to humans and aquatic ecosystems through cyanotoxin production. The aim of this study was to evaluate the cyanobacterial composition and toxins in five reservoirs and two natural lakes in the Küçük Menderes River Basin, all with varying trophic statuses. Within this scope, samples were collected in autumn 2017 and spring 2018. Cyanobacterial species were enumerated according to the Utermöhl method. Cyanotoxin samples were analyzed using HPLC. To find the trophic status of the water bodies, the Trophic State Index (TSI) developed by Carlson (1977) was used and Total Phosphorus (TP), Secchi Depth (SD), and Chlorophyll-*a* (chl-*a*) measurements were performed. Cyanobacterial abundance, species composition, and cyanotoxin production differed significantly between the lakes and reservoirs. A total of 13 cyanobacteria species were identified including potential cyanotoxin producers such as *Microcystis*, *Aphanizomenon*, and *Dolichospermum*. According to the TSI, three reservoirs were mesotrophic and the other four waterbodies had eutrophic-hypereutrophic conditions. *Microcystis* is the most common bloom-forming freshwater cyanobacteria in the Küçük Menderes River Basin. However, microcystin concentrations were relatively low and the highest microcystin concentration was detected in the Tahtalı Reservoir at 9 µg/L. The Küçük Menderes River Basin is under water-stressed conditions and the cyanobacteria blooms in the region might pose another threat for wildlife and humans.

Keywords: Cyanobacteria, Cyanotoxin, Reservoir, Lake, Algal Blooms, Eutrophication

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INTRODUCTION

Cyanobacteria commonly occur in many regions throughout the world from the polar regions to the tropics (Giani et al. 2020). In addition to favorable environmental conditions such as excessive nitrate/phosphate loading and temperature, urbanization, agriculture and inappropriate water policies also contribute to the occurrence of cyanobacteria. In USA and China, heavy rainfalls and floods, led to the distribution of the hepatotoxin-producer *Microcystis* into several estuaries (Preece et al. 2017).

Cyanobacteria-contaminated water bodies used for recreational activities, irrigation, or drinking water resources may cause health problems for both animals and humans since some of them produce toxic secondary metabolites including hepatotoxins, neurotoxins, and dermatotoxins (Janssen 2019). Among these toxin groups, hepatotoxic microcystin is the most commonly reported and studied toxin globally (Svirčev et al. 2019). Similar to other countries, Turkiye faces the major challenge of maintaining the quality of the available water resources. As a result of changing environmental conditions, the increasing presence of harmful algal blooms is an emerging issue (Akcaalan et al. 2009; Albay et al. 2003; Mariani et al. 2015; Wan et al. 2020). Several species of *Microcystis, Anabaena, Dolichospermum, Cylindrospermopsis*, *Aphanizomenon,* and *Nodularia* are known to dominate blooms in Turkiye (Albay et al. 2005, 2009; Koker et al. 2021; Oğuz et al. 2020). As regards the health risks associated with these groups, the guidelines for cyanobacterial toxins were prepared for drinking and recreational waters (Anonim 2019a; 2019b).

The Küçük Menderes River Basin is located in the western part of Turkiye and covers an area of about 3.490 km^2. It has the lowest total precipitation area in Turkiye (Ministry of Agriculture and Forestry 2019). There are three natural lakes (Belevi, Çatal, and Gebekirse) in the basin. Additionally, a number of reservoirs were built for drinking water supply and irrigation to support the increase in the population of the metropolitan city, İzmir (Sac et al., 2020).

This study aims to assess the levels of eutrophication in seven freshwater bodies, to identify potentially toxic cyanobacterial genera, and to evaluate the occurrence of microcystins in the Küçük Menderes River Basin.

MATERIAL AND METHODS

Study sites

Surface water samples were collected from five reservoirs and two natural lakes in the Küçük Menderes River Basin in November 2017 and May 2018 (Fig. 1). Sampling stations were chosen to represent water bodies relative to the size of the surface area. Two sampling stations were selected for water bodies with a surface area up to 3 km^2 and three sampling stations for those larger than 3 km^2 (Tahtalı and Beydağ reservoirs). Some brief information about the studied water bodies is given in Table 1.

There are nine dams in operation in the Küçük Menderes River Basin and the largest of them is the Tahtalı Reservoir (İzmir). This reservoir supplies approximately 40% of İzmir's drinking water (İspirli, 2009). The Alaçatı Reservoir, which is located on Hırsız Stream, is a drinking water supply with a volume of 16.61 hm^3 (Tosun, 2018). Although the Beydağ Reservoir was built for irrigation purposes, with the increase in urbanization in the region, it is also used as a domestic water source with a water consumption capacity of 108.9 hm^3 (Sac et al., 2021). Also, there are several dams and ponds such as the Kavakdere and Seferihisar Reservoirs on the Küçük Menderes river that were built for general irrigation and flood control purposes .

Çatal Lake and Gebekirse Lake, which are the closest lakes to the Aegean Sea, are brackish waters. Gebekirse Lake is under protection due to its ornithological and vegetation values (Somay et al., 2008). Çatal Lake is a small shallow lake, situated to the west of Gebekirse Lake.

Physical and chemical analysis

Water temperature (WT), pH, Dissolved Oxygen (DO), and Electrical Conductivity (EC) were measured *on-site* with a portable multiparameter (6600, YSI, USA). Water transparency was measured using Secchi Disc (SD). Nitrite+Nitrate (NO_2-N + NO_3-N) and Total Phosphorus (TP) were measured spectrophotometrically following APHA-AWWA WPCF (1989). Chlorophyll-*a* (chl-*a*) was performed using the ethanol extraction method (ISO 10260, 1992).

To determine the trophic state of the water bodies, the trophic state index (TSI) developed by Carlson (1977) was used and total phosphorus (TP), chlorophyll-*a* (chl-*a*), and Secchi depth (SD) measurements were used to calculate the index. The TSI $_{(SD)}$

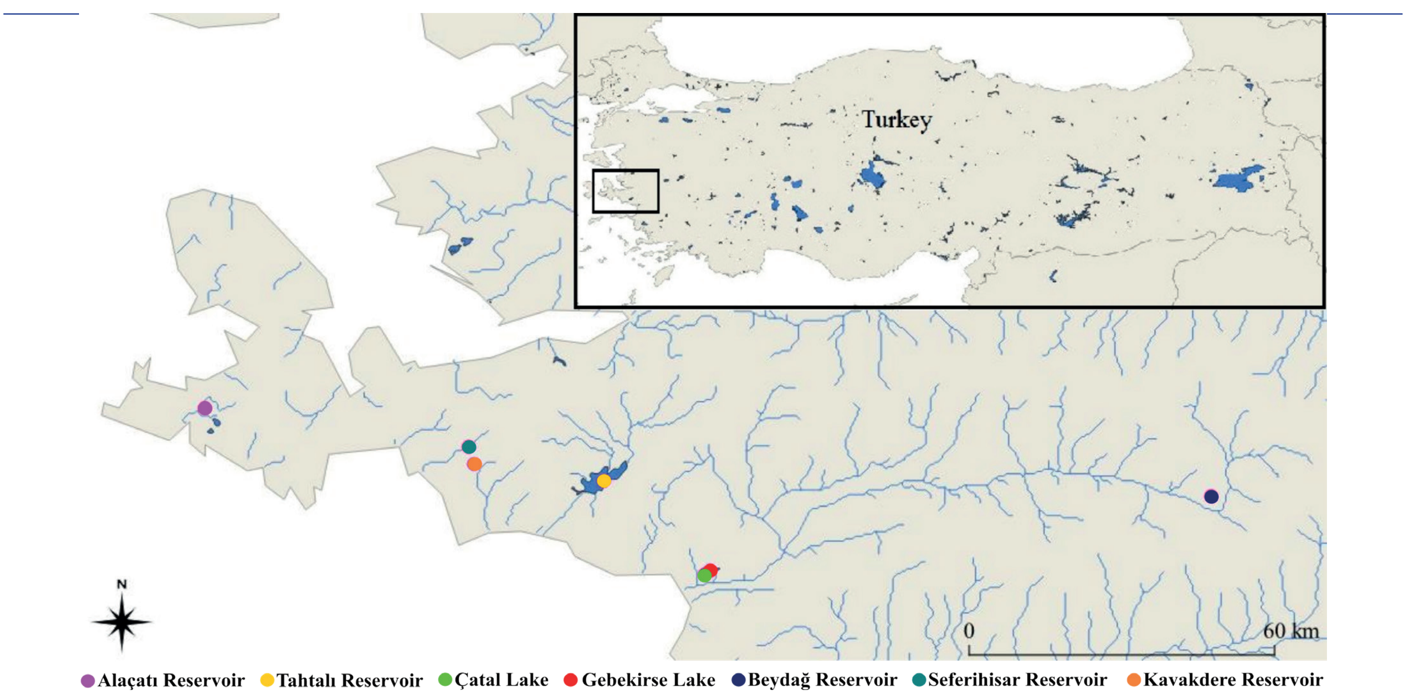

Figure 1. Sampling Sites in Küçük Menderes River Basin.

Table 1. Location and characteristics of the studied reservoirs and lakes in the Küçük Menderes River Basin.

	Coordinates	Surface Area (km²)	Maximum depth (m)	Purpose of use
Alaçatı Reservoir	38°17′20.12″N 26°24′47.29″E	2.6	4.5	Drinking supply
Beydağ Reservoir	38°7′ 2.74″ N 28° 13′ 10.4″E	12	22	Irrigation
Tahtalı Reservoir	38° 8′ 33.91″N 27° 6′ 42.81″E	23	55	Drinking supply
Seferihisar Reservoir	38°13′0.62″N 26°52′16.06″E	1.8	24	Irrigation
Kavakdere Reservoir	38°10′50.47″N 26°54′19.98″E	0.96	8	Irrigation
Çatal Lake	37° 59′ 25.38″N 27° 19′ 1.57″ E	0.74	4.5	Recreational
Gebekirse Lake	37° 59′ 12.96″N 27° 18′ 16.31″E	0.75	5	Recreational

values of the Alaçatı Reservoir, and Çatal Lake and Gebekirse Lake were not used to calculate the trophic status since they were shallow (~3-5m depth) and mixing throughout the year (Zou et al., 2020).

Microscopy analysis
Samples for the determination of phytoplankton composition were fixed by Lugol's Iodine solution. Phytoplankton was enumerated using a Zeiss Axiovert (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) inverted microscope according to Utermöhl (1958). Identifications of the sampled taxa were based on relevant literature (John et al. 2002; John 2005; Komarek and Anagnostidis 1989; 2007; 2008; Krammer & Lange-Bertalot, 1986). The phytoplankton biovolume was calculated according to the geometric equations of Hillebrand et al. (1999).

Microcystin analysis
The microcystin concentrations were measured using high-performance liquid chromatography (HPLC) with a photodiode array (PDA) detector (Perkin Elmer, USA) according to Lawton (1994). Elution mode was used: injection volume 25 µL, flow rate 1 mL min$^{-1,}$ and column temperature 40°C. The mobile phases were Milli-Q water and acetonitrile both containing 0.1% (v/v) TFA. All the reagents were of high-performance liquid chromatographical (HPLC) grade. The eluent absorbance was monitored from 200 to 300 nm and microcystins were detected at 238 nm.

RESULTS & DISCUSSION

The trophic status of a waterbody is generally a good indicator for the possibility of cyanobacteria blooms. The trophic statuses of the water bodies are given in Table 2. The Tahtalı, Seferihisar, and Kavakdere reservoirs were classified as mesotrophic. Aksu et al. (2015) reported that the Tahtalı Reservoir was found oligotrophic according to TSI $_{(chl-a)}$ between 2006 and 2007. The calculated TSI $_{(chl-a)}$ and TSI $_{(TP)}$ values measured in the waterbodies indicated both nutrient- and phytoplankton-rich conditions (Fernandez et al., 2021). Based on TSI $_{(chl-a)}$, only the Seferihisar Reservoir showed no sign of eutrophication.

In the Küçük Menderes River Basin, three of the seven lakes and reservoirs were classified as eutrophic, three of the reservoirs were mesotrophic and Çatal Lake was hypereutrophic (Figure 2).

Figure 2. Cyanobacterial blooms in the reservoirs.

Table 2. Trophic Status of studied water bodies in the Küçük Menderes River Basin.

	Alaçatı Reservoir	Beydağ Reservoir	Tahtalı Reservoir	Seferihisar Reservoir	Kavakdere Reservoir	Çatal Lake	Gebekirse Lake
TSI$_{(TP)}$	65.2	59.8	56.8	45,8	41.4	79.9	54.3
TSI$_{(chl-a)}$	49.5	63.5	41.8	39.2	43.5	69.0	57.8
TSI$_{(SD)}$	-	53.9	43.7	40.4	51.8	-	-
TSI	57.4	59.1	47.4	41.8	45.6	74.5	56.1

Oligotrophy Mesotrophy Eutrophy Hypereutrophy

Table 3. Environmental variables and chl-*a* values of studied water bodies.

		Alaçatı Reservoir		Beydağ Reservoir		Tahtalı Reservoir		Seferihisar Reservoir		Kavakdere Reservoir		Çatal Lake		Gebekirse Lake	
		Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018	Autumn 2017	Spring 2018
WT	**°C**	15.5	24.6	15.3	24.2	16.5	24.2	15.9	24.3	15.2	18.5	15.8	26.8	16.7	25.3
TP	**µg/L**	31.7	69.0	30.4	59.6	20.2	38.9	14.9	22.6	14.2	25.6	103.9	268.6	22.6	32.4
TN	**µg/L**	1037	787	1620	934	882	699	752	282	675	284	8475	3284	430	1110
TN:TP		32.7	11.4	53.3	15.7	43.7	18.0	50.6	12.5	47.7	11.1	81.6	12.2	19.1	34.3
SDD	**m**	0.43	0.15	0.87	2.21	2.73	3.23	0.9	2.7	3.4	4.5	1.00	0.50	1.23	0.83
Chl-*a*	**µg/L**	13.5	10.7	94.0	28	16.7	2.9	1.4	2.8	3.4	3.7	176.4	22.5	55.5	15.8

WT: Water Temperature; TP: Total Phosphorus; TN: Total Nitrogen; Secchi Disk Depth: SDD; Chl-α: Chlorophyll-α

TN:TP ranged from 11.1 to 81.6. Based on these ratios, in spring, all water bodies were potentially co-limited with a range of 10<TN:TP<17, except for Gebekirse Lake (34.3) and the Tahtalı Reservoir (18.0). In autumn, the ratios were significantly high and found to be potentially P-limited (TN:TP > 17) with the range of 19.1-81.6 (Wan et al., 2020) in all water bodies. The maximum TN:TP ratio was recorded during autumn in Çatal Lake (81.6) where the chl-*a* concentration was the highest, which reflects a significantly higher nutrient level (Table 3).

The phytoplankton compositions were generally dominated by Cyanobacteria, Bacillariophyta, and Chlorophyta (Fig. 3). In both sampling periods, phytoplankton composition in the Seferihisar Reservoir consisted of Ochrophyta, Cryptophyta, and Bacillariophyta, covering ~95% of the phytoplankton community. Similarly, in the Kavakdere Reservoir ~85% of the phytoplankton community was comprised of Bacillariophyta, Charophyta, Ochrophyta, and Miozoa. Cyanobacteria was not detected in both reservoirs. In other water bodies, the detected cyanobacterial genera were *Anabaenopsis, Aphanizomenon, Aphanocapsa, Chrococcus, Dolichospermum, Limnothrix, Merismopedia, Microcystis, Planktolyngbya, Pseudanabaena,* and *Snowella*. Data showed that *Microcystis aeruginosa* was the dominant cyanobacteria species in the studied water bodies in the Küçük Menderes River Basin. Sixteen cyanobacterial species were detected and 10 of them were potentially microcystin-producing species. As a general phytoplankton seasonal succession pattern in freshwater ecosystems, Bacillariophyta dominated in spring and cyanobacteria were relatively high in autumn in the studied basin. The presence of cyanobacteria was generally strongly affected by physicochemical variables such as temperature and nutrient availability (Yang et al., 2019, Huisman et al., 2018). The dominance of cyanobacteria was generally found in eutrophic water bodies in the Küçük Menderes River Basin. Xu et. al (2019) demonstrated that under conditions of medium nutrient availability (30 < TSI < 50), as in the Tahtalı Reservoir (TSI=49.3), cyanobacteria dominated. On the other hand, with the highest TN and TP concentrations, Chlorophyta dominated the phytoplankton community (Wei et al., 2020) and in Çatal Lake, which had the highest nutrient concentrations, Chlorophyta was the dominant group.

Generally, the concentration of microcystin was influenced by both water temperature and the abundance of cyanobacteria (Walls et al., 2018). In autumn, the water temperature varied between 15.3 to 16.7 °C, while in spring it measured between 24.2-26.8 °C (Fig. 4). Cyanobacterial blooms have been reported in temperate zones in summer and autumn, but with global warming, they have also been reported in spring and winter (Ma et al., 2016). Especially in the Tahtalı and Beydağı reservoirs, cyanobacterial biomass was higher in spring (Fig. 4). Apart from the Tahtalı Reservoir and Çatal Lake, microcystin concentrations were generally low, ranging from undetectable to 1.4 µg/L throughout the studied period. The highest microcystin value was measured at 9.1 µg/L in the autumn when the water level reached the lowest level in the Tahtalı Reservoir.

Even when the cyanobacterial population is high, it is important to evaluate the abundance of potentially toxin-producing species. In the Beydağ Reservoir, 61.5% of the phytoplankton community consisted of cyanobacteria which were dominated by *D. mendotae* (81.7%) in spring and *M. wesenbergii* (99.1%) in autumn, however, microcystin was not detected (Fig. 4). *Microcystis* blooms in freshwater ecosystems generally resulted in microcystin production, on the contrary, *M. wesenbergii* blooms generally have no microcystin production or low toxin production (Honma and Park, 2005). Via-Ordorika et al. (2004) could not detect microcystin-producing genes in *M. wesenbergii* colonies (n=21) in their study conducted in 13 water bodies in 9 European countries. Co-existing toxic and non-toxic *Microcystis* genotypes are common around the world and the proportion of microcystin production was affected by diverse factors such as nutrient concentrations, level of eutrophication, and also differences in morphotypes (Via-Ordorika et al. 2004).

In the Tahtalı Reservoir, cyanobacteria constituted 67.6% and 40.3% of the phytoplankton community in autumn and spring, respectively, and *M. aeruginosa* was the only dominant cyanobacteria in the reservoir. In autumn, microcystin concentration was the highest with 9.1 µg/L and in spring it was as low as 0.13 µg/L. This could be due to the seasonal dynamics of potentially toxic genotypes (Joung et al. 2011). *Microcystis*

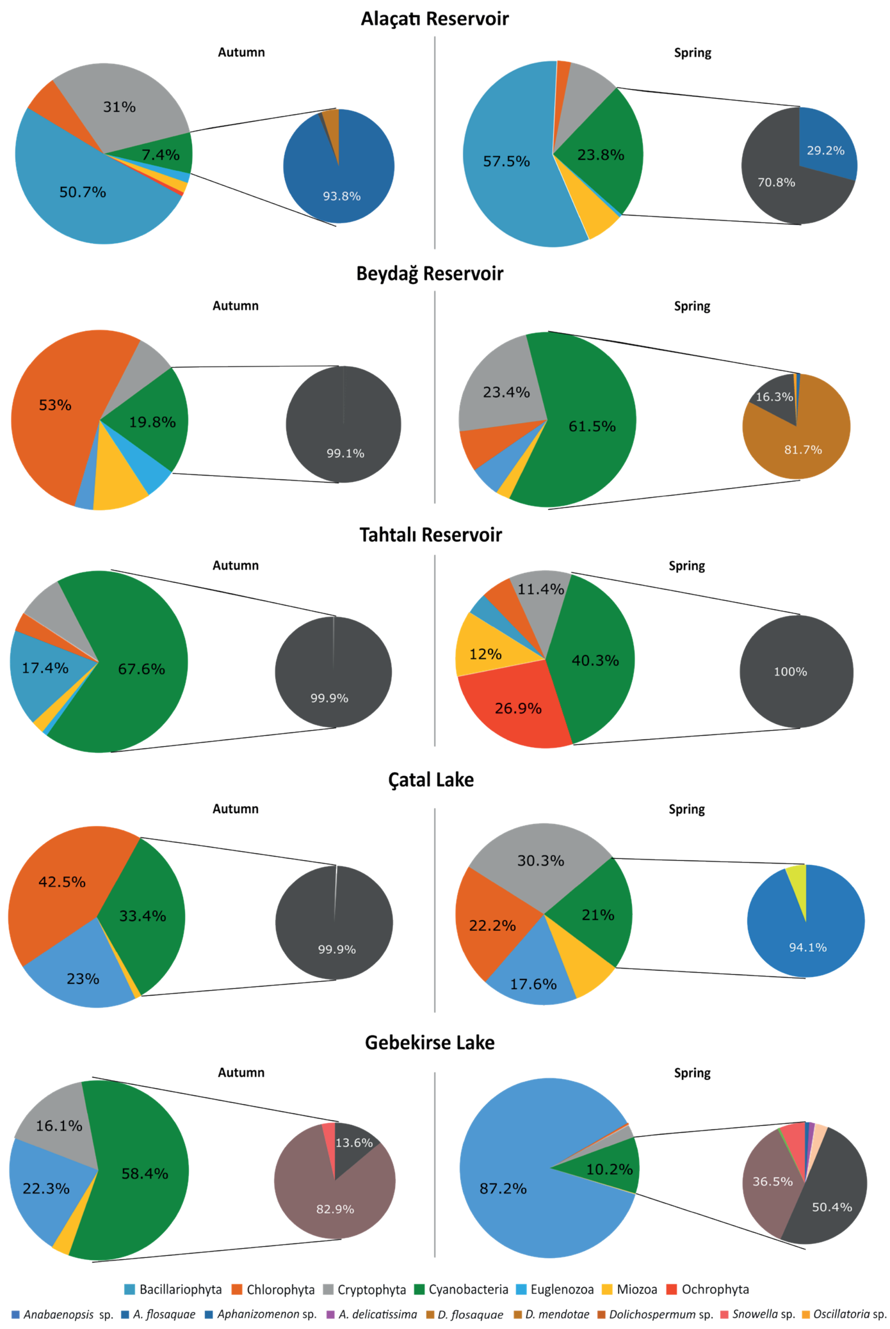

Figure 3. Biomass of phytoplankton community and cyanobacteria species in the studied water bodies.

species are dominant during the summer season with favorable water temperatures which could last through autumn (Carrasco et al., 2006; Koker et al., 2017). However, in 2006-2007, Ispirli (2009) did not detect microcystin above 1 μg/L in the Tahtalı Reservoir.

In the Alaçatı Reservoir, the phytoplankton communities were dominated by Bacillariophyta (more than 50%) in both sampling periods (Fig.2). The cyanobacteria biomass consisted of *A. flosaquae* and *Microcystis* sp. In spring, *Microcystis* sp. comprised 70.8% of the total cyanobacteria biomass which in turn composed 23.8% of the total phytoplankton biomass. *A. flosaquae* decreased and the abundance of *Microcystis* sp. increased in spring. Together, they contributed >93.8% of cyanobacteria biomass and formed a bloom. Microcystin production was detected 0.14 μg/L in autumn and 0.1 μg/L in spring. Generally, in the Alaçatı Reservoir, the microcystin production was low and co-occurrence with *Microcystis* sp. in both sampling periods may have been the reason for microcystin production (Lyon-Colbert et al. 2018).

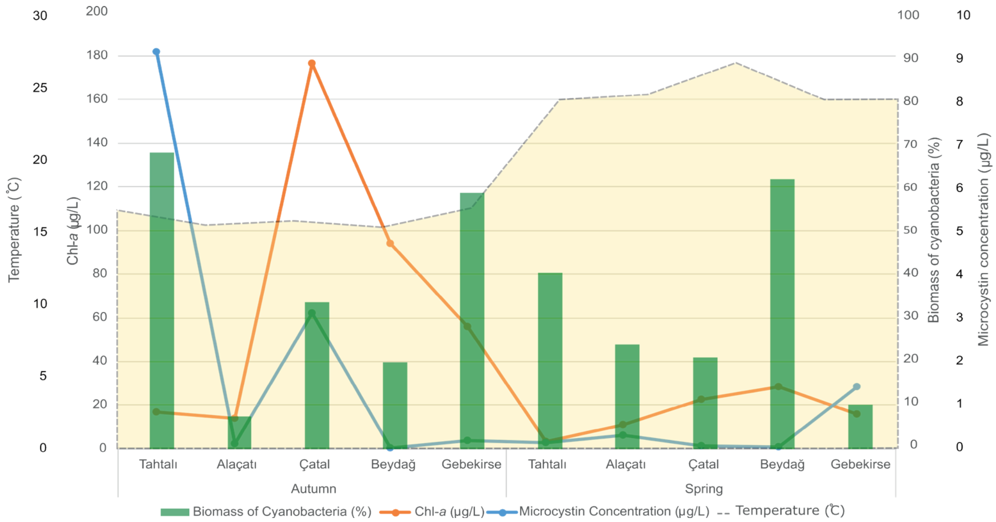

Figure 4. The relationship between water temperature, cyanobacterial biomass, chl-*a*, and microcystin concentrations in the water bodies.

The second highest microcystin concentration was detected in Çatal Lake (3 μg/L) in autumn. The potential microcystin-producing taxa *M. aeruginosa* was the dominant cyanobacterial species, accounting for more than 90% of cyanobacterial biomass in autumn.

In Gebekirse Lake, while the cyanobacteria biomass was 10.2% in spring with 50.4% of *M. aeruginosa*, the microcystin concentration was 1.4 μg/L. In autumn, the cyanobacteria biomass was 58.4% of the total phytoplankton community and 82.9% of the cyanobacteria biomass was dominated by *Planktolyngbya* sp. and 13.5% was *Microcystis aeruginosa*. Although microcystin production is directly related to the presence of cyanobacteria, the presence and abundance of potential microcystin-producing species have crucial importance (Sinang et al., 2013).

In many sites in the Mediterranean basin, cyanobacterial blooms start to occur in the spring-summer periods and last through autumn depending on water temperature (Mariani et al. 2015). Carrasco et al. (2006) demonstrated that from September to November, potentially toxic cyanobacteria were present in seven reservoirs in Madrid which is in line with our results. Furthermore, *Microcystis* spp. was dominant in Mediterranean countries including Greece, Spain, Portugal, France, and Turkiye (Cook et al., 2004; Koker et al., 2017). Although previous studies focused on the temperature effects on cyanobacterial abundances, multiple environmental factors, synergistic interaction between increased nutrients and temperature also promotes cyanobacterial dominance and persistence in water bodies (Carrasco et al., 2006; Gkelis, et al. 2015).

CONCLUSION

This study revealed that potentially toxin-producing cyanobacteria species were dominant in the Küçük Menderes River Basin, although the microcystin production was not high. Most of the harmful cyanobacteria blooms were dominated by the *Microcystis aeruginosa, M. wesenbergii, Anabaenopsis* sp., and other cyanobacterial taxa (*Dolichospermum* and *Aphanizomenon*). However, further data are needed for detection of other cyanotoxins such as cylindrospermopsin, saxitoxin, or anatoxins. Increased input of nutrients and global warming could have stimulated cyanobacterial blooms and cyanotoxin production in the Küçük Menderes River Basin.

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Ethics committee approval: Ethics committee approval is not required.

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Research Article

Evaluation of different scale-up strategies for *Haematococcus pluvialis* cultivation in airlift photobioreactors

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ABSTRACT

Large scale algal biomass production can be very challenging due to the potential issues of sustainability, environmental ethics, and economic concerns. A strategic approach to the transition from the laboratory to the industrial scale allows the prediction of process characteristics, design and analysis of large scale systems, and reduction of extra costs. In this study, a scale-up procedure that considered different approaches was carried out by selecting the *Haematococcus pluvialis* as a model organism. Three scale-up parameters (constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La)) were tested for biomass production in a 2-L airlift photobioreactor and they were compared with those obtained from a 1-L aerated cultivation bottle. Among three strategies, the maximum cell concentration, $4.60\pm0.20\times10^5$ cells/mL, was obtained in a constant volumetric power consumption rate experiment. Also, total carotenoid amount showed similar changes with the cell concentration and reached the maximum concentration of 2.02±0.11 mg/L under constant P/V experiment. However, the cultivation bottle presented the highest biomass amount of 0.62 g/L and specific growth rate of 0.38 day^{-1} of all of the photobioreactors. This result might be attributed to the low aeration rates or improper configuration of the system, which created a non-homogenous culture medium and led to ineffective mass transfer.

Keywords: *Haematococcus pluvialis*, Scale-up, Airlift photobioreactor, Biomass production, Carotenoid

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INTRODUCTION

The mass cultivation of microalgae has recently aroused interest because of several advantages, including the rich content of algal biomass, potential beneficial effects on the environment and their great capacity to create renewable energy. Large scale cultivation systems for microalgae production are highly preferable due to the increased growth rate, higher biomass productivity, and requirement of small areas. In addition, it has great potential in a clean and renewable environment by capturing atmospheric CO_2 and treating wastewater with the recovery of nutrients and pollutants (Bendetti et al., 2018). Industrial microalgae cultivation can be done in open ponds and in controlled, closed systems, which are called photobioreactors (PBR). The major advantages of open ponds include low cost, high production capacity, and ease of building and operating. Despite these advances, there are some drawbacks, such as the risk of contamination, low mass and heat transfer efficiency, ineffective mixing, water loss, and poor process control (Wang et al., 2013). Therefore, numerous closed PBRs, such as flat plate, airlift, bubble column, membrane, etc., have been designed and used instead of open ponds. An airlift PBR is a common closed cultivation system that has a cylindrical transparent bubble column and a draft tube located through the center of the PBR (Aslanbay Guler et al., 2020). This draft tube creates two regions, the downcomer and riser, and gas is sparged through the riser by moving the liquid in the riser zone upwards. The movement of the liquid with the driving force of gas bubbles creates a recirculation between the riser and downcomer regions and provides an effective flow pattern. In this way, airlift PBR has the characteristic advantage of creating a circular mixing pattern and thus, good heat and mass transfer (Ding et al., 2021). Furthermore, airlift PBRs provide proper operating conditions for many microalgae species by causing less shear stress than other types of PBRs (Azhand et al., 2020).

Haematococcus pluvialis is a freshwater green microalga commonly characterized by its high astaxanthin content (up to 5% of dry weight) (Deniz, 2020). Astaxanthin is a red-colored pigment that has strong antioxidant properties owing to its molecular structure. Its biosynthesis from *H. pluvialis* involves two major steps, which are green biomass production and red astaxanthin induction under specific stress conditions (Ranjbar et al., 2008). In order to reach high metabolite content, the concentration of green cells should be maximized during the first step of cultivation by optimizing conditions and performing large scale production. Astaxanthin production from *H. pluvialis* is mainly carried out in open ponds because the applied stress conditions reduce the probability of contamination. However, the first green stage is highly susceptible to the different contaminants and thus, PBRs are better choices for the mass production of *H. pluvialis* due to the ease of process control (Deniz, 2020; Wang et al., 2013). The applications of different types of PBRs for *H. pluvialis* production can be enhanced, integrating the proper scale-up strategy for the transition from lab-scale to commercial systems.

Effective scale-up is an essential and complex procedure for the successful mass cultivation of microalgae. It is highly important to choose the most suitable operation conditions depending on the cultivation system, microalga species, optimal growth conditions, and economic feasibility. According to these points, several scale-up procedures, including volumetric power consumption rate, impeller tip speed, light energy, stirrer rate, specific oxygen transfer rate, etc., are carried out during the transition from lab-scale to the industrial level. The aim of the scale-up process is enhancement of biomass and target-product yield, avoiding high cost and time consumption in the industrial scale (Aslanbay Guler et al., 2019). A number of studies have been conducted to improve the PBR production of *H. pluvialis* by integrating scale-up strategies, but more experiments are needed to understand and design an effective, large-scale cultivation system.

In this paper, the biomass production of microalgae *Haematococcus pluvialis* was studied in a 2-L airlift PBR by conducting different scale-up strategies for the transition from lab scale to the pilot scale. In this context, the main objective of this study was to investigate the use of constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La) as scale-up methodologies under laboratory conditions for the scale-up process from 1-L aerated cultivation bottle to the 2-L airlift PBR, considering whether an increase in the cell concentration and total carotenoid amount can be achieved. According to the literature, this is the first report that compares three different scale-up strategies for biomass production from *H. pluvialis* microalgae in an airlift PBR and investigates suitable conditions for higher biomass productivity than obtained in an aerated cultivation bottle.

MATERIAL AND METHODS

Microalgae and inoculum preparation

H. pluvialis (EGE MACC-32) was provided from the Ege-MACC from University of Ege, Izmir, Turkey. Stock culture was maintained in BG11 medium (Rippka et al., 1979) under light intensity of 65 μE/m^2 s at 24 ± 2 °C in a 2-L aerated sterile bottle for 15 days. At the end of the 15th day, the cells from the stock culture were harvested and inoculated to a 250 mL Erlenmeyer flask containing BG11 medium to use as inoculum in experiments. The cells were incubated under the continuous illumination of 65 μE/m^{2}s in an orbital shaker at 120 rpm at 26 ± 2 °C for five days and this culture was used as inoculum for all experiments.

Biomass production in cultivation bottle

H. pluvialis cells were cultivated in a 1-L aerated sterile bottle (8.2 cm internal diameter and 12.5 cm height) for 8 days. The bottle was continuously illuminated with the white LED downlight lamps (10 W CT-5254) and light intensity was adjusted to 65 μE/m^{2}s. Cultivation was maintained at 26 ± 2 °C in a temperature-controlled cabinet and sterile air was fed into the system at the aeration rate of 3 vvm.

Biomass production in airlift PBRs

A 2-L internal loop airlift PBR was used for the scale-up productions, with the following specifications: 1.6-L working volume, 6.4 cm diameter, 55.0 cm height, and a ratio of the cross-sectional area of the downcomer zone to the riser zone (A_d/A_r) of 5.4. More detailed design parameters and hydrodynamic properties were reported in a previous study (Aslanbay Guler et al., 2020). The PBR was constructed with transparent glass with an illuminated surface area of 0.088 m^2. Mixing and aeration were achieved by bubbling sterile air through a sparger with 6 nozzles located in the base of the column. The PBR was illuminated with a fluorescent daylight lamp along the vessel from one side, applying a light intensity of 70 μE/m^{2}s. Prior to inoculation, the system was sterilized at 121 °C and 1 atm for 15 min using an autoclave. Then *H. pluvialis* culture was inoculated into the PBR and it was operated at batch mode for 8 days at 26 ± 2 °C.

Scale-up procedures

The transition from aerated bottle to the 2-L airlift PBR (Figure 1) was carried out with three different scale-up strategies individually by changing the aeration rate. These strategies were constant mixing time (t_m), volumetric power consumption rate (P/V), and oxygen mass transfer coefficient (k_La). The mixing time was experimentally determined using the pH-response technique proposed by Van't Riet and Tramper (1991). The oxygen mass transfer coefficient was measured using the unsteady state method (Shuler & Kargi, 2002). The P/V value was calculated using the following equation (1) (Chisti & Jauregui-Haza, 2002),

$$\frac{P_G}{V_L} = \frac{\rho_L g U_{Gr}}{1+{}^{A_d}/_{A_r}} \quad (1)$$

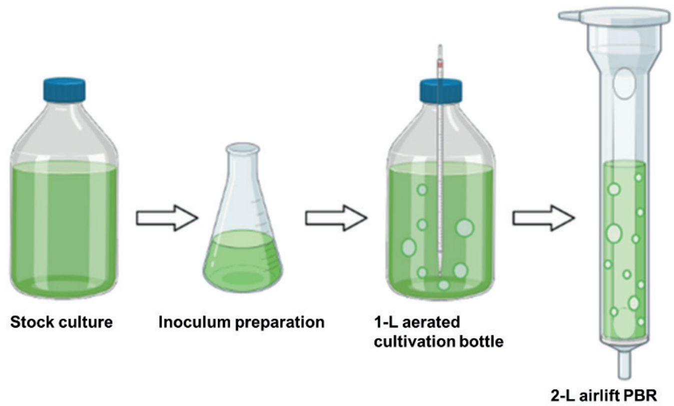

Figure 1. Scale-up process for *Haematococcus pluvialis* cultivation.

where P_G is the power input due to aeration (W), V_L is the culture volume (m^3), g is the gravitational acceleration (9.81 m/s^2), U_{Gr} is the superficial gas velocity (m/s), A_d is the cross-sectional area of the downcomer region (m^2), and A_r is the cross-sectional area of the riser region (m^2). In the aerated cultivation bottle, t_m, k_La, and P/V values were found to be 18 s, 0.01 s^{-1}, and 60 W/m^3, respectively. According to these experiments and calculations, PBR production was carried out only by varying the aeration rate while keeping the production parameters constant and to reach the calculated values in the cultivation bottle. Consequently, the aeration rates of 0.9, 1.24, and 1.8 L/min were used for the constant t_m (18 s), constant P/V (60 w/m^3), and constant k_La (0.01 s^{-1}) strategies in 2-L PBRs, respectively.

Analytical measurements and calculations

Cell growth was determined by measuring cell concentration and dry weight. Cell concentration was measured by counting samples in a Neubauer hemocytometer using an optical microscope. The dry weight content was determined by taking 5 mL aliquot and filtering it through pre-weighed GF/C filter. Then it was dried at 60°C for 12 h and allowed to cool in a desiccator before being re-weighed. The total content of carotenoids in the microalgal biomass was determined using the spectrophotometric method. Briefly, 5 ml of cells were harvested at via centrifugation (6000 rpm – 5.0 min) and extraction was carried out with 4:1 (v/v) dimethyl sulfoxide (DMSO): water at 55 °C for 1 h in the dark. The amount of total carotenoid (mg/L) was determined by measuring the light absorption at wavelengths of 480, 649, and 665 nm and calculated using the following equations (Wellburn, 1994):

$$Chlorophyll - a = 12.47\, A_{665} - 3.62\, A_{649} \quad (2)$$

$$Chlorophyll - b = 25.06\, A_{649} - 6.5\, A_{665} \quad (3)$$

$$Total\ carotenoid = (1000\, A_{480} - 1.29\, Chl\_a - 53.78\, Chl\_b)/220 \quad (4)$$

The specific growth rate (μ) (day^{-1}) of the microalgae was calculated using the equation (5);

$$\mu = \frac{lnX_2 - lnX_1}{t_2 - t_1} \quad (5)$$

where X_1 and X_2 (cells/ml) are the cell number at time 1 (t_1) (day) and time 2 (t_2) (day), respectively (Bailey & Ollis 1986). Furthermore, doubling time (DT) (day) was calculated as

$$DT = \frac{ln2}{\mu} \quad (6)$$

The biomass productivity was calculated (g/L/day) using Equation (7)

$$Biomass\ productivity = \frac{N_f - N_i}{\Delta t} \quad (7)$$

where N_i and N_f (g/L) are the initial and final biomass concentrations, respectively, and Δt (day) is the time of cultivation (Zhu et al., 2016).

All the experimental analyses were repeated at least two times and are presented in the figures and tables with the average values.

RESULTS AND DISCUSSION

One of the most critical aspects concerning mass cultivation of microalgae is the possibility of transition from lab-scale to the industrial level, and the first step in this process is performing an efficient scale-up strategy to enhance cultivation yield over that obtained at the lab-scale in terms of product and biomass yield. In this study, three different scale-up strategies, including mixing time (s), volumetric power consumption rate (W/m^3), and oxygen mass transfer coefficient (s^{-1}) were used for the biomass production from *H. pluvialis* cells in an airlift PBR. In order to provide high amount of green biomass, cultivation was transferred from 1-L cultivation bottle to the 2-L airlift PBR by taking into account geometric similarity.

Once the cells were cultivated in the aerated cultivation bottle, cell concentration significantly increased from the 4th day of cultivation and reached the highest amount (7.90 ± 0.15 × 10^5 cells/mL) at the end of production (Figure 2a). Among the PBR cultivations, the maximum cell concentration, 4.60 ± 0.20 × 10^5 cells/mL, was achieved in the constant P/V experiment. The cell concentration of 2.10 ± 0.20 × 10^5 cells/mL in constant t_m experiment was much lower than other strategies, and this result may be an effect of the lowest aeration rate of 0.9 L/min. This result was also sup-

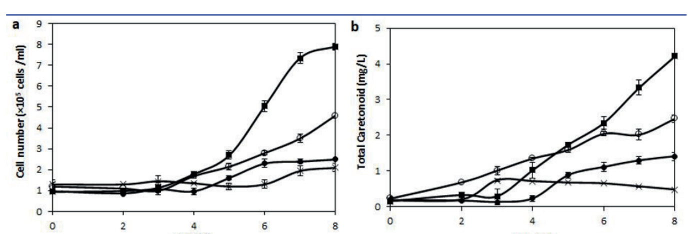

Figure 2. Growth profile and total amount of carotenoids for *H. pluvialis* cells in aerated cultivation bottle (■) and 2-L airlift PBR for constant volumetric power consumption rate (o), constant oxygen mass transfer coefficient (●) and constant mixing time (×), a) cell number; b) total carotenoid amount.

ported by the observation that *H. pluvialis* cells formed aggregates and collapsed at the bottom of the airlift PBR during cultivation in the constant t_m experiment because of insufficient aeration. According to the results obtained, the moderate aeration rate of 1.24 L/min in the constant P/V experiment led to higher cell concentration than was obtained in the other two scale-up strategies. This was due to the fact that a homogenous culture medium could not be provided because of low aeration in the constant t_m experiment, and during the constant k_La experiment, cells might have been exposed to shear stress and lost their flagella through bubble burst at the liquid-gas interface because of the higher aeration rate of 1.8 L/min. A similar result was observed for *Haematococcus alpinus*, an alpine strain of *Haematococcus*, where the moderate aeration rates did not affect cell growth negatively but further increases in airflow slowed cell growth and caused cell enlargement (Mazumdar et al., 2019). In addition, the transformation of vegetative green cells into red cysts was observed towards the end of culture in most PBR productions (results not shown). This may be associated with the fact that some cells became inactive and no further cell growth was achieved.

Total carotenoid amount showed similar changes with the cell concentration, as expected (Figure 2b). In the cultivation bottle, the total carotenoid amount obtained was 4.21 ± 0.11 mg/L at the 8th day of production. However, all of the PBR cultivations resulted in a decrease in total carotenoid amount and concentrations were found to be between the ranges of 0.5 - 2.5 mg/L. Among the PBR productions, maximum carotenoid concentration of 2.47 ± 0.10 mg/L was obtained in constant P/V criterion. It was an expected result because the chlorophyll and carotenoid contents of *H. pluvialis* cells show parallel changes with the growth profile during the green phase. Also, it is important to note that carotenoid concentration is strongly related to the lighting efficiency of cultivations due to the light harvesting role of the pigments during photosynthesis (Shah et al., 2016). Although airlift PBRs have a high illuminated surface area to volume ratio and thus more efficient lighting, cells may be exposed to light heterogeneously due to ineffective mixing. Homogenous cell distribution can be achieved by the increase of gas flow rate, but excessive hydrodynamic forces by aeration may cause shear stress to cells (Choi et al., 2018). In order to provide effective mixing, enhance the mass transfer, and prevent mechanical stress, air flow rate should be adjusted carefully together with light intensity considering biomass yield and accumulation of target product.

Table 1 shows the calculated kinetic parameters of cultivation bottle and airlift PBRs operating with three different scale-up strategies for green *H. pluvialis* cells. During the bottle experiment, cells showed significant growth, with the dry mass value of 0.62 g/L within eight days and the specific growth rate reaching 0.38 day^{-1}, which corresponds to a culture doubling time of 1.79 days. Among three scale-up strategies, maximum biomass amount (0.49 g/L) and maximum growth rate (0.31 day^{-1} (doubling time of 2.27 days)) were found in the constant P/V criterion. In a similar study, the effect of the airflow rate on the growth of *H. pluvialis* cells in an internal-loop airlift PBR was investigated and

Table 1. Kinetic parameters for the cultivation of *Haematococcus pluvialis* cells.

Cultivation	Aeration rate (L/min)	Dry weight (g/L)	Biomass productivity (g/Lday)	Specific growth rate (day^{-1})	Doubling time (day)
Cultivation bottle	3	0.62	0.077	0.38	1.79
t_m	0.9	0.17	0.021	0.07	9.35
P/V	1.24	0.49	0.061	0.31	2.27
k_La	1.8	0.35	0.044	0.17	3.99

four different aeration rates, including 0.25, 0.5, 0.75, and 1.1 vvm, were used. Cell growth decreased when aeration was increased above a certain value and maximum specific growth rate of 0.23 day^{-1} was obtained at 0.5 vvm airflow rate (Vega-Estrada et al., 2005). In another study, *H. pluvialis* cells were cultivated in an airlift PBR supplemented with CO_2 and gave a maximum specific growth rate of 0.317 day^{-1} (Haque et al., 2017), which is comparable to that reported in the present study. Biomass productivity values obtained were in parallel with the dry mass amount and the minimum productivity of cells was recorded in the constant t_m strategy as 0.021 g/L day. Overall, obtained kinetic values were in parallel with cell growth profile and carotenoid accumulation where constant P/V strategy was the most effective cultivation than other scale-up procedures.

CONCLUSION

Mass cultivation of microalga in controlled PBR provides remarkable advantages compared with open ponds, considering process control, contamination risk, and operation conditions. From the engineering and biological points of view, a systematic scale-up procedure is essential to selecting the most suitable conditions depending on the cultivation system, microalga species, optimal growth conditions, and economic feasibility during transition to the industrial scale. In the present study, scaling up from an aerated cultivation bottle to the airlift PBR was evaluated for the biomass production of *H. pluvialis* using three different scale-up strategies: constant t_m, constant P/V, and constant k_La. According to the findings obtained, constant P/V strategy provided the most efficient production for biomass production and total carotenoid accumulation due to enhanced mixing, mass and heat transfer, and dispersion of light. However, enlarging the system from 1-L to 2-L caused a decline in performance in terms of biomass and carotenoid productivity. The decrease in growth rate and carotenoid amount in PBR cultivation might be related to the insufficient aeration rate, high shear stress due to bubble coalescence, or improper reactor configuration in terms of the column and draft tube length, which led to uneven densities of fluid flow. These data were also supported by the observations of *H. pluvialis* cells forming a conglomeration and collapsing at the bottom of the PBR, and the transformation of vegetative green cells into red cysts towards the end of culture. The results obtained in this study will be considered in order to design optimal PBR configuration and determine more suitable operation conditions for the large-scale production of *H. pluvialis* biomass. In this context, draft tube structure, illuminated surface area to volume ratio, and A_d/A_r value may be modified and different scale-up strategies may be applied to find the proper aeration rate in order to achieve more effective cultivation.

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Research Article

Population Dynamics, Current Trends and Future Prospects of the Black Goby (*Gobius niger*) in the Eastern Part of the Black Sea (Turkiye)

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ABSTRACT

With its adaptation to the Black Sea, the black goby *Gobius niger* (Linnaeus, 1758) has increased in biological and ecological importance in recent years. Despite previous studies, up-to-date information on population status in the Black Sea is still lacking. Specimens were collected monthly from April 2020 to March 2021 from different commercial fishing landings on the Turkish coast of the eastern Black Sea. The total length of sampled individuals (n=630) ranged from 7.20 to 14.0 cm. Males were dominant throughout all size classes and the overall sex ratio was significantly different from the expected ratio of 1:1. The length-weight relationship indicated isometric growth (b=3) for both sexes. Spawning occurred from March to June. The observed maximum age was 4 and both males and females were dominant in age group 3. The black goby appeared to have relatively low growth rates (Φ'=1.74) in the Black Sea, but longer asymptotic length ($L\infty$=16.94 cm) data were obtained in the study area. The total mortality rate (Z) estimated by means of the catch curve method was 1.43 yr^{-1} and the fishing mortality (F) was 0.88 yr^{-1}. The estimated exploitation rate (E) was 0.61 yr^{-1} which was higher than the optimum value of 0.5. Updated biological parameter estimates show that black goby populations in the Black Sea are now more exploited than previously thought. Additional studies are recommended to ensure sustainable management of black goby populations and national regulations to reduce bycatch.

Keywords: Sex ratio, age, growth, mortality, fisheries management, black goby

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INTRODUCTION

The Gobiidae (Perciformes) family comprises at least 2000 species (Nelson, 2006) and is well known for its successful adaptation to different environmental conditions. A few gobiid species are also fully adapted to marine and freshwater ecosystems (Freyhof, 2011). The goby species are distributed in various seas carried by ballast water (Skora & Storlarski, 1993). There are 74 known species of goby in the Black Sea and the Mediterranean (Engin & Bektaş, 2010; Sezgin, Bat, Ürkmez, Arıcı, & Öztürk, 2017; Aydın & Bodur, 2018; Aydın, 2021a; Karadurmuş & Aydın, 2021). The black goby *Gobius niger* (Linnaeus, 1758), a gobid species, has recently reached the Black Sea and has adapted to this area in a short time. It is distributed commonly in the Eastern Atlantic and the Mediterranean Sea and is common in the Black Sea, Baltic Sea, and the Red Sea (Miller, 1986; Whitehead, Bauchot, Hureau, Nielsen, & Tortonese, 1986). It prefers muddy and sandy bottoms up to 80 m in depth (Miller, 1986; Kara & Quignard, 2019) and frequently enters estuaries and lagoons (Whitehead et al., 1986). Rocky substrates, sea grasses, and mussel shells serve as spawning habitats and refuges to hide from predators (Malavasi et al., 2005). It mainly feeds on crustaceans, bivalves, gastropods, polychaetes, and small fishes (Miller, 1986; Filiz & Toğulga, 2009; Bengil & Aydın, 2020).

It is suitable as an indicator for monitoring marine pollution (Ramsak et al., 2007), and also occupies an essential position in the food chain (Rigal et al., 2008). It is classified as a discard in the coastal fisheries of the Black Sea. Recently, gobies have become a more abundant species in fishing operations, and they have started to be sold commercially in fish markets (Aydın, 2021b). There is no fishing regulation due to their non-commercial value in Turkiye. Because of its biological and ecological importance, this species has attracted the attention of many scientists over the years. Several studies on the distribution, genetics, ecology, behavior, and biology of this species have been carried out in different regions (Vaas, Vlasbom, & De Koeijer, 1975; Nash, 1984; Joyeux, Bouchereau, & Tomasini, 1991; Silva & Gordo, 1997; Immler, Mazzoldi, & Rasotto, 2004; Hajji, Ouannes-Ghorbel, Ghorbel, & Jarboui, 2013; Locatello, Mazzoldi, & Rasotto, 2021; Matern, Herrmann, & Temming, 2021). Limited studies focused on the population status (sex ratio, age, growth, mortalities) and biological aspects (reproduction, feeding) of the black goby on the coast of Turkiye (Özaydın, Taşkavak, & Akalın, 2007; Kınacıgil et al., 2008; Filiz & Toğulga, 2009; İlkyaz, Metin, & Kınacıgil, 2011; Kırdar & İşmen, 2018).

The status of black goby populations in the Black Sea was examined by different researchers between 2008 and 2013. Unlike this study, Kasapoğlu (2016) studied a lower number of samples, a study carried out by Bilgin & Onay (2020) included only the province of Rize, and the age determination was made according to the computer-based length frequency distribution analysis. Van & Gümüş (2021) studied in the same region as the current study and examined more samples, but more than ten years have passed since their study. Up-to-date information about the latest status of these populations is not available. The present paper aims to assess the current status of the black goby population in the Black Sea (Turkish coasts), by determining growth and mortality rates based on age readings from the otolith. Past and present data were evaluated in terms of fisheries management and inferences were made about the future status of the populations. In addition, the sex ratio, length-weight parameters, spawning season, and condition of the population were also evaluated separately by sex.

MATERIAL AND METHODS

The sampling studies were conducted along the Turkish coast of the eastern Black Sea (from 41°21′N−36°14′E to 40°55′N−38°31′E) (Figure 1) between April 2020 and March 2021. The black goby was caught by commercial fisheries using artisanal gillnets with different mesh sizes, at depths ranging from the shoreline to 50 m. After sampling, the samples were stored and transferred to the laboratory in a cooler for further analysis. For each sample, the total length (TL) was measured to the nearest 0.01 cm and the total weight (TW) was weighed to the nearest 0.01 g. The samples were sexed based on the macroscopic observation of the gonads – which are tubular with capillaries and a yellow-orange color in females, and soft-textured, flat, and white-cream in color in males (Guellard, Sokołowska, & Arciszewski, 2015).

The datasets of the total number of specimens by sex were used to calculate the sex ratio per month. In order to determine

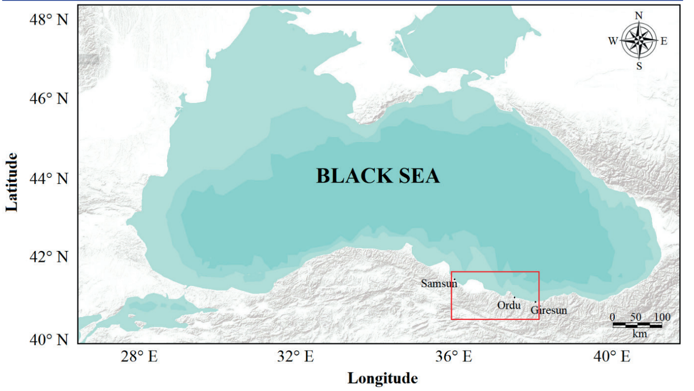

Figure 1. Location of sampling area in the eastern part of the Black Sea, Turkiye. The red line shows the limit of the study area.

variations in the sex ratio, data were categorized into 1 cm TL size classes. Statistically, deviations from a theoretical sex ratio of 1:1 were determined by the Chi-square test (χ^2) (Zar, 1996).

The length-weight relationship (LWR) was estimated using the expression, $W=aL^b$ (Le Cren, 1951) where L is the total length (cm), W is the total weight (g), a is the intercept and b is the slope of regression. The regression coefficients (a and b) were computed using a least-square linear regression on log-transformed data (Froese, 2006). The growth type of the population was evaluated using the t-test to analyze whether the b slope was significantly different from the theoretical value of 3 (Sokal & Rohlf, 1969).

The spawning season was determined from the monthly variations of the gonadosomatic index (GSI) of the specimens and was calculated with the following formula GSI=GW×100/TW (Bagenal, 1978) where GW is the gonad weight (g) and TW is the total weight of specimen (g). Gonads were weighed to 0.001 g precision. Fulton's condition factor (Kn) was calculated with the formula $Kn=W/L^3\times100$ (Fulton, 1904), where L is the total length (cm) and W is the total weight (g).

Sagittal otoliths were removed, cleaned, and stored for further processing and readings (Chugunova, 1963). Otoliths were placed in alcohol and investigated under a stereo binocular microscope by 30× magnification and reflected light connected to a computer. The association of one opaque zone and one translucent zone was regarded as an annulus (Silva & Gordo, 1997; Florin et al., 2018). Only compatible otoliths were included in the reading's analysis. Age readings were performed by two independent readers. The error in the counting of the annuli between readers was evaluated by the coefficient of variation and percentage of agreement (Campana, 2001). Moreover, the index of average percent error was calculated according to Beamish & Fournier (1981). The length-at-age datasets were used to estimate von Bertalanffy (VBGF) parameters by sexes:

$$L_t = L_\infty\,(1 - e^{-K(t-t_0)})$$

where L_t is length at age t (yr), L_∞ is asymptotic length (cm), K is the intrinsic growth rate (yr^{-1}), and t_0=hypothetical age at which the length of the fish is zero (von Bertalanffy, 1938). Phi-prime growth index (Φ′) was calculated using the formula $\Phi' = \log K + 2\log L_\infty$ (Munro & Pauly, 1983).

The total mortality (Z, yr^{-1}) was estimated using a length-based converted catch curve analysis (Chapman & Robson, 1960). Natural mortality (M, yr^{-1}) was estimated using VBGF parameters and annual mean seawater temperature (16.4 °C; quoted from the Turkish State Meteorological Service) based on the equation of Pauly (1980). The fishing mortality (F, yr^{-1}) was obtained from the difference between Z and M. The exploitation rate was estimated using equation E=F/Z (Gulland, 1971).

Statistical analysis was performed at p = 0.05 using the statistical software SPSS v0.26. The normality of the data was checked using the Kolmogorov-Smirnov test, depending on the sample size (Sokal & Rohlf, 1969).

RESULTS

Sex ratio

The population consisted of 19.2% females and 80.8% males. The overall sex ratio (F:M) was 1:4.21 and was statistically significant departing from the expected ratio of 1:1 (X^2=238.96; n=630; df=1; p<0.001), indicating that males dominated the population. Although males predominated in all seasons, an almost equal sex ratio was observed in summer and autumn (Table 1). The sex ratio was balanced in the lower size interval (7 cm TL) and males significantly dominated in the upper size classes (8 cm TL) (Table 1).

Population status

The TL distribution range of black goby specimens was 7.20-14.0 cm (11.36±0.04), and the TW distribution range was 3.60-30.91 g (16.46±0.17) (Table 2). The majority of the samples (89.4%) were found in the size range of 10-13 cm. Males were abundant in longer length groups (Figure 2). The mean values of TL (U test; U=36695.0; Z=-2.280; p<0.05) indicated significant differences between males and females. No significant differences were identified for the mean TW between males and females (U test; U=29890.5; Z=-0.502; p>0.05). A significant difference was determined both in the mean TL (H test; H=77.205; df=11; p<0.001) and TW (H test; H=90.948; df=11; p<0.001) between sampling months.

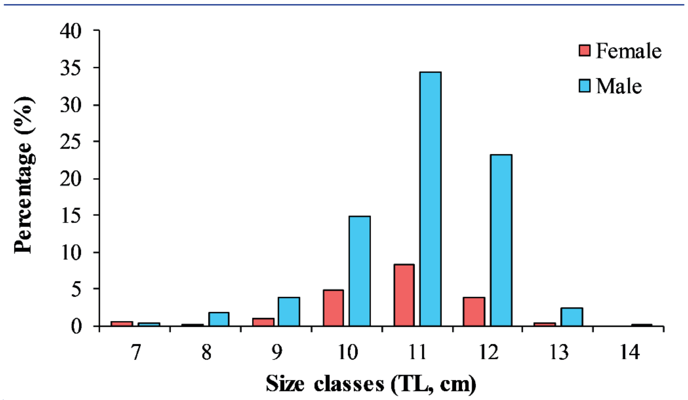

Figure 2. Length–frequency distribution of the studied *Gobius niger* population by sex.

Table 1. Sex ratios of *Gobius niger* according to months and size classes (TL, cm).

Key variables	Number of specimens: Female	Number of specimens: Male	Sex ratio: Female:Male	Sex ratio: χ^2	Sex ratio: Significance
Months					
Apr 20′	17	134	1:7.88	90.66	***
May 20′	53	227	1:4.28	108.13	***
June 20	12	42	1:3.50	16.67	***
July 20	9	14	1:1.56	1.09	n/a
Aug 20′	8	12	1:1.50	0.80	n/a
Sep 20′	1	2	1:2.00	0.33	n/a
Oct 20′	2	5	1:2.50	1.29	n/a
Nov 20′	2	10	1:5.00	5.33	*
Dec 20′	4	17	1:4.25	8.05	**
Jan 21′	3	7	1:2.33	1.60	n/a
Feb 21′	4	18	1:4.50	8.91	**
Mar 21′	6	21	1:3.50	8.33	**
Size classes (cm)					
7	4	3	1:0.75	0.14	n/a
8	1	11	1:11.00	8.33	**
9	6	24	1:4.00	10.8	**
10	31	93	1:3.00	31.00	***
11	53	216	1:4.08	98.77	***
12	24	146	1:6.08	87.55	***
13	2	15	1:7.50	9.94	**
14	0	1	–	–	–
Total	121	509	1:4.21	238.96	***

Note: χ^2: Chi-square value, –: not calculated, n/a: not significant, *: p<0.05, **: p<0.01, ***: p < 0.001

Length-weight relationship (LWR)

The estimated LWR for the combined sexes was $TW=0.010TL^{3.019}$ (N=630; r^2=0.870) and the population exhibited isometric growth (t-test; t_{pauly}=0.411; p>0.05) (Table 2). Significant differences were determined between the predicted slopes (b) for male and female gobies (ANCOVA; F=19.973; p=0.031).

Gonadosomatic index (GSI) and condition factor (Kn)

The reproductive period extends from March to June, and spawning takes place intensively in May (Figure 3). The GSI values increased markedly between winter and spring when the evolution of gonadal development in the ovaries began. The highest GSI values were observed for May with 12.73% for females and for April with 1.03% for males. The resting period starts in September with low GSI values for females (0.68%) and in July for males (0.10%). Temporal variation of GW presents significant changes among months (H test; H=132.53; df=11; p<0.001).

Table 2. Descriptive statistics and estimated parameters of the length-weight relationship of *Gobius niger.*

	TL (cm)	Regression parameters			Confidence intervals (CI)		Growth parameters		
	Mean±SE (Range)	a	b±SE	r^2	Cla	Clb	$t_{(pauly)}$	Sig.	Growth
Female	11.17±0.10 (7.40-13.10)	0.011	3.029±0.09	0.905	0.007-0.016	2.851-3.208	0.319	ns	I
Male	11.40±0.04 (7.20-14.00)	0.010	3.040±0.05	0.865	0.008-0.013	2.935-3.145	0.747	ns	I
Combined	11.36±0.04 (7.20-14.00)	0.010	3.019±0.05	0.870	0.08-0.013	2.927-3.110	0.411	ns	I

Note: TL: total length, a: regression intercept, b: regression slope, r^2: coefficient of determination, Cl: 95% confidence intervals, sig: significance, I: isometric growth

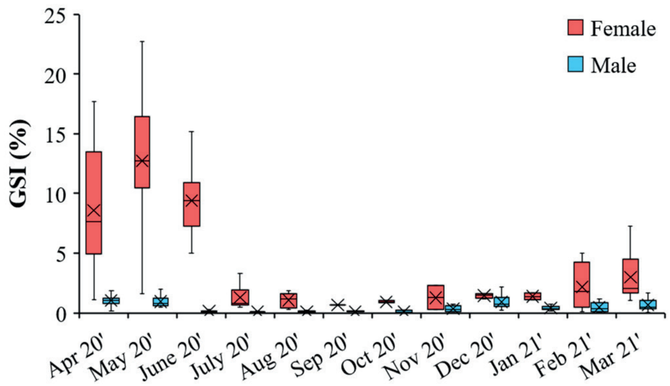

Figure 3. Box-plot of the gonadosomatic index (GSI%) for females and males of *Gobius niger.* Seawater temperatures along the study; Apr 20: 10.5 °C, May 20: 14.6 °C, June 20: 20.3 °C, July 20: 24.1 °C, Aug 20: 25.7 °C, Sep 20: 23.8 °C, Oct 20: 19.9 °C, Nov 20: 16.2 °C, Dec 20: 12.6 °C, Feb 21: 11.5 °C, Jan 21: 9.8 °C, Mar 21: 9.1 °C.

The mean Kn was calculated as 1.14±0.11, 1.09±0.01, and 1.10±0.01 for females, males, and combined sexes, respectively. The mean Kn was significantly different between females and males (t-test; t=20.286; df=1; $p<0.05$). The lowest Kn was observed in November for females (0.87) and in February and July for males (0.99) (Figure 4). The monthly variations of the Kn were not significantly different during all months (t-test; t=5.232; df=11; $p<0.05$).

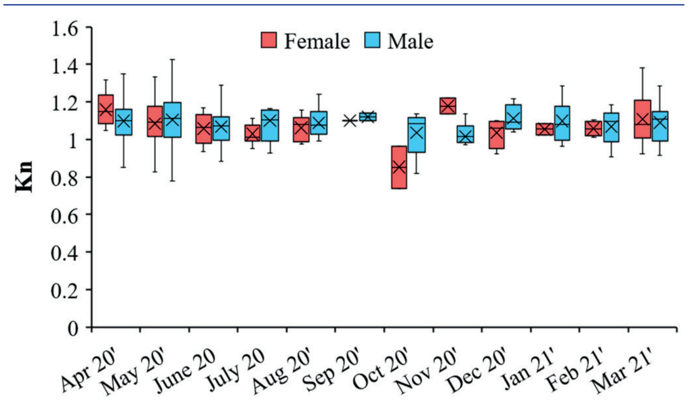

Figure 4. Box-plot of the condition factors (Kn) for females and males of *Gobius niger.*

Age and growth

The age of black goby specimens varied between 0 and 4 for both males and females. The otolith of a 13 cm long, four-year-old black goby is shown in Figure 5. Both females (42.1%) and males (55.4 %) were dominant in age group 3. The ratio of females (15.7%) and males (14.3%) at the maximum age (L_{max}) of 4 in the population was close together (Table 3).

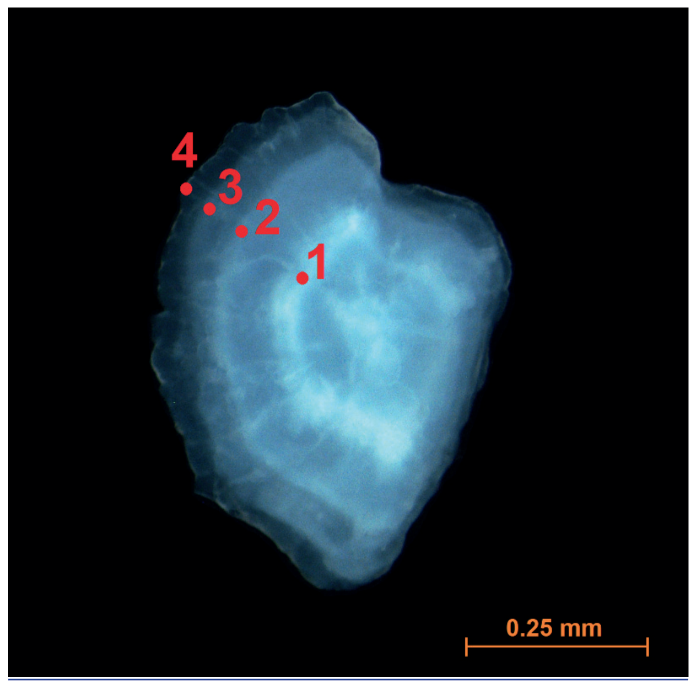

Figure 5. Sagittal otolith of a four-year-old black goby. Age rings are shown with red dots.

The VBGF equations were calculated as $L_t=14.55(1-e^{-0.29(t+2.45)})$, $L_t=18.05(1-e^{-0.16\ (t+3.23)})$, and $L_t=16.94(1-e^{-0.19(t+3.05)})$ for females, males and combined sexes, respectively. The predicted growth curve is illustrated in Figure 6. The **Φ'** was found to be 1.78, 1.73, and 1.74 for females, males, and combined sexes, respectively.

Mortality rates

The Z was estimated as $1.41 yr^{1}$ and $1.46\ yr^{1}$ for females and males, respectively. The other mortality estimates and exploitation rates are given in Figure 7. The E was calculated as higher than the optimum level (E~0.5) for the combined sexes (Patterson, 1992). The black goby stock in the study region appears highly exploited (E>50%, F/M>1).

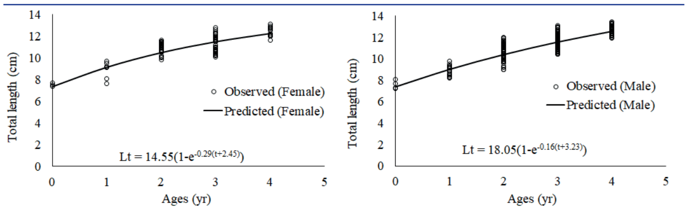

Figure 6. Estimated von Bertalanffy growth curves with observed (○) and predicted (—) length of *Gobius niger.*

DISCUSSION

The sex ratio may differ from the theoretical ratio of 1:1 by size class and gender in the same population, being influenced by reproductive behavior, the adaptation of the population, environmental conditions, and food availability (Clarke, 1983). In other studies of *Gobius niger* in the Black Sea, females were found to predominate males (Kasapoğlu, 2016; Bilgin & Onay, 2020; Van & Gümüş, 2021). However, in this study we report more males. The ratio obtained by the researchers in this study can be associated with the behavior of individuals during the reproduction period.

Table 3. Length-at-age key of *Gobius niger* for females and males based on otolith age readings.

Size classes (cm)	Age groups										Total	
	0		1		2		3		4			
	F	M	F	M	F	M	F	M	F	M	F	M
7	3	3	1								4	3
8		1	1	10							1	11
9			5	9	1	15					6	24
10					16	54	15	39			31	93
11					24	61	28	154	1	1	53	216
12						1	8	86	16	59	24	146
13								3	2	12	2	15
14										1		1
Total	3	4	7	19	41	131	51	282	19	73	121	509
TL_{avr} (cm)	7.53	7.58	8.91	8.90	10.97	10.74	10.79	11.62	12.36	12.61	11.17	11.40
±SE	0.09	0.21	0.29	0.10	0.07	0.05	0.04	0.03	0.10	0.04	0.10	0.04
TW_{avr} (g)	5.04	4.28	7.67	7.54	15.35	13.68	14.08	17.29	20.85	21.44	16.36	16.49
±SE	0.05	0.36	0.84	0.31	0.37	0.26	0.22	0.17	0.74	0.30	0.42	0.19

Note: F: female, M: male, TL_{avr}: average total length, TW_{avr}: average total weight, ±SE: standard error

Table 4. Parameter estimates of the von Bertalanffy and mortality rates of *Gobius niger* reported from various Turkish seas (modified and updated from Kasapoğlu, 2016; Kırdar & İşmen, 2018; Van & Gümüş, 2021).

Region	Sampling year	L_{max} (cm)	t_{max} (cm)	L_∞ (cm)	K (yr^{-1})	t_0 (yr)	Φ′	Z (yr^{-1})	M (yr^{-1})	F (yr^{-1})	References
BS[a]	2010-2011	13.4	5	13.26	0.31	-1.43	1.73	1.26[a]	0.65[a]	0.61[a]	Van & Gümüş, 2021
BS[b]				13.96	0.29	-1.42	1.75	1.01[b]	0.66[b]	0.35[b]	
BS	2020-2021	14.0	4	16.94	0.19	-3.05	1.74	1.43	0.55	0.88	This study
BS	2012-2013	14.6	4	12.10	0.68	-0.54	2.00	-	-	-	Bilgin & Onay, 2020
BS	2008-2011	15.8	5	17.95	0.27	-1.50	-	0.68	0.54	0.14	Kasapoğlu, 2016
MS	2011-2014	14.2	4	15.31	0.36	-1.77	1.92	1.34	0.82	0.51	Kırdar & İşmen, 2018
AS	2004-2007	16.3	7	17.18	0.39	-0.13	-	-	-	-	İlkyaz et al., 2011
AS	2003-2004	15.2	5	17.6	0.26	-2.17	1.90	-	-	-	Filiz & Toğulga, 2009
AS	-	-	-	16.8	0.39	-0.04	2.04	0.81	0.72	0.09	Kınacıgil et al., 2008
AS	1995-1996	-	3	14.6	0.46	-1.54	1.99	-	-	-	Özaydın et al., 2007

Note: BS: Black Sea, MS: Sea of Marmara, AS: Aegean Sea, L_{max}: maximum length, t_{max}: maximum age, L_∞: asymptotic length, K: growth rate, t_0: hypothetic age at zero length, Φ′: growth performance index, Z: total mortality, M: natural mortality, F: fishing mortality, [a]: Kızılırmak-Yeşilırmak shelf area, [b]: Melet River shelf area.

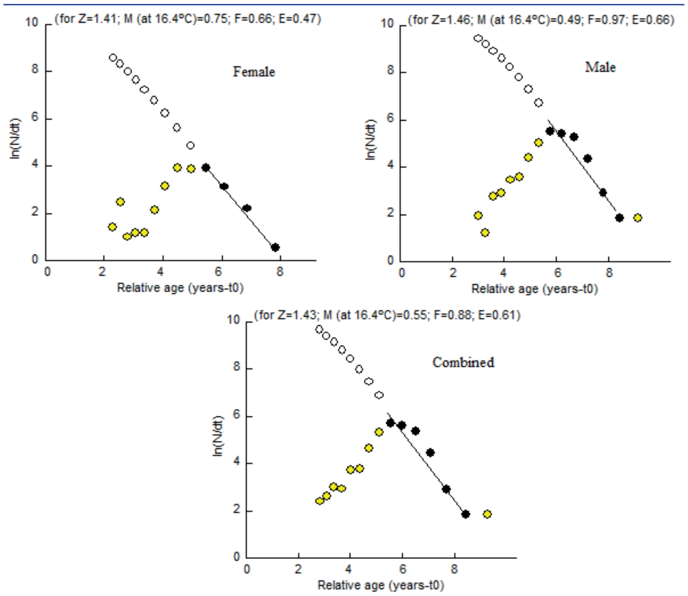

Figure 7. Parameter estimates of the mortality rates for the female, male, and combined *Gobius niger.* Derived from raw data based on a 0.5 cm size-class interval. Black dots represent the data used in the analysis.

The parental males of the black goby guard the nest until the eggs hatch (Mazzoldi, 1999). In this study, however, males were dominant during the reproduction period, and in other periods, sex ratios were almost equal (Table 1). Researchers in previous studies (Kasapoğlu, 2016; Bilgin & Onay, 2020; Van & Gümüş, 2021) used bottom trawls for sampling and this indicates non-selective sampling and a sampling site away from the shore. In this study, gill nets were used and sampling was made in a narrow depth range. In order to clarify this contradiction, it is recommended to examine the seasonal and reproductive migration behaviors of male and female black gobies. Males may have an advanced survival chance than females, especially in excessive environmental conditions, which explains the increasing male dominance with increasing size (Table 1). The studies reported that males become dominant during the spawning period (Hajji et al., 2013; Van & Gümüş, 2021). Males undertake burrow construction and egg maintenance (Filiz & Toğulga, 2009), and therefore the ratio of males caught during the spawning season is expected to decrease as they are not more easily caught (Miller, 1984). A male-biased sex ratio during the spawning season (May to June) does not mean that there is no burrow or egg maintenance. One of the reasons is probably the sampling method using gill nets. Since this study was mostly carried out with gillnets in areas close to the shore, males who took care of the nests may have been caught more.

The average TL of females was significantly lower, due to the predominance of males in the higher length classes (Figure 2). In contrast, different populations have been described where there is no difference in TL between males and females of black goby (Kasapoğlu, 2016; Kırdar & İşmen, 2018; Bilgin & Onay, 2020). The L_{max} of the black goby in the study area is close to that in previous studies in the Black Sea (Table 4). Differences in mean sizes between intra-specific populations may be due to several factors such as the relative abundance of the species, the selectivity of the fishing gears, sampling methods, or environmental variations (Baganel & Tesch, 1978; Edgar & Shaw, 1995; Gonçalves et al., 1997; Basilone et al., 2006).

The estimated *a* value for black goby was in accordance with the expected range of 0.001-0.05 for the natural fish populations (Froese, 2006). The b value of LWR is generally expected to be in the range of 2.5-3.5 for fish in general (Carlander, 1969) and the estimated b values of black goby were within expected ranges (Table 2). The black goby displayed an isometric growth (b=3) which may be concerned with the environmental factors of its natural habitat or its typical phenotype (Tsoumani, Liasko, Moutsaki, Kagalou, & Leonardos, 2006). This means that the length and weight of the individuals increase at the same rate. The LWR results in the Turkish coasts of the Black Sea show that the calculated b values were changed between 2.86 (Kasapoğlu, 2016) and 3.54 (Bilgin & Onay, 2020). LWR parameters may differ significantly due to environmental or biological factors, and temporal, geographical, and sampling factors (Bagenal & Tesch, 1978; Froese, 2006). Therefore, it is usual to obtain different results from different studies and regions.

Spawning of black gobies was observed in spring and at the beginning of summer in the study area (from March to June). This period coincided with the range where the seawater temperature was above 10°C and below 20°C. Partially similar spawning seasons are observed in the Black Sea (Bilgin & Onay, 2020), the Sea of Marmara (Kırdar & İşmen, 2018), the Aegean Sea (Özaydın et al., 2007; Kınacıgil et al., 2008) and in different countries (Arruda, Azevedo, & Neto, 1993; Immler et al., 2004; Hajji et al., 2013). Filiz & Toğulga (2009) reported a long spawning season from March to October in the Aegean Sea. Bilgin and Onay (2020) suggested that the extent of the black goby spawning period was mainly determined by daylight length and water temperature. Kara and Quignard (2019) linked the spawning cycle of the land goby with feeding. Due to the errors that are prone in macroscopic observations of gonads (West, 1990), it is usual for the spawning season to differ between regions. GSI values were lower in males than in females. The lower GSI value for males is a general characteristic of most gobies (Miller, 1984) including the black goby (Hajji et al., 2013). Females commonly invest more in reproduction than males. The testes are lighter than the gonads and constitute a lower ratio of the total fish weight (Wootton, 1990).

High conditions estimated for the black goby (Kn>1) may be signs of favorable environmental conditions and good nutrition (Le Cren, 1951). There was no significant difference between mean Kn and monthly seawater temperature (t-test; p>0.05) for these fish species (Figure 4). Fish feeding activities are reduced during spawning, they use up their lipid reserves, and their condition is significantly reduced (Lloret, Demestre, & Sánchez-Pardo, 2007). On the contrary, as the spawning season of the black goby begins in the cold season, the sudden increase in Kn observed in the spring can be explained by the increase in gonadal mass. The Kn of females was significantly higher than males only in spring, while males had higher Kn in other seasons (Figure 4). Males undertake critical tasks such as burrow construction, egg maintenance, and intraspecific competition

(Clark, Stoll, Alburn, & Petzold, 2000; Dinh, Qin, Dittmann, & Tran, 2014). During this time, females have more feeding opportunities because they need more energy to prepare for reproduction. These behaviors are thought to be the main factor in the low Kn values of males.

Males were more dominant in all age groups and females revealed a younger age profile (Table 3). The maximal life span was determined as 4 years in this study. Similar age groups (Kırdar & İşmen, 2018; Bilgin & Onay, 2020) and older individuals were reported from the Turkish coasts (Filiz & Toğulga, 2009; Kasapoğlu, 2016; Van & Gümüş, 2021). The longest lifespan of the black goby reported so far was 7 years in İzmir Bay, in the Aegean Sea (İlkyaz et al., 2011).

L_∞ calculated in this study is higher than noticed in previous studies on the Black Sea coasts (Table 4). The differences may be due to the selectivity of gill nets that can select especially smaller individuals of black goby (Wootton, 1990). However, high L_∞ values indicate that black gobies in the study area have a longer chance of living. Van and Gümüş (2021) reported a younger age profile for the black goby in the Black Sea related to high fishing pressure due to bottom trawls. The sexes have different growth rates, as is similarly recorded in different studies (Silva & Gordo, 1997; Filiz & Toğulga, 2009; Van & Gümüş, 2021). The Φ′ value was estimated lower than in other regions, but similar to studies carried out in the Black Sea (Table 4). The results indicated that the growth performance of the black goby is relatively low in the Black Sea. Geographical differences in the growth parameters of fishes may be based upon various factors such as environmental conditions, fishing pressure, sampling methodology (Blanchard et al., 2005; Mouine-Oueslati, Ahlem, Ines, Ktari, & Chakroun-Marzouk, 2015), the physicochemical contents of the water, environmental factors (Özeren, 2009), and genetics and social interactions (Helfman, Collette, & Facey, 1997).

In this study, the E are mostly higher than in previous studies performed on the Turkish coasts (Kınacıgil et al., 2008; Kasapoğlu, 2016; Kırdar & İşmen, 2018; Van & Gümüş, 2021) (Table 4). The black goby does not have commercial value but is captured together with target species, and it is categorized as ‘discard’ prey in gill nets (Aydın, Karadurmuş, & Kontaş, 2015) and trawling (Bilgin & Onay, 2020). Small-scale fishing in the Black Sea is an important source of livelihood and gillnets are frequently used in coastal areas (Aydın et al., 2015). In addition, rapa whelk fishing with beam trawl is carried out intensively in areas close to the coast of the Black Sea (Aydın, Düzgüneş, & Karadurmuş, 2016). It is inevitable that the black goby distributed in the shallower area where small-scale fishing and beam trawling are densely carried out is subject to death due to fishing activities. This unreported mortality rate explains the high fishing mortality in the Black Sea (Table 4). For the sustainable management of black goby populations, it is recommended to investigate the exploitation effect of different fishing gear. The higher mortalities in males (Z=1.46 yr^{-1}) than in females (Z=1.41 yr^{-1}) may be due to their sociobiological and ecological behaviors. Males prepare nests in a seagrass area in shallow coastal territory. Females lay their eggs in the nest and the males guard them until hatching (Poli et al., 2021). Black goby can compete with native fish for nesting spots during the guarding period (Janssen & Jude, 2001). This competition makes males more sensitive to survival.

CONCLUSION

The broad adaptive abilities and the successful reproductive strategy of the black goby are expected to result in further colonization and competition with other species in the Black Sea. Even though the population status of black gobies (in terms of size distribution, Kn, L_{max}, L_∞ , and Φ′) has not fluctuated much over the years in the Black Sea, this should not be considered a sign of potential resilience. It can be concluded that the black goby exhibited a high degree of tolerance in the Black Sea ecosystem, but there was an adverse effect of the fishing pressure based on discard on its population. There are no known species-specific protection programs for the black goby in the Black Sea. The living areas are open for commercial fisheries, including trawling, in the Black Sea. The response of the population to intense fishing pressure may depend on additional factors, such as vulnerability to capture, selectivity of fishing gear, and the survival of species in their natural environment. Further studies on catch per unit effort will provide more concrete data on prey pressure and species behavior. Bilgin and Onay (2020) reported the size at sexual maturity of the black goby as 8.9 cm for combined sexes in the Black Sea. Approximately 97% of the individuals in the population had reproductive potential (>9 cm). To make the sustainability of this population possible, a more restrictive arrangement is suggested, where only adult individuals are caught. Such an arrangement would allow successful spawning activities and recruitment in the subsequent season. Recently, goby species have started to be sold commercially in fish markets (Aydın, 2021b). The black goby may potentially become a commercial fish for coastal fisheries on the Black Sea coast of Turkiye and become an important source of income for fishermen. Although the black goby is not yet consumed as primary human food, its sustainable management is required because it forms the food of other commercial species such as turbot and whiting. In this case, long-term monitoring studies (stock and biomass estimation, spawning, and recruitment) and good management strategies (locational and seasonal prohibitions, catch quota, and regulation of selective fishing gears) will be needed to maintain stocks in the Black Sea. Updated parameter estimates and results serve as important indicators of fisheries management, and should be documented and monitored for changing trends.

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Research Article

Fish Biodiversity at Kawadighi *Haor* of Northeastern Bangladesh: Addressing Fish Diversity, Production and Conservation Status

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ABSTRACT

This research was conducted to explore the status of fish species diversity and production in the Kawadighi *Haor* of northeastern Bangladesh. Data were collected biweekly through direct catch assessment surveys, focus group discussions, and personal interviews using a questionnaire. A total of 87 fish and prawn species belonging 14 orders and 30 families were identified in the *Haor*, where 18% species were abundantly available, 20% were commonly available, 42% were moderately available and 20% were rarely available. Among the recorded species, Cypriniformes, having 34 species, had the most species, followed by Siluriformes (20), Anabantiformes (11), Ovalentaria (4), Synbranchiformes (4), Clupeiformes (3), Decapoda (3), Osteoglossiformes (2), Anguilliformes (1), Beloniformes (1), Cyprinodontiformes (1), Gobiiformes (1), Mugiliformes (1), and Tetraodontiformes (1). The values of Shannon-Weaver diversity (H), Margalef's richness (*d*), and Pielou's evenness (*J*) indices were 2.98, 7.72 and 0.67 in Hawagulaia, 2.97, 7.52 and 0.67 in Patasingra and 2.61, 7.30 and 0.59 in Salkatua *beel*, respectively. The *haor's* average yearly fish production was 704.09 kg/ha. Small indigenous species (SIS) of fish dominated the *haor's* total production, accounting for 51.8 to 70.57 percent of the total contribution. The highest portion of fish produced in the non-stocked *beel* was SIS of fish but per hectare SIS of fish production of non-stocked *beel* was lower than the fingerling stocked *beels*. Aquaculture might have a good effect on fish production and biodiversity. The findings showed that Kawadighi *Haor* is a very productive and biodiversity-rich inland open waterbody that may function as a mother fishery. For the protection of current fisheries resources, multiple approaches including public awareness campaigns might be beneficial.

Keywords: Kawadighi *Haor*, species diversity, diversity indices, aquaculture, conservation

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INTRODUCTION

Bangladesh is one of the countries facing challenges both in adequately feeding its burgeoning population and improving the living standards in its below standard population. To meet the challenge, the development of an indigenous food production system for local use and also for foreign earnings is now seen as critical for achieving higher living standards. Among the different production systems, aquaculture is an important one. Floodplain, *beel* and *Haor* resources should be included for horizontal expansion for aquatic food production. Although these resources have been stopped altogether or controlled by sluice gates, embankments, communication roads, pumps, FCD (Flood control and drainage) and FCDI (flood control, drainage and irrigation) project activities for five decades (Alam et al., 2015, 2017), these have colossal potential for huge increments in fish production through aquaculture. *Haor*, a marshy wetland ecosystem in Bangladesh's northeastern region, is literally a bowl or saucer-shaped depression with some different deeper areas (locally called *beels*) that resembles an inland sea during the monsoon (BHWDB, 2012; Pandit et al., 2015, 2021) and which becomes segregated into *beels* during the dry season.

Generally, open water natural fish diversity and production are decreasing gradually except for certain fingerling stocked and co-managed waterbodies (Jannatul et al., 2015; Aziz et al., 2021; Talukder et al., 2021). Some species are disappearing from individual waterbodies (Pandit et al., 2015). Still *Haor* is richer with various resources than other parts of the country. It has a great importance in the national economy, nutrition, and rural livelihoods (Hasan, 2007). However, it is reducing gradually. Now, the degradation of biodiversity of the aquatic environment is the prime concern to the environmentalists (Jannatul et al., 2015). Leaseholders of some *beels* of the *Haor* usually stock carp fingerling as part of aquaculture in their *beels* to increase fish production. However, no sufficient information is available on the impacts of fingerling stocking on the status of fish production and biodiversity for the Kawadighi *Haor*. To fill up some of the infprmation gaps, the current study is designed to analyze the existing status of biodiversity and richness of fish fauna in the Kawadighi *Haor*, as well as to estimate the impact of aquaculture activities on fish productivity and biodiversity. The Kawadighi *Haor*, formerly a mother fishery, is now a multifunctional (FCDI) project with a gross area of 22700 hectares, bounded by the Kushiyara River in the north, the Monu River in the south and west, and the foot of the Bhatera hills in the east. In the project area, the *Haor* covers about 12,295 ha with 63 *beels* and connecting canals located in the Rajnagar upazila under the Moulvibazar district, which is further connected to the Kushiara River by Koradoyer khal (canal). Prior to gathering this knowledge, we should be conversant on the level of fish production and species diversity and the thinking of different stakeholders about aquaculture for conservation and maximum sustainable yields (Galib et al., 2009). However, very limited information is available, infact, there is no available information for Kawadighi *Haor* concerning the above matters. The present research is focused on the current biodiversity situation, production, and conservation status of Kawadighi *Haor* fish species and people's perception of the impact of aquaculture.

MATERIAL AND METHODS

Study sites

Three out of the 63 *beels* (Hawagulaia, Salkatua and Patasingra) were selected as sampling sites with Hawagulaia being a non-stocked (no fingerlings were released) *beel* and Salkatua and Parasingra being stocked (fingerlings released) *beels*. The locations of the study sites are depicted on the map (Fig. 1).

Preparation of questionnaire

A preliminary questionnaire was created and pre-tested with a focus group discussion with a small sample of respondents to ensure that the study's goals are met. During pre-testing, special care was taken to include any additional information that was not intended to be requested and completed in the draft questionnaire. The questionnaire was updated, adjusted, and reorganized based on the feedback received during pre-testing.

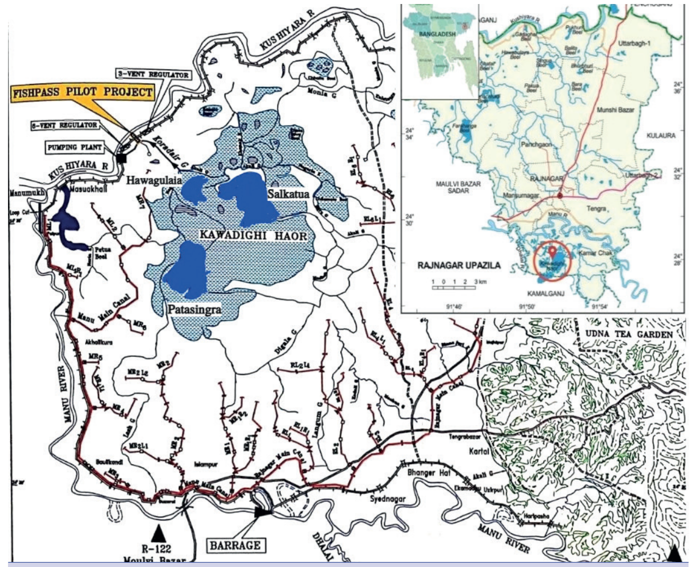

Figure 1. Kawadighi *Haor* indicating sampling sites (Modified from NERP, 1998).

Data collection procedure

Direct interviews with fishers, fish traders, and local communities were conducted to gather primary data. Cross checking was done through an interview with the upazila (sub-district) fisheries officer of Rajnagar upazila. Some focus group discussions were held using a semi-structured and structured questionnaire. Catch assessment surveys were conducted bi-weekly in each of the sampling sites during fishing from January to December 2014. The catches were identified by species and were recorded species-wise and by the number of specimens according to Rahman (2005), Shafi & Quddus (2001), and Talwar & Jhingran (1991). Based on public opinion and occurrence frequency (Percent of surveys where the researcher recorded the particular species), identified fish species were divided into four groups. The following are the categories: Abundantly Available (AA): Species seen on a regular basis all through the year (frequency > 75%); Commonly Available (CA): Species seen regularly but in limited numbers throughout the year (frequency from 51 to 75%); Moderately Available (MA): Species found occasionally in the research zone (frequency from 26 to 50%); and Rarely Available (RA): The species that are only seen infrequently and in limited quantities (frequency ≤ 25%) (Pandit et al., 2020, 2021).

Biodiversity tools and production measurement

Species diversity was analyzed using the Shannon-Weaver index (H) (Shannon & Weaver, 1963), species richness using the Margalef index (*d*) (Margalef, 1968) and evenness using Pielou's index (*J*) (Pielou, 1966).

The Shannon-Weaver index (H) is defined as:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Where, H =Shannon-Weaver index, S = Number of species, $p_i = n_i/N$, n_i = Number of individuals of a species and N = Total number of individuals.

Margalef richness index (*d*) was calculated with the following formula:

$d = (S - 1)/\log(N)$

Where,

S= Total number of species,

N= Total number of individuals.

Pielou's evenness index (*J*) is defined as:

$J = H_{(S)}/H_{(max)}$

Where,

$H_{(S)}$ =The Shannon-Weaver diversity index,

$H_{(max)}$ =The maximum possible value of the Shannon-Weaver index if all the values are identical.

Monthly net catches were determined using average catch rates and daily fishing effort for each of the gear types. The name of the species, the quantity, and weight of fish of various species in the daily catch, as well as the CPUE, were all reported on a monthly basis. Based on the monthly data, annual yield was calculated. The total fish production of each sampling site was calculated from the modified formula of Hurst & Bagley (1992) as:

Total catch from sampling sites for a specific gear = N × f × CPUE

Where, N is the number of fishing days per year,

f is the daily mean number of individual fishing unit and

CPUE is the mean daily catch per gear unit.

For monthly production, N was counted as days per month. In this way, the total catch was estimated summing the amount of catch by different gears monthly.

Data analysis

To minimize all possible errors and contradictions, the data was summarized, processed, and verified. Microsoft Excel, version 2010, was used to analyze the data. Different fish diversity indices, tables, pie charts, column diagram etc. were used to analyze and represent the data respectively.

RESULTS AND DISCUSSION

Fish biodiversity

A total of 87 fish and freshwater prawn species belonging 30 families under 14 orders was recorded from the Kawadighi *Haor* where 78 were indigenous fish, 6 were exotic fish and 3 were prawn species (Table 1). Among the families, Cyprinidae dominated with 20 species followed by Danionidae (10), Danionidae (10), Bagridae (7), Ambassidae (4), Channidae (4), Osphronemidae (4), Siluridae (4), Ailiidae (3), Clupeidae (3), Mastacembelidae (3), Palaemonidae (3), Botiidae (2), Cobitidae (2), and Notopteridae (2). Anabantidae, Aplocheilidae, Badidae, Anguillidae, Belonidae, Chacidae, Clariidae, Heteropneustidae, Gobiidae, Horabagridae, Mugilidae, Nandidae, Pangasiidae, Synbranchidae, Sisoridae, and Tetraodontidae contributed one species each.

In terms of orders, Cypriniformes had the most, with 34 species, followed by Siluriformes (20), Anabantiformes (11), Ovalentaria (4), Synbranchiformes (4), Clupeiformes (3), Decapoda (3), Osteoglossiformes (2), Anguilliformes (1), Beloniformes (1), Cyprinodontiformes (1), Gobiiformes (1), Mugiliformes (1), and Tetraodontiformes (1) (Figure 2).

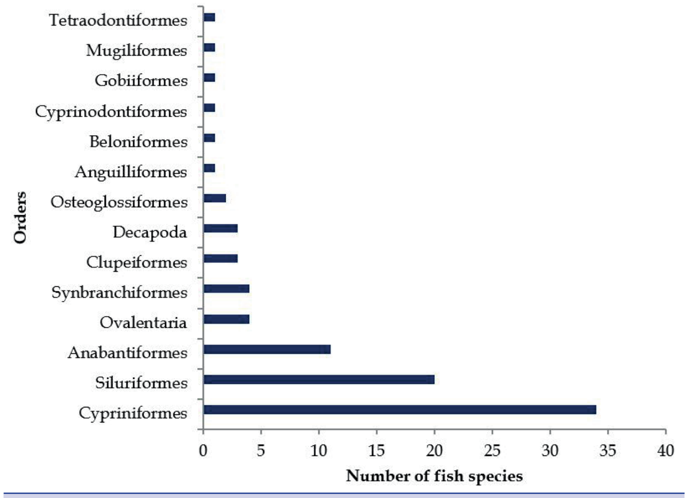

Figure 2. Fish species under different orders identified from Kawadighi *Haor*.

Any comparison of current data is difficult because there has been no previous research on fish biodiversity in the Kawadighi *Haor*. While assessing the fish biodiversity in various wetlands in Bangladesh, many other researchers had a similar experience (Galib et al., 2013; Pandit et al., 2015; Talukder et al., 2021). A total of 93 bony fish species and 2 prawn species belonging to eight orders were found in *Beel* Kumari and Hilna *beel* of Northwestern Bangladesh (Alam et al., 2017). Similarly, 92 different fish and prawn species were recorded in the Sylhet-Mymensingh basin (Haroon et al., 2002). In the 2013-14 fiscal years, in the Dekar *Haor* of Sunamganj District, a total 65 species belonging to 23 families were recorded (Pandit et al., 2015). A total of 57 species from 20 families were found in the Tilai River (Ahmed et al., 2020) and 47 fish species were found in the Borulia *Haor* of Nikli, Kishoreganj (Nath et al., 2010), which are much less than the current study. These studies indicated the Kawadighi as being a fish harbor.

Present status of fish biodiversity

The present study found 18% AA, 20% CA, 42% MA, and 20% RA fish species in the study area (Figure 3). The local fishing community assumes that this is due to declining population trends. The maximum fish species of the Gurukchi River of the Sylhet District was RA (29.82%), followed by CA (28.07%), MA (22.81%), and AA (19.30%) (Pandit et al., 2020). Another study found 17.4% AA, 27.5% CA, 31.9% MA, and 23.1% RA fish species in the Dhanu River and adjacent *Haor* ecosystems (Pandit et al., 2021). Among 87 species, *Macrobrachium lamarrei* showed the highest relative abundance (38.476%) in the Kawadighi *Haor*, followed by *Puntius sophore*, *Macrobrachium malcolmsonii*, and others (Table 1).

Table 1. List of fish and prawn species recorded from the Kawadighi *Haor* during the study period.

Order	Family	Local name	Species	Relative abundance (%)	Present availability status	Conservation status	
						BD	World
Anabantiformes	Osphronemidae	Boro kholisha	*Trichogaster fasciata* (Bloch & Schneider, 1801)	4.036	AA	LC	LC
		Choto kholisha	*T. chuna* (Hamilton, 1822)	0.032	MA	LC	LC
		Lal kholisha	*T. lalius* (Hamilton,1822)	2.297	AA	LC	LC
		Naptani	*Ctenops nobilis* McClelland, 1845	0.007	RA	LC	NT
	Anabantidae	Koi	*Anabas testudineus* (Bloch, 1792)	0.773	CA	LC	LC
	Nandidae	Meni/Bheda	*Nandus nandus* (Hamilton, 1822)	1.016	AA	NT	LC
	Badidae	Napit koi	*Badis badis* (Hamilton, 1822)	0.024	MA	NT	LC
	Channidae	Shol	*Channa striata* (Bloch, 1793)	0.060	MA	LC	LC
		Taki	*C. punctata* (Bloch, 1793)	0.820	CA	LC	LC
		Cheng	*C. orientalis* (Bloch & Schneider, 1801)	0.052	MA	LC	VU
		Gozar	*C. marulius* (Hamilton, 1822)	0.007	RA	EN	LC
Beloniformes	Belonidae	Kaikka	*Xenentodon cancila* (Hamilton, 1822)	0.812	CA	LC	LC
Mugiliformes	Mugilidae	Khorsula	*Rhinomugil corsula* (Hamilton, 1822)	0.008	RA	LC	LC
Gobiiformes	Gobiidae	Baila	*Glossogobius giuris* (Hamilton, 1822)	0.158	CA	LC	LC
Ovalentaria	Ambassidae	Gol chanda	*Pseudumbassis ranga* (Hamilton, 1822)	1.723	AA	LC	LC
		Lomba chanda	*Chanda nama* (Hamilton, 1822)	3.867	AA	LC	LC
		Kata chanda	*Pseudumbassis baculis* (Hamilton, 1822)	0.671	MA	NT	LC
		Lal chanda	*Parambassis lala* (Hamilton,1822)	0.051	CA	LC	NT
Synbranchiformes	Mastacembelidae	Tara baim	*Macrognathus aculeatus* (Bloch, 1786)	0.075	MA	NT	LC
		Boro baim	*Mastacembelus armatus* (Lacepède, 1800)	0.151	CA	EN	LC
		Chirka baim	*M. pancalus (Hamilton,1822)*	0.164	CA	LC	LC
	Synbranchidae	Kuchia	*Monopterus cuchia* (Hamilton, 1822)	0.018	MA	VU	LC
Cyprinodontiformes	Aplocheilidae	Kanpuna	*Aplocheilus panchax* (Hamilton, 1822)	0.128	CA	LC	LC
Cypriniformes	Danionidae	Chela	*Salmostoma phulo* (Hamilton, 1822)	0.167	CA	NT	LC
		Chela	*Securicula gora* (Hamilton, 1822)	0.003	RA	NT	LC
		Chela	*S. bacaila* (Hamilton, 1822)	0.061	MA	LC	LC
		Kash khaira	*Chela laubuca* (Hamilton, 1822)	0.002	RA	LC	NE
		Darkina	*Rasbora daniconius* (Hamilton, 1822)	0.103	CA	LC	LC
		Chebli	*Devario devario* (Hamilton, 1822)	0.027	MA	LC	LC

Table 1. Continue.

Order	Family	Local name	Species	Relative abundance (%)	Present availability status	Conservation status	
						BD	World
Cypriniformes	Danionidae	Darkina	*R. rasbora* (Hamilton, 1822)	1.578	AA	NT	LC
		Darkina	*Esomus danricus* (Hamilton, 1822)	3.204	AA	LC	LC
		Piali	*Cabdio morar* (Hamilton, 1822)	0.006	RA	VU	LC
		Mola	*Amblypharyngodon mola* (Hamilton, 1822)	2.174	AA	LC	LC
	Cyprinidae	Goinna	*Labeo gonius* (Hamilton, 1822)	0.040	MA	NT	LC
		Bata	*L. bata* (Hamilton, 1822)	0.177	CA	LC	LC
		Boga	*L. boga* (Hamilton, 1822)	0.160	CA	CR	LC
		Kalibaush	*L. calbasu* (Hamilton, 1822)	0.203	CA	LC	LC
		Mrigal	*Cirrhinus cirrhosus* (Bloch, 1795)	0.066	MA	NT	VU
		Katla	*Gibelion catla* (Hamilton,1822)	0.048	MA	LC	LC
		Rui	*L. rohita* (Hamilton, 1822)	0.066	MA	LC	LC
		Silver carp	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	0.045	MA		NT
		Dhela	*Osteobrama cotio* (Hamilton, 1822)	0.026	MA	NT	LC
		Lachu	*Cirrhinus reba* (Hamilton, 1822)	0.085	MA	NT	LC
		Sarpunti	*Systomus sarana* (Hamilton, 1822)	0.021	MA	NT	LC
		Thaipunti	*Barbonymus gonionotus* (Bleeker, 1849)	0.059	MA		LC
		Titpunti	*P. ticto* (Hamilton, 1822)	3.092	RA	VU	LC
		Punti	*P. phutunia* (Hamilton, 1822)	0.154	CA	LC	LC
		Punti	*P. chola* (Hamilton, 1822)	1.999	AA	LC	LC
		Jatipunti	*P. sophore* (Hamilton, 1822)	17.174	AA	LC	LC
		Carpio	*Cyprinus carpio* Linnaeus, 1758	0.111	AA		VU
		Bangna	*Gymnostomus ariza* (Hamilton, 1807)	0.003	RA	VU	LC
		Grass carp	*Ctenopharyngodon idella (Valenciennes, 1844)*	0.017	MA		NE
		Bighead carp	*Hypophthalmichthys nobilis* (Richardson, 1845)	0.005	RA		DD
	Cobitidae	Gutum	*Lepidocephalichthys guntea* (Hamilton, 1822)	0.046	MA	LC	LC
		Pahari gutum	*Canthophrys gongota* (Hamilton, 1822)	0.035	MA	NT	LC
	Botiidae	Rani	*Botia dario* (Hamilton, 1822)	0.037	MA	EN	LC
		Putul	*B. lohachata* Chaudhuri, 1912	0.026	MA	EN	NE
	Clariidae	Magur	*Clarias batrachus* (Linnaeus , 1758)	0.073	MA	LC	LC
	Siluridae	Boal	*Wallago attu* (Bloch & Schneider, 1801)	0.019	MA	VU	VU
		Boali pabda	*Ompok bimaculatus* (Bloch, 1794)	0.025	MA	EN	NT

Table 1. Continue.

Order	Family	Local name	Species	Relative abundance (%)	Present availability status	Conservation status	
						BD	World
Siluriformes	Siluridae	Madhu pabda	*O. pabda* (Hamilton, 1822)	0.037	MA	EN	NT
		Pabda	*O. pabo* (Hamilton, 1822)	0.009	RA	CR	NT
	Heteropneustidae	Shingi	*Heteropneustes fossilis* (Bloch, 1994)	0.263	CA	LC	LC
	Chacidae	Chaca/ kaua	*Chaca chaca* (Hamilton, 1822)	0.001	RA	EN	LC
	Ailiidae	Garua	*Clupisoma garua* (Hamilton, 1822)	0.008	RA	EN	LC
		Bacha	*Eutropiichthys vacha* (Hamilton, 1822)	0.036	MA	LC	LC
		Kazuli	*Ailia coila* (Hamilton, 1822)	0.048	MA	LC	NT
	Horabagridae	Batashi	*Pachypterus atherinoides* (Bloch, 1794)	0.074	MA	LC	LC
	Bagridae	Air	*Sperata aor* (Hamilton, 1822)	0.010	RA	VU	LC
		Tengra	*Mystus vittatus* (Bloch, 1794)	2.928	AA	LC	LC
		Tengra	*Batasio tengana* (Hamilton, 1822)	0.031	MA	EN	LC
		Guizza	*S. seenghala* (Sykes, 1839)	0.004	RA	VU	LC
		Kabasi tengra	*Mystus cavasius* (Hamilton, 1822)	0.075	MA	NT	LC
		Gulsha	*M. bleekeri* (Day, 1877)	0.829	CA	LC	LC
		Buzuri tengra	*M. tengara* (Hamilton, 1822)	1.018	AA	LC	LC
	Pangasiidae	Thai pangas	*Pangasianodon hypophthalmus* (Sauvage, 1878)	0.009	MA		EN
	Sisoridae	Jainzza	*Gogangra viridescens* (Hamilton, 1822)	0.010	MA	LC	LC
Osteoglossiformes	Notopteridae	Foli	*Notopterus notopterus* (Pallas, 1769)	0.011	MA	VU	LC
		Chital	*Chitala chitala* (Hamilton,1822)	0.003	RA	EN	NT
Clupeiformes	Clupeidae	Chapila	*Gudusia chapra* (Hamilton, 1822)	0.445	CA	VU	LC
		Ilish	*Tenualosa ilisha* (Hamilton, 1822)	0.001	RA	LC	LC
		Ketchki	*Corica soborna* (Hamilon, 1822)	3.407	AA	LC	LC
Tetraodontiformes	Tetraodontidae	Potka	*Leiodon cutcutia* (Hamilton, 1822)	0.005	RA	LC	LC
Decapoda	Palaemonidae	Golda chingri	*Macrobrachium rosenbergii* (de Man, 1879)	0.069	MA	LC	LC
		Icha	*M. lamarrei* (H. Milne-Edwards, 1837)	38.476	AA	LC	LC
		Kalo icha	*M. malcolmsonii* (H. Milne-Edwards, 1844)	4.146	AA	LC	LC

BD = Bangladesh, MA = Moderately Available, AA = Abundantly Available, RA = Rarely Available, CA = Commonly Available, NE = Not Evaluated, DD = Data Deficient, LC = Least Concerned, NT = Near Threatened, CR = Critically Endangered, EN = Endangered, VU = Vulnerable

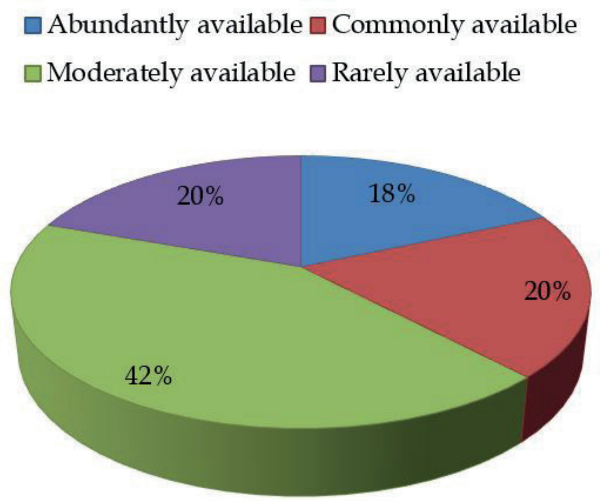

Figure 3. Current status of fish biodiversity.

Conservation status of fish

According to the worldwide conservation status (IUCN, 2021), the least concerning category (79 %) made up the largest proportion of the fish species, followed by near threatened (10 %), vulnerable (5 %), not evaluated (4 %), endangered (1 %), and data deficient (1%) (Figure 4). It is worth noting that in the research reason, globally vulnerable fish species such as *Channa orientalis*, *Cirrhinus cirrhosus*, and *Wallago attu* were found to be MA while *Cyprinus carpio* were found to be AA. The availability status of *Pangasianodon hypophthalmus* were recorded as moderately available and is considered as endangered species globally. A recent study found a very similar result: 84.6% were least concerned, 9.9% were near threatened, 3.3% were vulnerable, and 2.2% were not evaluated (Pandit et al., 2021).

The least concerned category occupied the highest position in terms of national conservation status with 45 species 52%, near

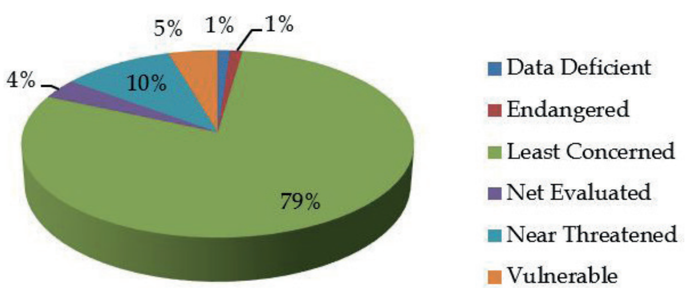

Figure 4. Global conservation status of fish species.

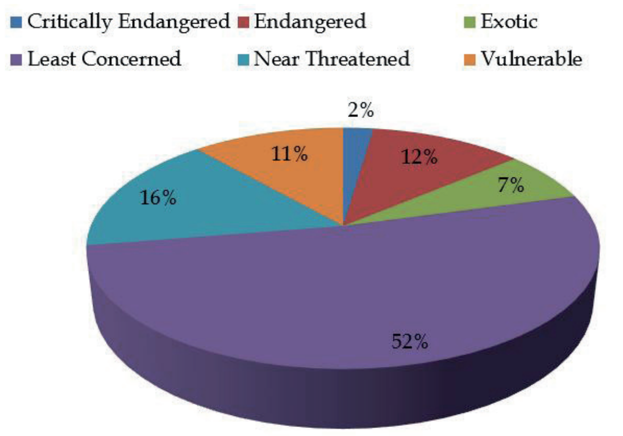

Figure 5. National conservation status of fish species.

threatened 16%, endangered 12%, vulnerable 11%, exotic species 7%, and critically endangered 2% (Figure 5). The highest 53.8% species were occupied by least concerned category, followed by 17.6% near threatened, 12.1% endangered, 11.0% vulnerable, 3.3% critically endangered , and 2.2% data deficient in the Dhanu River and its adjacent area by Pandit et al. (2021) which is very similar to the current result.

Species diversity indices

The values of Shannon-Weaver diversity (H), Margalef's richness (*d*) and Pielou's (*J*) evenness indices are presented in Table 2. As shown in Table 2, H, *d* and *J* were 2.98, 7.72 and 0.67 in Hawagulaia, 2.97, 7.52 and 0.67 in Patasingra and 2.61, 7.30 and 0.59 in Salkatua *beel*, respectively.

In floodplain lakes of India, values of H ranging from 3.61- 3.95, *J* ranging from 0.85- 0.94 and *d* ranging from 0.08- 0.12 were recorded by Mondal et al. (2010). SIS of fishes were dominant in the present study area, *d* was higher, and *J* was lower than Mondal et al. (2010). A similar H index (3.145-2.789) was found in the Meghna River estuary (Hossain et al., 2012). In the Ratargul freshwater swamp forest of Bangladesh, a higher H, *d*, and *J* value were found as 3.690±0.191, 9.497± 1.314, and 0.971±0.003, respectively (Das et al., 2017). H ranging from 3.12-2.9, *d* 3.02-2.70 and *J* 0.82-0.88 were found in Konoskhai *Haor* of Northeastern Bangladesh (Iqbal et al., 2015). In the Surma River of Sylhet district of Bangladesh, mean values of H 2.30±0.14, *d* 6.99±0.86 and *J* 1.93±0.23 were recorded (Chowdhury et al., 2019). The biodiversity parameters of the present study are in line with the previous records and any differences are maybe due to associated spatial, hydrological, and biological combined conditions of the concerned area.

Table 2. Shannon-Weaver diversity, Margalef's richness and Pielou's evenness indices of fishes of the three *beels*.

Study Area	Number of species (S)	Total Number of individuals (N)	LnN	Diversity, $H=\sum PilnPi$	Richness, $d=\frac{S-1}{lnN}$	lnS	Evenness, $J=\frac{H}{lnS}$
Patasinghra	86	81958	11.31	2.97	7.52	4.45	0.67
Shalkatua	86	115376	11.65	2.61	7.30	4.45	0.59
Hawagulia	83	41011	10.62	2.98	7.72	4.42	0.67

Species composition and production

Fish and prawns were divided into four categories: SIS of fish, large indigenous fish, large exotic fish, and prawn. SIS of fish contributed the most in all *beels*, accounting for 70.57%, 51.8%, and 63.43% in the Hawagulaia, Patasingra, and Salkatua *beel*, respectively. Large exotic fish contributed 13.72%, 26.52% and 16.11% in Hawagulaia, Patasingra and Salkatua *beel*, respectively whereas large indigenous fish was 11.4%, 26.52% and 16.11% in Hawagulaia, Patasingra and Salkatua *beel*, respectively (Fig 6). Prawn occupied the lowest position. The results show that SIS governs *Haor* production, even in the fingerling stocked *beels*, and revealed that SIS has a dominant capacity over the total fish production. Although SIS has taken over the majority of the non-supplied Hawagulaia *beel*, SIS production per hectare is lower than that of fingerling stocked *beels* (Table 3). It's possible that fingerling stocking has a good effect.

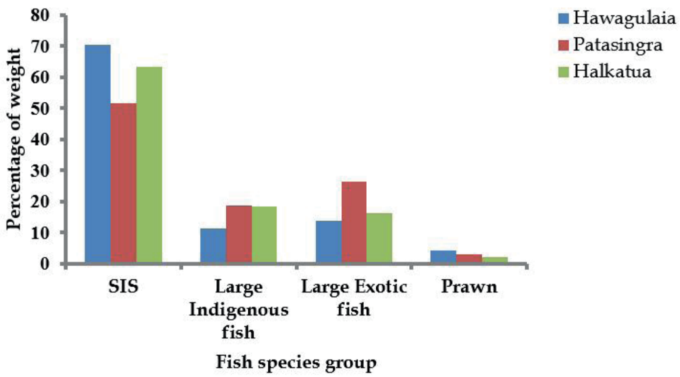

Figure 6. Composition of different fish groups and prawn.

Table 3. Production (kg/ha) of different fish groups and prawns.

Fish group	Hawagulaia	Patasingra	Salkatua
SIS of fishes	176.48	351.18	472.9
Large indigenous fish	28.5	151.87	137.1
Large exotic fish	34.3	220.92	104.1
Prawns	10.8	21.63	15.5
Total	250.08	745.6	729.6

Average annual SIS of fishes, large indigenous fish, large exotic fish, and prawn production of the *Haor* were 356.59, 139.84, 187.85, 19.81 and 704.09 kg/ha, respectively (Table 4). During the survey, the total fish and prawn production of Kawadighi *Haor* was 8656.789 MT. (Table 4).

Table 4. Annual production of different fish groups and prawns in the *Haor*.

Fish group	Haor total (MT)	Haor average (kg/ha)
SIS	4384.262	356.59
Large indigenous fish	1719.305	139.84
Large exotic fish	2309.62	187.85
Prawns	243.6018	19.81
Total	8656.789	704.09

Stakeholders' perception on the impact of aquaculture on fish production and biodiversity

There were positive and negative impacts on fish production and biodiversity. When the survey was implemented, 449 persons in the area (lease holders, fishermen, and the general public) expressed their opinions on stocking. The stakeholders' reactions are shown in Figure 7. Of the respondents, 54.12 percent made a positive comment, 35.86 percent expressed a negative comment, and the remaining 10.02 percent refused to comment. When asked whether they had complete freedom in collecting fish, the negative respondents said "no," and when asked if there is any chance of successful SIS breeding as a consequence, they said "yes", but they dewater the water bodies over the winter. From the preceding remark, it may be deduced that aquaculture may have a positive impact on boosting fish production and biodiversity in Kawadighi *Haor*.

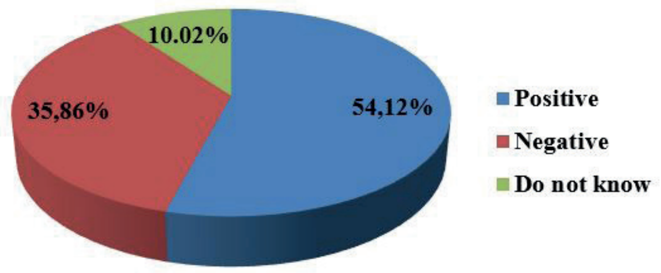

Figure 7. Stakeholders' perception on the impact of aquaculture on fish production and biodiversity in Kawadighi *Haor* (n=449).

Table 5 also indicates that in non-stocked *beel*, three large fish occupied third, fourth and ninth position among the top ten species, the rest were SIS. In the Patasingra *beel*, five large fish and the rest five were SIS and in the Salkatua *beel*, six were SIS. The freshwater shark fish *Wallago attu* occupied the third position in the non-stocked *beels* and second position in the stocked *beels* indicating a more or less successful recruitment. On the contrary, the number of exotic fish among the top ten species was highest in the Patasingra *beel* (4 species out of 10), followed by the Salkatua *beel* (3), and the Hawagulaia *beel* (2), which indicated that fingerling stocked *beels* have higher availability of non-native fishes. Alien fish species may control the local one, triggering elimination and disrupting original ecosystems.

Positive impacts of floodplain aquaculture on ecology and fish biodiversity were recorded by (Hossain et al., 2014). A positive impact of aquaculture on aquatic production and both a positive and negative impact on aquatic biodiversity were observed (Diana, 2009). All leaseholders claimed to be performing admirably in terms of fish production and biodiversity richness, both physically and ecologically. Aquaculture technology has a beneficial impact on fish productivity and biodiversity in the seasonal floodplain of Bangladesh (Rahman et al., 2010). However, there might be other factors that come into play, *viz.* depth, size, or management. Therefore, more in-depth research is necessary to find out if there are any other factors responsible.

Table 5. Top ten fish species (by weight) of the studied *beels*.

SL No	Hawagulaia beel	Patasingra beel	Salkatua beel
1	*Puntius sophore*	*Cyprinus carpio*	*Puntius sophore*
2	*Pethia ticto*	*Wallago attu*	*Wallago attu*
3	*Wallago attu*	*Puntius sophore*	*Cyprinus carpio*
4	*Cyprinus carpio*	*Pethia ticto*	*Pseudumbassis ranga*
5	*Pseudumbassis ranga*	*Hypophthalmichthys molitrix*	*Mystus vittatus*
6	*Trichogaster fasciata*	*Pseudumbassis ranga*	*Barbonymus gonionotus*
7	*Mystus vittatus*	*Ctenopharyngodon idella*	*Gudusia chapra*
8	*Mystus cavasius*	*Pangasianodon hypophthalmus*	*Hypophthalmichthys molitrix*
9	*Hypophthalmichthys molitrix*	*Trichogaster fasciata*	*Trichogaster fasciata*
10	*Nandus nandus*	*Mystus vittatus*	*Mystus cavasius*

Table 6. Factors affecting species diversity.

Serial Number	Components affecting species diversity	Number of respondents (%) n=449
1	Dewatering *beels* every year	93.98
2	Overfishing	90.86
3	Use of destructive fishing gear	86.85
4	Intensification of agricultural activities	68.81
5	Construction of road and embankment in and around the *Haor*	67.92
6	Use of pesticides	58.79
7	Sedimentation from adjacent river	56.79
8	Construction of barrage in adjacent river	52.78
9	Lack of proper fish ranching by Govt./NGO	28.95
10	Drought	22.93

Factors affecting fish biodiversity

Despite the abundance of fish species in the Kawadighi *Haor*, there are rising concerns about the long-term viability of fish biodiversity due to various anthropogenic and natural processes that are reducing biodiversity and habitats for the fishes in the *Haor* area and surrounding *beels*. Dewatering *beels* (93.98%), and overfishing (90.86%), followed by the use of destructive fishing gear (86.85%), the intensification of agricultural activities (68.81%), construction of roads and embankment (67.92%), use of pesticides (58.79%), sedimentation (56.79%), construction of barrage (52.78%), lack of proper fish ranching (28.95%), and drought (22.93%) are the most significant anthropogenic factors that have contributed to the reduction of species diversity in the Kawadighi *Haor* (Table 6). Each year lease holders dewater their *beels* and sublet them to another person who again dewaters the *beels* as some water is retained in the *beel* after the initial dewatering. A similar management problem was also found in the Dhanu River and adjacent *Haor* wetlands in the Kishoreganj district of Bangladesh (Pandit et al., 2021). Drying the *beels*, heavy rainfall, overfishing, siltation, use of destructive gear, temperature fluctuation, and the application of urea fertilizer to harvest fish all have a destructive effect on all fish fauna in Hakaluki *Haor* in northeast Bangladesh (Aziz et al., 2021). Furthermore, the absences of other income-generating opportunities for fishers, tourism, navigation, invasive fish species, and revenue-oriented leasing schemes have directly or indirectly affected fish biodiversity. Invasive fish species, in particular, may have severe effects on native species, triggering extinctions and changing natural ecosystems that were previously unknown owing to the fishermen's lack of awareness.

CONCLUSION

This research largely focused on the impact of aquaculture on the fish biodiversity and production of the Kawadighi *Haor*. Species diversity and production were found to be higher in the fingerling stocked *beels* than in the non-stocked *beel*. In contrast, the availability of many non-native fish species and their invasive tendencies indicated a worrisome current state of the fisheries resources in the *Haor*. Furthermore, ecosystem-based management of common aquatic resource pool with local community engagement is strongly advocated for the *Haor* in order to conserve fish diversity and sustainable fisheries production.

Conflicts of interest: The authors declare no conflict of interest.

Ethics committee approval: All procedures used in experiments involving human and animals (fish) were in compliance with the "Sylhet Agricultural University Ethical Committee's" ethical standards. Informed consent was obtained from all survey respondents.

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Research Article

Improving the Reproductive Yield of Black Sea Salmon (*Salmo labrax* PALLAS, 1814) with a Selective Breeding Program

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ABSTRACT

This study aimed to improve some culture characteristics of Black Sea salmon (*Salmo labrax*) culture generations through a classical selective breeding program. Thus, the success of the applied program was examined by comparing the reproduction time and proportional distribution of the wild broodstock (F_0) individuals with the F_1, F_2, F_3 and F_4 generation broodstocks adapted to the culture conditions. According to the results, gamete uptake from the new generations occurred between October and February. The highest egg uptake was determined for all generations in December. While the difference between wild (F_0) broodstock and F_1, F_2, F_3, and F_4 generations was statistically significant in favor of new generations (P<0.05), the difference was insignificant between hatchery origin-new generations. Mean egg diameters were low in F_2 and F_3 generations, and F_0, F_1 and F_4 generations were found to be higher than the others (P<0.05). It was calculated that the fertilization rate was higher in F_3 and F_4 generations, similar in F_1 and F_2 generations, and lower in F_0 generation than the others (P<0.05) at the end of the study. Through the selection program, it was determined that the adaptation of the species to the culture conditions improved, the reaction to human activities declined, and homogeneous distribution in tanks/ponds was relatively achieved from the F_2 generations. As a result, it has been determined that F_4 generation broodstock have higher culture performance than other generations. Producers of this species should use F_4 broodstock for efficient and economical production.

Keywords: *Salmo labrax*, Black Sea salmon, breeding characteristics, broodstock management, selection program

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INTRODUCTION

Black Sea salmon (*Salmo labrax* PALLAS, 1814), also known as Black Sea trout, is an endemic species to the Black Sea from the brown trout family (Tabak et al., 2001). Black Sea salmon, as an anadromous fish, migrate between sea and rivers for reproduction and feeding (Slastenenko, 1956; Svetovidov, 1984; Geldiay & Balık, 1996; Solomon, 2000). The natural distribution area of the Black Sea salmon is the Black Sea and the rivers pouring into it (IUCN, 2016). However, Black Sea salmon is listed as an endangered species according to several local Black Sea countries' databases (GRID, 1999; Lusk et al., 2004; Vassilev & Pehlivanov, 2005; Peev et al., 2011). The decrease in the natural stocks of Black Sea salmon has also directed researchers to aquaculture studies for ex-situ conservation of the species.

The first cultivating study of this species was initiated by the Central Fisheries Research Institute (SUMAE) in 1998. Tabak et al. (2001) created a wild (F_0) broodstock born in rivers in Turkiye, discharged it into the Eastern Black Sea in 1998, and obtained F_1 progeny in the first reproduction season in the same year. In 2001, a selective breeding program was initiated with studies to determine the species' culture characteristics, with high consumer demand for starting commercial cultivation. When creating the broodstock for each generation, some specific characteristics of the species and culture conditions were considered primary targets. These characteristics were rapid growth, feed utilization efficiency, body form, late maturity (3 years), appearance (silver-spotted coloration for marine ecotype), reproductive efficiency, survival rate, and domestic behavior. After 15 years of research and study, the F_4 generation broodstock was achieved. The private sector supported the study with the F_3 generation broodstock to expand the species' cultivation (Çakmak et al. 2011). Along with these studies, Aksungur et al. (2013) conducted a study called "The use of molecular genetic analyses in stock management of Black Sea salmon." In that study, molecular genetic analyses (microsatellite, sequence analysis of mtDNA genes (cyt-b, dloop and 16S)) were performed on 600 tissue samples taken from 18 private farms producing Black Sea salmon, including SUMAE, and it has been determined that natural genetic variation continues in the broodstock of SUMAE within some farms. With the success of these studies, nowadays Black Sea salmon has great economic importance (Kasapoğlu et al., 2020) due to its rich nutritional value and high consumer demand (Çankırılıgil et al., 2020; 2022). According to the data from the Turkish Statistical Institute, 19 private aquaculture facilities are culturing the species professionally, and 63 private enterprises have trial production permits to raise the species in Turkiye. The total production reached 2311 tons/year (TSI, 2021).

This study discusses the selective breeding program, which plays a significant role in bringing Black Sea salmon into the aquaculture sector and making it a significant part of the seafood industry. The success of the program was examined by comparing the spawning time and proportional distribution of the wild broodstock (F_0) individuals and F_1, F_2, F_3 and F_4 generation broodstocks during breeding season, which were caught from some streams in the Eastern Black Sea Region and adapted to the culture conditions. In the literature review that was conducted, no previous study was found regarding the selection program for this species. This research study is the first and most comprehensive broodstock selection study, starting from the wild stock and progressing for 4 generations of Black Sea salmon.

MATERIAL AND METHODS

Sampling studies and adaptation

The wild broodstock individuals were caught from the Black Sea salmon's natural habitats, such as Kapistre, Çağlayan, Fırtına, İyidere, Baltacı, and Solaklı streams. To form the broodstock pool, 3000 fish (±5) at approximately 2+ (34 months) years old were caught and transferred to adaptation units located in Trabzon, Türkiye. Sampling stations and locations of research units are shown in Figure 1. The adaptation study was conducted in the Central Fisheries Research Institute (SUMAE) marine cages research unit, which is composed of 4 m x 4 m square cages with 6 m net depths, in Yomra/Trabzon (salinity 0.17% and water temperature ranging from 5 to 20°C). In this study, 2328 of the 3000 individuals that adapted to culture conditions were used as the first broodstock candidates.

Broodstock selectivity program

In forming F_0 broodstock, 650 promising individuals were selected from the 2328 adapted fish, and the first stock was achieved. F_1 generation juvenile fish were obtained by stripping wild individuals (F_0) in 1998. These juveniles were used in the establishment of the F_1 broodstock. The F_2 generation broodstock was created from the juveniles obtained by stripping 3-year-old F_1 broodstock in 2001. Individuals obtained from the second breeding season (4 years old) from F_2, F_3, and F_4 generation broodstocks were used as the broodstock material for the next generation. Broodstock reaching 7 years of age were removed from the breeding stock (Çakmak et al., 2018). Fish with low sperm quality were not used in the subsequent studies. Sperm quality was determined visually, considering sperm volume and sperm activity via a light microscope. In addition, in each generation, some individuals were discarded from the pool due to the reproductive performance of the previous year (egg yield, gonad size, fertilization ratio, etc.) and observations. New broodstock candidates were added to the stock to maintain the number of 650 individuals in each generation. Finally, some individuals with low body condition and low egg quality who did not develop gonads or developed late gonads in the stripping studies were also excluded from the study. Thus, in this study, the data of 59 females (1360.4±821.06g) and 45 males (1526.8±150.50g) from F_0 broodstock, 167 females (1392.6±780.22g) and 182 males (1526.8±150.50g) from F_1 broodstock, 118 females (1210.8±555.66g) and 136 males (1252.62±85.45g) from F_2 broodstock, 159 females (1663.4±566.87g) and 171 males (1268.44±113.65g) from F_3 broodstock, and 132 females (1252.1±707.01g) and 148 males (1509.6±122.46) from F_4 broodstock were used. The broodstock selectivity program is shown in Table 1.

Stripping studies and rearing

In stripping studies, firstly, fish were marked using individual markers to determine their reproductive and growth performance. While alphanumeric markers (Visible implant tags, Northwest Marine Technology) were used to mark the F_0, F_1, and F_2 generations, electronic markers (Biomark, 12 mm, 134 kHz) were used for the F_3 and F_4 generations in the reproduction season, which spanned October to January. The alphanumeric markers were applied to the transparent tissue above the eyes, whereas electronic markers were applied to the muscle tissue located below the dorsal fin. Markers and the application procedure are shown in Figure 2.

The breeding season of the Black Sea salmon in the natural environment starts in mid-October and continues until the end of December (Tabak et al., 2001). In this study, broodstock control for reproduction started in the first week of October each year following the natural reproduction cycle. Egg maturity controls were made weekly during the breeding season. Individuals that had matured gonads were taken into separate ponds for stripping. Breeding studies were conducted in the SUMAE marine cages research unit (salinity 0.17% and water temperature 5-20°C) and in the stream research unit, which has circular ponds with 6m diameter and 1.2 m depth in the Maçka/Trabzon freshwater unit (water temperature 4-22°C), between 1998 and 2016. Changes in the water temperatures were shown in Figure 3. Broodstock was

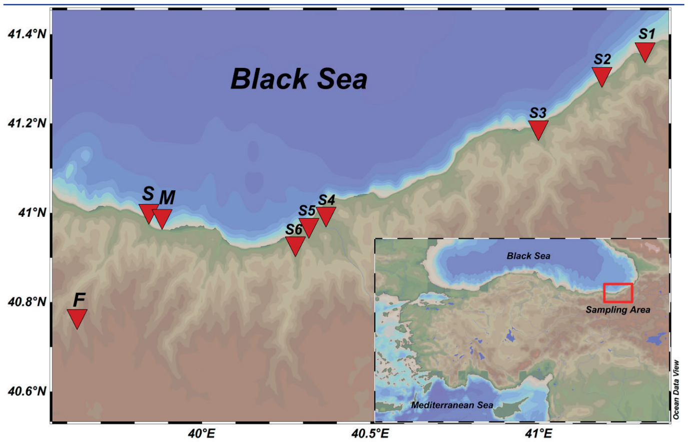

Figure 1. Locations of facilities and sampling stations. S: Central Fisheries Research Institute (SUMAE), M: Marine cage unit, F: Freshwater aquaculture unit, S: Sampling stations 1 to 6 (From east to west, Kapistre, Çağlayan, Fırtına, İyidere, Baltacı and Solaklı streams, respectively). The sampling station map was prepared with Ocean Data View software (Schlitzer, 2021).

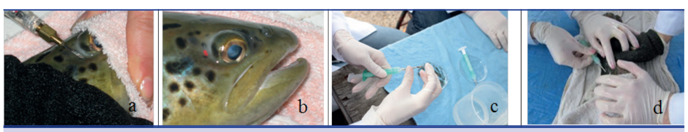

Figure 2. Markers used in the marking of broodstock and their application areas (a-b: alphanumeric markers, c-d: electronic markers).

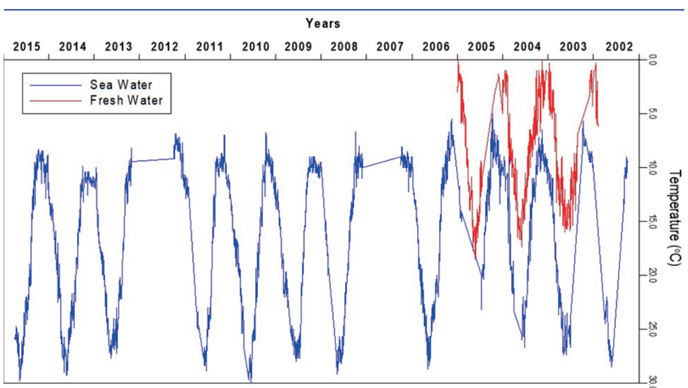

Figure 3. Water temperature changes on the marine cage research unit and Altındere freshwater unit.

anaesthetized by applying 50 ppm benzocaine (Oswald, 1978) for height-weight measurements and effortless stripping. Stripping was done via the dry stripping method (Billard, 1992). Multiple mating method (3 (F):3 (M)) was applied in fertilization. 25 minutes after fertilization, the eggs were washed with hatching water, and the residues were removed. Fertilized eggs were transferred to the vertical incubators in the freshwater system with the 4-6 egg/cm² stock density at 8-9.5 mg/lt saturated O_2

Table 1. The broodstock creation program was implemented between 1998 and 2016.

Year	FG	LS	Stripping Year																		
			1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
1998	F_0	B	III*	IV	V	VI	VII	VIII													
1999	F_1	J																			
2000	F_1	S																			
2001	F_1	B				III*	IV	V	VI	VII											
2002	F_2	J																			
2003	F_2	S																			
2004	F_2	B							III	IV	V	VI	VII								
2005	F_2	B								IV*	V	VI	VII								
2006	F_3	J																			
2007	F_3	S																			
2008	F_3	B											III	IV	V	VI	VII				
2009	F_3	B												IV*	V	VI	VII				
2010	F_4	J																			
2011	F_4	S																			
2012	F_4	B															III	IV	V	VI	VII
2013	F_4	B																IV*	V	VI	VII
2014	F_5	J																			
2015	F_5	S																			
2016	F_5	B																			III

FG: filial generation (F_0, F_1, F_2, F_3, F_4, F_5). LS: life stage of the fish, which were B: broodstock, J: juvenile, S: selection. III, IV, V and IV: Age of the individuals.
The asterisk (*) indicates the individual's stripping age at which the next broodstock was formed.

and 12°C until hatching within 38-40 days. Fry were raised in freshwater units up to smolt length (11.5 cm, 17gr). In the freshwater research unit, water exchange was provided 18-20 times a/day in the breeding ponds. After stripping, the fish were transferred to marine cage units and kept until June. In June, fish were transferred to the freshwater units due to increasing sea water temperature (≥18°C) till the reproduction period. In this stage, *Salmo labrax* reached approximately 30.30±1.63cm in length and 335.50±44.39g in weight in the 8 month span (Çakmak et al. 2007). The stock density was applied as 15 kg/m³ in both marine cages and ponds. The cultivation procedure was carried out according to the study of Çakmak et al. (2010), based on observations gained in domestication studies of the *Salmo labrax*.

Determination of gamete quality and growth parameters

Total length of broodstock was measured with a ruler with a precision of 0.1, while body weight (W) and total egg weights were measured with a precision scale. Total egg weight was determined by weighing the dehydrated eggs for each broodstock. Average egg diameters were determined by scaling 20 eggs for each broodstock in a Von Bayer vessel (Von Bayer, 1910) and calculated by dividing the total number of eggs. Total fecundity was determined using Arıman Karabulut's method (2005). The fertilization rate was calculated by proportioning the remaining eggs (fertilized eggs) to the total number of eggs (Çakmak et al., 2018). Eggs were placed in incubators fed with spring water filled through vertical flow, using separate trays for each broodstock. Eggs that became opaque one day after fertilization were considered unfertilized and were counted and removed. Condition factor was calculated according to Ricker (1975). Commercial trout feed was used for fish consumption. The equations of fecundity, fertilization rate, and condition factor are shown below:

Total fecundity = n/W
n: Total egg count, W: Weight of the individual after stripping (g).

Fertilization rate = $(W_1/W_0) \times 100$
W: Weight (g), L: Length (cm).

Condition factor = $(W \div L^3) \times 100$
W: Weight (g), L: Length (cm).

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Obtained data were analyzed by performing one-way ANOVA using the SPSS 15 statistical analysis program. Differences between means were compared using Duncan's multiple range test. The homogeneity was determined by the Levene test, while normality was determined by the Anderson-Darling test. Mean values between groups were accepted as statistically significant when the probability value found smaller than 0.05 was accepted as ($P<0.05$).

RESULTS AND DISCUSSION

The temporal and proportional distribution of F_0, F_1, F_2, F_3, and F_4 generation broodstocks stripped during the reproduction period is shown in Figure 4. Stripping of wild individuals adapted to the culture conditions (F_0) started at the end of October and continued until mid-February. During the reproduction season,

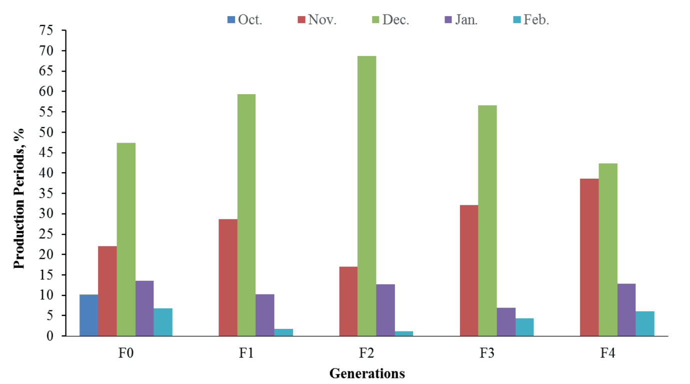

Figure 4. Temporal and proportional distribution of F_0, F_1, F_2, F_3 and F_4 generation broodstocks stripped during the reproduction period.

10.17% of the wild broodstock were stripped in October, 22.04% in November, 47.45% in December, 13.56% in January and 6.78% in February (mean values of the years of 1998-2001). The breeding period of F_1, F_2, F_3, and F_4 generation broodstocks was completed in a shorter period than the wild broodstock. F_1, F_2, F_3, and F_4 generation broodstocks were stripped at the rate of 28.74%, 16.94%, 32.08%, and 38.63% in November, 61.07%, 68.64%, 56.60%, and 42.42% in December, 10.17%, 12.71%, 6.91%, and 12.87% in January, 1.79%, and 1.69%, 4.40%, and 6.06% in February, respectively. Brown trout spawn between October and December in the northern hemisphere (Needham, 1945; Horton, 1961; Thomas, 1964; Moyle, 1976). Tabak et al. (2001) stated that the reproductive season of the Black Sea salmon starts in November and continues to mid-December in nature. Salihoğlu et al. (2013) report that the spawning season of the rainbow trout (*Oncorhynchus mykiss*) is between late December and late February. Similarly, Kurtoğlu et al. (1998) report that stripping of rainbow trout broodstock started in mid-January and continued until the first quarter of March. In this study, stripping of wild individuals (F_0) adapted to the culture conditions started at the end of October and continued until mid-February. The breeding season of F_1, F_2, F_3, and F_4 generation broodstocks started in November and ended in February. F_4 generation broodstock was mature enough to be stripped at the rate of 38.63% in November, 42.42% in December, 12.87% in January, and 6.06% in February. Unlike the wild population, the stripping season of the F_4 generation broodstock obtained through the selection program starts one month after the stripping season of the brown trout in the northern hemisphere and continues for a long period of four months. The reproduction time of most of the F_4 generation broodstock (81.05%) is between November and December, which does not overlap with the rainbow trout production period. In this case, businesses can have an advantage in using hatcheries. In addition, the preference of compressing the breeding period to a shorter time interval or spreading it over a long period in the natural environment can be possible by implementing good management plans.

Some biological parameters of broodstock individuals are shown in Table 2 and Table 3. Fecundity was found to be lower than others in native broodstock (F_0) adapted to culture conditions and similar in F_1, F_2, F_3, and F_4 generation broodstocks (P<0.05). There is no statistical difference between egg yields of F_0, F_1, and F_2 generation broodstocks (P>0.05). However, the total egg yield of F_3 and F_4 generation broodstocks were statistically higher than that of the others (P<0.05). The largest egg diameters were observed in F_0, F_1, and F_4 generation broodstocks among all groups (P<0.05). Egg yield and size of fish are affected by various factors. The most important of these are broodstock size, age, genotypic structure, and feeding conditions (Bromage et al. 1990, 1992). In salmonids, larger females generally produce larger eggs especially in the culture environment (Sargent et al., 1987; Hatcher et al., 1995). Total egg production and egg diameter values were similar to the values found for wild Black Sea salmon broodstock, but they were different from the other studies mentioned above. In all broodstock groups, total egg production increased in direct proportion to fish size. Heinimaa & Heinimaa (2004) stated that the size of female Atlantic salmon had a positive effect on the total number of eggs, as expected. Furthermore, Şahin et al. (2007) stated that there is a positive correlation between fish size and total fecundity in cultured Black Sea salmon, while a negative correlation exists in wild individuals. In a study by Iwamoto et al. (2017) about selective breeding of the Coho salmon (*Oncorhynchus kisutch*), egg yield is increasing parallel with weight increase of the individuals, especially in the 16th and 17th generations. Thus, in this study, the high egg yield of F_3 and F_4 generation broodstocks was because the broodstock used was larger than the others. Brown & Kamp (1941) determined the egg yield as 1285 eggs/broodstock, and the egg diameter was 4.64 mm for brown trout in their study. Toledo et al. (1993) studied the reproductive data of 24 brown trout broodstock and found that the egg yield was 1176 eggs/broodstock and the egg diameter was 4.67 mm. Estay et al. (2004) determined the total egg production, relative egg production, and egg diameter of the culture from brown trout as 1904±595 eggs/broodstock, 2591±900 eggs/kg, and 4.77±0.27 mm, respectively. Tabak et al. (2001) report that the total egg yield of wild Black Sea salmon broodstock was 3226±320 eggs/broodstock, the relative egg yield was 1747±70 eggs/kg, and the egg diameter was 5.48±1.10 mm. Serezli et al. (2010) reported that Black Sea salmon has 1404 eggs/kg total fecundity and 4.51±0.67 egg mm diameter. In another study, total fecundity and egg diameter were determined to be 3524.6±2106.9 and 5.2±0.20 in wild fish and 1931.3±915 and 5.0±0.24 in cultured fish, respectively (Şahin et al., 2007). Our results are similar to other studies.

The fertilization rates of F_1 and F_2 generation broodstocks were statistically the same (P>0.05), and the lowest fertilization rate was determined in the wild broodstock eggs (P<0.05). However, in the F_3 and F_4 generations, fertilization rate was determined to

Table 2. Some biological parameters of male individuals were used in the study.

Parameters	Filial Generations				
	F_0 (n=45)	F_1 (n=182)	F_2 (n=136)	F_3 (n=171)	F_4 (n=148)
L	49.60±1.58	44.8±0.99	46.68±1.04	46.88±1.38	50.4±1.74
(Min-Max)	(34.8-68.2)	(35.5-56.5)	(35.2-58.6)	(34.7-56.9)	(43.1-59.1)
W	1526.8±150.50	1526.8±150.50	1252.62±85.45	1268.44±113.65	1509.6±122.46
(Min-Max)	(562-3603)	(562-3603)	(561-2326)	(479-2207)	(1058-2210)
CF	1.13±0.01	1.12±0.02	1.19±0.01	1.17±0.02	1.17±0.06
(Min-Max)	(0.97-1.26)	(0.93-1.27)	(0.99-1.31)	(1.01-1.31)	(1.00-1.64)

L: mean length (cm), W: mean weight (g), CF: condition factor.

Table 3. Reproduction yields of F_1, F_2, F_3 and F_4 generation Black Sea salmon broodstocks.

Parameters	Filial Generations				
	F_0 (n=59)	F_1 (n=167)	F_2 (n=118)	F_3 (n=159)	F_4 (n=132)
L	49.99±10.24^{bc}	50.13±0.78^{b}	47.84±7.44^{c}	53.98±6.17^{a}	52.48±7.06^{a}
W	1360.42±821.06^{b}	1392.60±780.22^{b}	1210.78±555.66^{b}	1663.42±566.87^{a}	1584.25±820.46^{a}
TEW	248.76±156.05^{c}	294.11±170.29^{b}	236.88±116.01^{c}	348.72±131.75^{a}	327.40±160.43^{a}
EW	0.096±0.01^{a}	0.096±0.01^{a}	0.088±0.01^{c}	0.091±0.01^{b}	0.097±0.01^{a}
ED	5.46±0.37^{a}	5.52±0.34^{a}	5.21±0.38^{b}	5.21±0.28^{b}	5.45±0.21^{a}
EC	2789±1756^{b}	3202±1665^{b}	2916±1472^{b}	3964±1405^{a}	3664±1220^{a}
TF	2159±739^{b}	2428±709^{a}	2512±898^{a}	2436±593^{a}	2417±586^{a}
FR	93.46±5.35^{c}	95.28±6.29^{b}	95.74±4.70^{b}	98.25±1.87^{a}	98.25±1.81^{a}
CF	0.990±0.158^{a}	1.010±0.087^{a}	0.988±0.098^{a}	1.021±0.102^{a}	0.992±0.076^{a}

L: mean length (cm), W: mean weight (g), TEW: total egg weight (g), EW: the weight of one egg (g), ED: egg diameter (mm), EC: egg count, TF: total fecundity, FR: fertilization rate (%), CF: condition factor. Different letters on the same line indicate the statistical difference in the mean values (P<0.05).

be highest (P<0.05). It is a fact that the fertilization rate of the Black Sea salmon was improved with the selective breeding program throughout the years. In addition, the fertilization rates of F_3 and F_4 generations' eggs were found to be similar to the cultured rainbow trout and brown trout, according to the literature. Tabak et al. (2001) found that the fertilization rate of wild Black Sea salmon eggs is 97.76%. In other research, Estay et al. (2004) reported the highest fertilization rate as 98.5% for cultured brown trout. Salihoğlu et al. (2013) found the average fertilization rate of eggs obtained from rainbow trout broodstock to be 98.7% in a study they conducted in a private enterprise in the Eastern Black Sea region of Turkiye.

Condition factor values were found to be statistically the same in all broodstock groups (P>0.05). Condition factor indicates the general fattening status of the fish, and it can be changed with feeding, gonadal development, and some abiotic factors (Lizama & Ambrosio, 2002). In this study, environmental conditions and feeding regime were kept as constant as possible, and broodstock candidate fish were selected from individuals with the high condition. Therefore, it was expected that there will be no difference between the groups in terms of condition factor values.

CONCLUSION

In the selective breeding programs applied to improve culture characteristics of the species, qualitative characteristics as well as quantitative ones are crucial. In the first generation, some undomestic behaviors, such as escaping from humans, feeding on the bottom rather than the water column, cannibalism, which rises with starvation, and the response to instant changes in the environment (transportation process, salinity, temperature) were improved gradually through the selective breeding study conducted over 15 years. Garner et al. (2010) stated that the breeding method and rearing environment directly affect salmon growth and behavior.

The broodfish displaying an overall reduced sensitivity to environmental conditions are likely to grow faster through feeding more, thereby propagating their traits to the next generation (Solberg et al., 2020). Thus, undomestic behaviors can be reduced with selective breeding programs, as in our study. It was also observed that timid behaviors in the F_0 generation in response to human activities decreased as the generation progressed over time and the adaptation to culture conditions increased. In addition, a relatively homogeneous distribution has been achieved in the tanks/ponds since the F_0 and F_1 generations. General movement of the fish to the feeder's side during feeding and feed intake from the water surface, water column, and bottom were also seen. The second stripping requirement was seen in the first generation broodstock in the stripping season, gradually decreasing as the new generations progressed. Feeding with the appropriate diet and establishing a broodstock from the eggs stripped in the first stripping in selective breeding may be effective for adapting to the culture conditions. Future studies should be carried out to clarify this situation. According to Chavanne et al. (2016), in addition growth and fecundity, feed efficiency, morphology, disease resistance, carcass yield, and product quality are also important traits for the salmonids. With the experience and knowledge gained through this study, future studies on salmon breeding can close the gaps in these areas.

In conclusion, it was determined that the selectivity program carried out with the Black Sea salmon caused a positive effect on the reproductive performance of the fish. Considering the high demand for the Black Sea salmon culture, due to high market value and consumer appreciation compared to other Salmonidae species, the progress gained through this study is vital. Applying a breeding program to improve the culture characteristics of this species, whose culture is spreading rapidly, is essential for business management and profitability. We believe that our findings play a beneficial role in future work, and that the results will interest all researchers while highlighting Black Sea salmon (*Salmo labrax*) as a valuable food source.

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Conflict of Interest: The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Ethics committee approval: Non applicable.

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Research Article

Seasonal Differences in Lipid and Fatty Acid Composition of European Eels (*Anguilla anguilla*, Linnaeus 1758) from Orontes River, Turkiye

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ABSTRACT

Seasonal differences in the lipid contents and fatty acid composition of European Eels (*Anguilla anguilla*, Linnaeus 1758) caught from the Orontes River (Hatay, Turkey) were determined. High lipid levels, as well as ω-3 and ω-6 fatty acids, are important factors in product quality. High-content lipid values of European eels from the Orontes River differed between seasons ($p<0.05$). The fatty acid compositions of eels ranged from 5.91-8.03 g/100 g in saturated fatty acids (SFA), 10.59-14.08 g/100 g in monounsaturated fatty acids (MUFAs), and 2.19-3.52 g/100 g in polyunsaturated fatty acids (PUFAs). Those present in the highest proportions were palmitic acid (C16:0, 62-67.51%), palmitoleic acid (C16:1, 15.67-19.07%), stearic acid (C18:0, 11.04-17.2%), oleic acid (C18:1ω-9, 70.67-72.44%), eicosapentaenoic acid (EPA) (C20:5ω-3, 12.77-22.83%), and docosahexaenoic acid (DHA, C22:6ω-3, 4.35-11.93%). Some fatty acids' composition differed significantly ($p<0.05$) between seasons. In addition, the ratio of ω-3/ω-6 PUFAs varied between 1.14 and 1.72, reaching the highest value in autumn. The highest EPA+DHA contents were recorded during summer. In conclusion, analysis parameters show that commercially important European eels from the Orontes River are quite good sources of high-quality lipids and fatty acids.

Keywords: EPA, DHA, lipids, nutritional value, Orontes

INTRODUCTION

It is known that eels have a high oil content and are rich in ω-3 lipids, which is a high-value fish species preferred in gourmet cuisine (especially smoked products) due to its high-value nutrient content. It constitutes an important foreign exchange source input for the countries where eel production and fishing are carried out. If the fish, classified according to their size immediately after being caught, are to be consumed fresh, they are transferred to cooled, oxygenated packaging so that the skin does not dry out. Fish transferred for processing are usually delivered to the processing plant alive in water tanks (FAO, 2009).

Generally classified as warmwater fish, eels have a total of 19 species, which are distributed worldwide. They are found in freshwater sources in the Atlantic Ocean and the Mediterranean and are considered commercially important species, especially in the Eastern and Southern coasts of Turkey (Arai, 1991; Özogul et al., 2005; El-Obeid et al., 2018). The total production of European eels in Europe was 4478 tons (FEAP, 2020). In 2020, 320 tons of European eel were caught in Turkey (TurkStats, 2022). European eels in Turkey are distributed in the Orontes River, which flows to the East Mediterranean basin (Genc et al., 2008; Özden et al., 2020). The market demand for eels, which is also defined as gourmet seafood, is high in Europe and Asia, due to their taste and high flesh yield, resulting in increased eel exports (Ersoy, 2011).

Eels are a good source of FAs such as EPA, DHA and docosapentaenoic (C22:5ω-3, DPA), which are essential for human health. Eels' high-quality fat content and composition are regarded as highly marketable quality traits that affect the wholesale price and consumer acceptance overall (Lie et al., 1990). Humans ingest long-chain ω-3-PUFAs, which cannot be synthesized inside the body, through diet (Alasalvar et al., 2002). Due to the link between human health and diet, consumers' interest in healthy food has increased, thus increasing the nutritional quality of food (Lie, 2001).

In this study, the seasonal differences in total fatty acid and lipid composition of European eels caught in the Orontes River, which are commercially important, were determined.

MATERIALS AND METHODS

Fish sampling

European eels *Anguilla anguilla* (Linnaeus, 1758) used in this study were caught from the Turkish waters of Orontes River, Samandag using fyke nets by professional local fishermen. Sampling was carried out monthly for a year and the values were examined seasonally (winter, spring, summer, and autumn). A total of 170 European eels of both sexes were investigated during this study. Samples were frozen in individual plastic bags immediately after capture. After transportation to the laboratory in a cold chain, physical data such as length and weight were recorded. Data concerning the sampling seasons, number of eels examined and their total body length and weight range are reported in Table 1. For fatty acid analyses, fish were eviscerated and filleted. Muscle tissues were analyzed after homogenization in the blender (Retsch, Grindomix GM200, Germany).

Lipid analysis

The modified acid hydrolysis method as in Erkan et al. (2020) was used for this analysis. Approximately 2 to 2.5 g of minced fillet were weighted into beakers and digested with 6 mL concentrated HCl by heating on a hot plate (approximately 80°C) for 90 minutes. The mixture was then transferred to a Mojonnier flask, after which the beaker was rinsed with 7 mL ethanol and 25 mL diethyl ether, and then vigorously shaken. Petroleum ether was added 3 times to the mixture and shaken again. The supernatant was taken and transferred to a pre-weighed flask. The petroleum ether in the flask was evaporated with a rotary evaporator and then dried in an oven for an hour at 105 °C. Flasks were allowed to cool and re-weighed. The amount of fat was calculated from the weight difference as below:

$$Fat\ (\%) = \frac{Fa - Fb}{W}$$

Fa: Weight of the flask after the oven

Fb: Weight of the flask before the oven

W: Weight of the sample

Fatty acid analyses

GC analyses were performed on Claurus 500 GC (Perkin-Elmer/Claurus 500 GC) equipped with an integrated autosampler and flame ionization detector (Awenud-Shelton, USA). Injector temperature was 220 °C and samples were injected with an autosampler (0.5 µL), in triplicate. The SGE BPX70 (SGE Analytic Science, Australia) capillary column had a length of 60 m, with an inner diameter of 0.25 mm, and with a film thickness of 0.25 µm. The temperature program was 120°C rising to 240 °C at a rate of 5 °C/min. The total program process time was 45 min. H_2 was used as carrier gas at a flow rate of 45 mL/min and air was at a flow rate of 450 mL/min. The flame ionization detector (FID) temperature was 240 °C. Lipids obtained after extractions (AOAC, 1998) were turned into corresponding methyl esters of fatty acids (FAMEs) (Ichihara et al., 2002). FAMEs were identified by analyzing a reference material (Menhaden Fish Oil, 47116 Supelco) and standards (Supelco 37 Component FAME Mixture, 47885-U Supelco). Results were expressed as g/100 g fish flesh. Greenfield and Southgate (2003) were used as a reference for the calculation of percent fatty acid contents, as in Özden et al. (2020).

Statistical analysis

Possible differences between means values were analyzed using ANOVA and *post-hoc* comparisons were done by Tukey›s test, with the statistical package SPSS version 28.0 (SPSS Inc., USA). The strength of lipid-length and lipid-weight relationship were evaluated from the determination coefficient (R^2).

RESULTS AND DISCUSSION

The muscle lipid content determined between seasons for the European eel from the Orontes River is shown in Table 2. Lipid values of eels were found to be significantly different in relation to seasons, especially in winter and summer (p<0.05).

Table 2. Lipid content of muscle from European eel caught from Orontes River.

Seasons	Lipids (w.w%)
Winter	30.32 ±0.54^{*a}
Spring	27.90 ±0.46^{ab}
Summer	24.09 ±0.35^{b}
Autumn	23.12 ±0.47^{b}

^{*a,b}:Mean ± standard deviation (S.D), p<0.05.

Table 1. Sample characteristics of the study (n_{total}=170).

Seasons	*n**	Length (Mean cm ± S.D.)	Weight (Mean g ± S.D.)
Winter	37	41.08±4.59^{a}	182.33±114.02^{a}
Spring	58	35.16±0.63^{a}	90.41±4.74^{a}
Summer	42	39.11±3.70^{a}	140.97±57.76^{a}
Autumn	33	38.63±1.01^{a}	130.06±30.19^{a}

* Fish sample (n); S.D. – standard deviation, different letters in the same column indicate differences (p<0.05).

The lipid content of eels tends to increase with age (Van Ginneken et al., 2018). In this study, lipid contents ranged between 23.12% ±0.47 and 30.32% ±0.54 in yearlong sampling. Our findings were comparable to those of Parzanini et al. (2021). According to Ackman (1990), samples with more than 8% fat content are classified as high fat. All eels sampled according to this classification are in the high-fat category. In this study, no correlations were found between lipid values and weight (R^2=0.03) or lipid values and length (R^2=0.06).

Table 3. Seasonal changes in the fatty acid composition of European Eels (*Anguilla anguilla)* from Orontes River.

Anguilla anguilla (g/100 g)	**Seasons (Mean ± SD)**			
Fatty acids	**Winter**	**Spring**	**Summer**	**Autumn**
Lauric acid C12:0	0.12±0.18^a	0.17 ± 0.08^a	0.14±0.20^a	0.06±0.05^a
Tridecanoic acid C13:0	0.05±0.04^a	0.04±0.01^a	0.02±0.03^a	0.03±0.00^a
Myristic acid C14:0	1.00±0.21^a	0.91±0.14^a	0.81±0.07^a	0.72±0.08^a
Pentadecanoic acid C15:0	0.09±0.03^a	0.07±0.02^b	0.07±0.02^a	0.03±0.00^a
Palmitic acid C16:0	5.19±0.32^a	4.65±0.47^b	4.02±0.49^{ab}	3.99±0.60^a
Heptadecanoik acid C17:0	0.13±0.05^a	0.12±0.01^a	0.11±0.03^a	0.09±0.00^a
Stearic acid C18:0	1.15±0.27^a	1.29±0.13^b	1.02±0.14^b	0.67±0.06^{ab}
Behenic acid C20:0	0.24±0.10^a	0.21±0.08^a	0.14±0.07^a	0.22±0.04^a
Tricosylic acid C22:0	*nd*	*nd*	0.03±0.06^a	*nd*
Lignoceric acid C24:0	0.07±0.09^a	0.06±0.04^a	0.04±0.05^a	0.11±0.03^a
SFAs (Total)	8.03±0.69^a	7.50±0.54^b	6.41±0.63^{bc}	5.91±0.58^{ac}
Myristoleic acid C14:1	0.11±0.16^a	0.13±0.11^a	0.14±0.21^a	0.03±0.04^a
Pentadecanoic acid C15:1	0.05±0.03^a	0.05±0.01^a	0.05±0.04^a	0.04±0.05^a
Palimiteloic acid C16:1	2.43±0.25^a	2.12±0.06^a	2.02±0.37^a	1.83±0.46^a
Heptadecanoic C 17:1	0.11±0.08^a	0.10±0.09^a	0.12±0.07^a	0.08±0.01^a
Oleic acid C18:1 (ω-9)	9.95±1.16^a	9.57±0.19^a	6.99±1.00^{ab}	8.07±2.13^{ac}
Vaccenic acid C18:1 (n-7)	0.88±0.05^a	1.10±0.35^a	0.89±0.27^a	0.71±0.04^a
Gadoleic acid C20:1 ω-9	0.30±0.03^a	0.29±0.01^{ab}	0.21±0.04^{ab}	0.26±0.05^{ac}
Erucic acid C22:1 (ω-9)	0.14±0.07^a	0.10±0.05^a	0.07±0.08^a	0.09±0.04^a
Nervonic acid C24:1 (ω-9)	0.10±0.04^a	0.05±0.06^a	0.10±0.07^a	0.03±0.01^a
MUFAs (Total)	14.08±1.25^{ab}	13.53±0.51^a	10.59±1.35^{ab}	11.14±2.62^b
Hexadecadienoic acid C16:2 (n-4)	0.15±0.08^a	0.13±0.04^a	0.10±0.05^a	0.06±0.09^a
Hexadeca-6,9,12-trienoic acid C16:3 (n-4)	0.01±0.01^a	*nd*	*nd*	0.03±0.00^b
Linoleic acid C18:2 (ω-6)	1.01±0.09^a	0.94±0.97^a	1.05±0.25^a	0.48±0.28^a
alfa-Linolenic acid C18:3 (ω-6)	0.04±0.01^a	0.08±0.02^{ab}	0.03±0.01^b	0.03±0.04^a
alfa-Linolenic acid C18:3 (ω-3)	0.18±0.09^a	0.13±0.06^b	0.17±0.03^a	0.05±0.03^b
Eicosadienoic acid C20:2 (ω-6)	0.16±0.04^a	0.07±0.0^a	0.17±0.07^a	0.10±0.03^a
Eicosatrienoic acid C20:3 (ω-6)	0.10±0.08^a	0.12±0.08^a	0.14±0.03^a	0.01±0.00^a
Eicosatrienoic acid C20:3 (ω-3)	0.29±0.17^a	0.28±0.09^{ab}	0.49±0.22^{ab}	0.09±0.03^b
Arachidonic acid C20:4 (ω-3)	0.18±0.31^a	*nd*	0.04±0.06^a	0.33±0.13^a
Docasadienoic acid C22:2 (ω-6)	0.01±0.00^a	0.01±0.01^a	0.01±0.00^a	0.01±0.01^a
Eicosapentaenoic acid C20:5 (ω-3)	0.42±0.11^a	0.56±0.34^a	0.49±0.03^a	0.50±0.09^a
Clupanodonic acid C22:5 (ω-6)	0.05±0.03^a	0.01±0.02^a	0.05±0.03^a	0.02±0.00^a
Clupanodonic acid C22:5 (ω-3)	0.36±0.15^a	0.30±0.17^a	0.37±0.19^a	0.32±0.03^a
Docosahexaenoic acid C22:6 (ω-3)	0.32±0.22^a	0.12±0.05^a	0.42±0.28^a	0.17±0.03^a
PUFAs (Total)	3.29±0.29^a	2.76±0.47^{ab}	3.52±0.69^{ab}	2.19±0.48^b
Unidentified	1.88±0.32^a	1.32±0.39^a	1.16±0.27^a	1.56±0.26^a
Fatty Acids (Total)	27.29±1.78^a	25.11±0.40^b	21.68±2.76^{ab}	20.80±2.98^a
EPA+DHA	0.74±0.31^a	0.68±0.39^a	0.91±0.26^a	0.67±0.06^a
ω-3 (Total)	1.57±0.47^a	1.40±0.52^a	1.94±0.60^a	1.12±0.09^a
ω-6 (Total)	1.37±0.19^a	1.23±1.03^a	1.44±0.35^a	0.65±0.35^a
ω-3/ ω-6	1.14^a	1.14^a	1.38^a	1.72^a

*a, c: Mean ± standard deviation (S.D.), different letters in the same column indicate differences (p<0.05).

As opposed to other fish species, eels store their lipids in between fibers of muscle tissue, and fat can comprise up to 55% of the eel's body (dry) weight (Lovern, 1938; Henderson & Tocher, 1987). Also, for the success of migration and spawning, eels require high-fat content in their muscular tissue (Larsson et al., 1990) and they accumulate these lipids before migration (Durif et al., 2005; Parzanini et al., 2021). This situation is also compatible with our lipid values. As seen in Table 2, before migration, in winter, eels accumulated more lipids (30.32% ±0.54) than in summer and autumn.

Seasonal differences in the total fatty acids composition of samples are presented in Table 3. The major fatty acids in European eels at all seasons were C18:0, C16:0, C22:6 ω-3 (DHA), C18:1 (ω-9), C18:2 (ω-6), and C16:1. In winter and summer, stearic acid (C18:0) values differed ($p<0.05$).

Similarly, McKenzie et al. (2000) stated that the most common FAs in muscle tissue were C16:0 and C18:1 (ω-9). According to our results, palmitic acid was the highest corresponding SFA, contributing approximately 62-67.51% to the total SFA content for eels in all seasons. The highest contents of MUFA was oleic acid [C18:1 (ω-9)] in winter, with 9.95 ± 1.16 g/100 g, and PUFA was linoleic acid [C18:2 (ω-6)] in summer, with 1.05 ± 0.25 g/100 g. Soriguer et al. (1997) reported lower values in European eels than in our study in ω-3 (0.71 g/100 g), ω-6 (0.61 g/100 g), MUFA (3.93 g/100 g), and SFA (2.23 g/100 g). Also, some significant variances in MUFA and SFA values between seasons ($p<0.05$) were observed.

The content of exogenous oleic acid is related to the content of fatty acids in the diet and depends on the metabolism of individual fish species (Ackman, 1990). Most of the MUFA in this study was identified as oleic acid, highest in winter. This is consistent with the hypothesis that in winter, the eels accumulate energy stores before migration (Parzanini et al., 2021). In contrast to this, total PUFAs were not as high as expected in winter. This decrease has been associated with eels' PUFA utilization for gonad maturation (Pérez et al., 2000; Zhou et al., 2011; Kaçar & Başhan, 2017). The contents of C16:3 (n-4) and alfa-Linolenic acid C18:3 (ω-6) PUFAs between winter and summer were significantly different ($p<0.05$). PUFAs such as EPA and DHA in eels are directly associated with their feeding behavior. Vasconi et al. (2019) also reported that lipids from a Mediterranean coastal lagoon appear to be richer in SFA and ω-6 PUFA if compared to fish from lagoons and the open seafood web, which are richer in ARA ($p<0.05$). The fact that some fatty acid values in our samples did not differ significantly according to the seasons is thought to be related to the nutritional habits throughout the year. This has been seen in the case of Japanese eels, which are characterized by the presence of MUFAs as the main fatty acid category instead of PUFAs (Oku et al., 2009).

Marine organisms have relatively smaller amounts of ω-3 PUFAs than freshwater organisms. Freshwater fish are rather better than marine fish at elongating and desaturating shorter fatty acids into longer DHA and EPA and upturning food of low nutritional value into higher value (Moreira et al., 2001). Specifically, freshwater fish contain higher amounts of C-16, C-18, EPA, and DHA and lower amounts of C-20 and C-22 acids compared with marine fish (Ackman, 1967; Wang et al., 1990). In this study, the lowest amount of EPA was in winter with 0.42 ± 0.11 g/100 g. EPA, DHA, linoleic acid and clupanodonic acid were dominant in the PUFAs in all seasons. EPA contributing to the total SFA in muscle tissue of European eels was found to be 12.77%, 20.29%, 13.92%, and 22.83% in winter, spring, summer, and autumn, respectively. Thus, among the ω-3 series, it was determined that European eels are good sources of EPA and DHA throughout all seasons. Gómez-Limia et al. (2021) reported lower values of EPA and DHA for European eels than our study. In this study, no significant difference was determined in the content of EPAs and DHAs between seasons. It was reported that there is also a coordinate relationship between eel size and the fat content of the muscle (Garcia-Gallego & Akharbach, 1998).

The ω-3/ω-6 ratio is used to compare the relative nutritional value of fish oils. Generally, this proportion is extended from 1 to 4 in freshwater fish species, and 5 to 10 in marine fish species (Valfré et al., 2003). This is mainly due to the fact that freshwater fish species feed on plants and marine fish species feed on zooplankton, which are rich in PUFAs (Vlieg and Body, 1988; Gómez-Limia et al., 2021). All of the eels in our study were caught in the freshwater Orontes River. The present data show that the ω-3/ω-6 ratio was 1.14 in winter and spring and 1.38 in summerm and that the highest value 1.72 was in autumn. Gómez-Limia et al. (2021) observed ω-3/ω-6 ratio levels between 1.80 to 2.07 depending on the eel size.

CONCLUSION

Changes in lipids and fatty acids were examined in this year-long study of European eel from the Orontes River. Lipid content was determined to be in the range of 23-30% between seasons and significant differences were observed ($p<0.05$). The ω-3/ ω-6 ratio differed between 1.14 to 1.72, from winter to autumn during sampling. The essential FAs, such as EPA and DHA, are important in a healthy human diet. There were some significant variances ($p<0.05$) between the SFA, MUFA, and PUFA profiles of the muscle tissues. The findings of our research showed that European eels from the Orontes River have high nutritional benefits with their high-quality lipid and fatty acid content.

The overall results of this study revealed that European eels caught from the Orontes River showed consistent high-quality lipid content throughout the year in terms of nutritional content. Stable nutrient content throughout the year is also important in terms of product quality in this fish, which offers delicious and attractive products with its high and quality lipid content. In particular, the measures taken against lipid oxidation enable products with a longer shelf life. In addition, the results of this study will be decisive and informative in terms of revealing the expected nutrient composition in eel culture, which is at the forefront of new species trials in rising trends in aquaculture.

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Research Article

Benthic Macroinvertebrate Fauna of Some High-Altitude Lakes in the Aladağlar Mountains (Niğde)

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ABSTRACT

This study was carried out in July and August 2019 as a preliminary study to determine the benthic macroinvertebrate fauna of Karagöl, Çömçe and Yıldız Lakes, which are high-altitude lakes in the Aladağlar mountains. As a result of the examination of the collected macrobenthic fauna, seven species belonging to three families from three orders (Diptera, Coleoptera and Trichoptera) in Karagöl Lake, four species belonging to three families from two orders (Diptera, Haplotaxida) in Çömçe Lake, and five species belonging to three families from three orders (Diptera, Trichoptera, Haplotaxida) in Yıldız Lake were determined. The taxa detected is a new record for the studied lakes. Shannon-Weaver diversity (H) and Shannon-Evenness density (EH) indices were applied in order to determine the species richness of the lakes and the density relationships among the species, respectively. Accordingly, the highest diversity was observed in Çömçe Lake with a value of 1.18, followed by Karagöl Lake, and Yıldız Lake with values of 0.87 and 0.83, respectively. While the most balanced distribution was observed in Çömçe Lake with a value of 0.81, this was followed by Yıldız Lake and Karagöl Lake, with values of 0.46 and 0.34, respectively. In order to determine the similarities between the stations according to the distribution of the detected taxa, a two-way clustering analysis based on the Bray-Curtis similarity index was applied. Accordingly, while the highest similarity was calculated between Karagöl Lake and Yıldız Lake, it was determined that there was no similarity between Yıldız Lake and Çömçe Lake.

Keywords: Benthic macroinvertebrate, diversity index, high-altitude lake, Aladağlar, Taurus Mountains

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INTRODUCTION

High mountains have extreme climatic conditions, and are important formations in terms of hosting endemic species adapted to these conditions. There are many high-altitude lakes in these systems, which are seasonal or permanent water bodies and important ecosystems in terms of biodiversity (Ustaoğlu et al., 2008).

High-altitude lakes in the Alpine belt are aquatic ecosystems that are very sensitive to environmental changes, as they are isolated from anthropogenic effects due to their location (Williamson et al., 2008). In the mountainous regions where these lakes are located, the temperature, atmospheric pressure and amount of usable land decrease with the increase in altitude. These climatic or spatial differences limit the distribution of populations of species in both terrestrial and aquatic ecosystems. (Dullinger et al., 2012). For this reason, these ecosystems are represented by relatively low numbers of endemic or adapted species to extreme environmental conditions (Galas, 2004; Krno et al., 2006; Sömek & Ustaoğlu, 2016). In this respect, these lakes are reference points in testing the effects of environmental degradation on the ecosystem (Galas, 2004; Taşdemir & Ustaoğlu, 2016).

Benthic macroinvertebrates, which are one of the most important indicators of biological productivity in aquatic systems, form a wide niche in terms of taking place at different nutritional levels. Most of these creatures have a key role in the transfer of matter and energy from the lower steps of the food pyramid to the upper steps in aquatic ecosystems. With these features, these living groups are important biological indicators used to determine the ecological structure, water quality, pollution and eutrophication in aquatic ecosystems (Dügel & Kazancı, 2004; Toksöz & Ustaoğlu, 2005; Akbaba & Boyacı, 2015; Şimşek, 2015). Therefore, the qualitative and quantitative distribution of benthic fauna and the determination of various factors affecting this distribution are important both within the scope of biodiversity and in ecosystem monitoring and index studies (Bayrak, 2015; Kıymaz, 2018; Baydar, 2020). However, limnological studies in our country have generally been carried out on lowland lakes, which provide convenience in terms of transportation. Very few studies have been carried out on glacial or other lakes of different origin located at high altitudes (Balık et al., 2003; Ustaoğlu et al., 2004; Yıldız et al., 2005, 2007; Ustaoğlu et al., 2008; Topkara et al., 2009, 2011; Zeybek et al., 2012; Taşdemir & Ustaoğlu, 2016; Topkara et al., 2018).

The aim of this preliminary study was to determine the benthic macroinvertebrate fauna of Karagöl, Çömçe Lake and Yıldız Lake, which are high-altitude lakes in Aladağlar mountains.

MATERIALS AND METHODS

Aladağlar is a mountainous areas located in the Central Taurus Mountains of the Taurus Mountains, stretching between the Ecemiş Stream in the west and the Zamantı Stream valley in the east. This mountain range is located on the natural border between the Central Anatolia Region and the Mediterranean Region and separates the provinces of Kayseri, Niğde and Adana from each other. It is a unique area of Turkey in terms of natural life and rare species due to its transitional position between these two regions and its highly diverse geomorphological structure (Gürgen et al., 2010; Zreda et al., 2011).

There are many peaks, such as Kızılkaya (3771 m), Demirkazık (3756 m), Kaldı (3734 m) and Emler (3726 m), in Aladağlar, an important mountain range where the highest peaks of the Taurus Mountains are located (Yence, 2019). There are many high-altitude lakes of various sizes on these peaks and these lakes have the characteristics of crater lakes that occur during orogenic and epyrogenic movements, they are very important ecosystems in terms of serving as a shelter for many living groups (Balık et al., 2003). Having a variable climatic structure, Aladağlar has a Mediterranean climate on its southern slopes and a typical continental climate on its northern slopes (Toroğlu & Ünaldı, 2008).

Within the scope of this study, field studies were carried out in July and August 2019 to determine the benthic macroinvertebrate fauna of three high-altitude lakes in the Aladağlar mountains. Location maps and general information about the study areas are given in Figure 1 and Table 1.

The benthic macroinvertebrate samples were made from the littoral parts of the lakes using a dip net with a 500 μm mesh, depending on the multihabitat sampling method. The benthic macroinvertebrate samples obtained were cleared of their mud and fixed in plastic drums containing 4% formaldehyde solution and transported to Nevşehir Hacı Bektaş Veli University Hydrobiology Research Laboratory. First, systematic separation of the samples brought to the laboratory was made under the stereomicroscope, and then diagnostic procedures were completed using the light microscope to the lowest possible systematic category. For taxonomical identification of the specimens, the following were used: for Coleoptera, Illies (1955) and Brauer (1909);

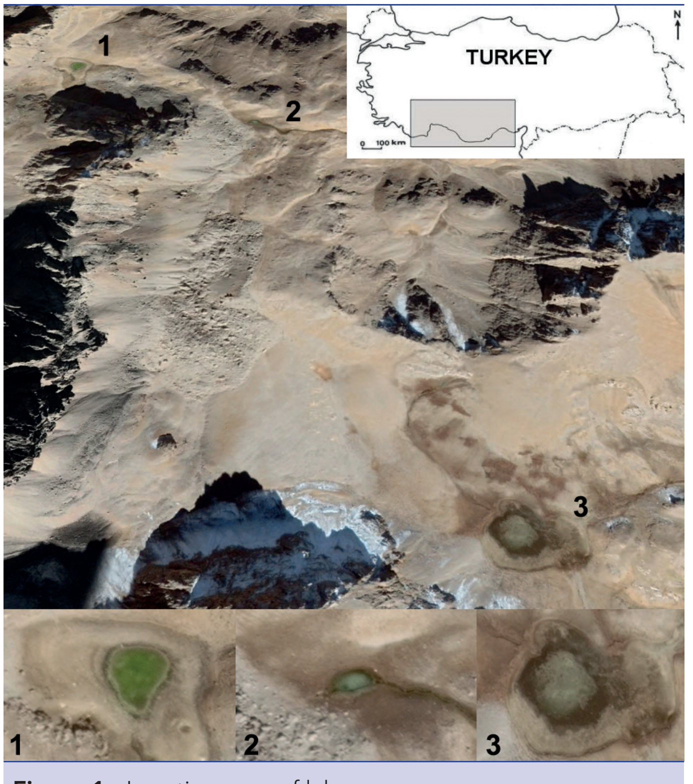

Figure 1. Location map of lakes.

Table 1. Information on the studied lakes

No	Lake	Altitude	Coordinate	Characteristic	Surface area (m²)
1	Yıldız Lake	3084	37°51′37.46″K-35°11′27.72″D	Seasonal-bottom structure muddy	16024
2	Çömçe Lake	2974	37°52′17.23″K-35°11′07.68″D	The permanent-bottom structure is muddy and aquatic plants are present.	356
3	Karagöl Lake	2876	37°52′25.94″K-35°10′48.17″D	Permanent-bottom structure muddy	1626

for Trichoptera, Ulmer (1961), Jansson & Vuoristo (1979), Brohmer (1979), Morse (1983) and Wallace et al., (1990); for Diptera, Şahin (1987, 1991) and Pennak (1989); for Haplotaxida, Brinkhurst & Jamieson (1971), Brinkhurst & Gelder (1991), Milligan (1997), Timm (1999) and Wetzel et al. (2000).

Regarding the benthic macroinvertebrate groups: Dominance analysis (Kocataş, 1997) was used to examine the community structure, and two-way cluster analysis based on the Bray-Curtis similarity index (Bray & Curtis, 1957) was applied to determine the distinctions between stations depending on the distribution of species. Shannon-Weaver diversity and Shannon-Evenness density indices (Shannon & Weaver, 1949) were applied, respectively, to determine species diversity and density relationships among species. Analyses were carried out using PAST 3.0, CAP 4.0 and PC-ORD software packages.

RESULTS AND DISCUSSION

As a result of the sampling studies, a total of 1297 individuals were examined and as a result of the diagnoses, 11 species belonging to five families and 11 genera were identified in four orders (Diptera, Coleoptera, Trichoptera, Haplotaxida) (Table 2).

Among the 1297 individuals examined, the highest dominances (914 individuals (70.47%); 211 individuals (16.27%); 157 individuals (12.10%); 15 individuals (1.16%)) were observed in Trichoptera, Haplotaxida, Diptera and Coleoptera orders, respectively.

According to the evaluation made in the species category, the most dominant species was *L. bipunctatus* (70.47%), followed by *U. uncinata* (13.88%). The lowest dominance was found in the taxa of *T. tubifex* (0.31%) and *P. limbatellus* (0.62%).

Regarding the distribution of macrobenthic fauna in the studied lakes: in Karagöl Lake, 465 individuals were examined and seven species were identified from three families (Chironomidae, Dytiscidae and Limnephilidae) in three orders (Diptera, Coleoptera and Trichoptera). Among the examined individuals, the highest dominance (78.71%) was observed in *L. bipunctatus*, while the lowest dominance (1.29%) was detected in *M. nebulosa*. In Çömçe Lake, 54 individuals were examined and four species were identified from three families (Chironomidae, Naididae, Enchytraeidae) in two orders (Diptera, Haplotaxida). Among them, the highest dominance was observed in *C. sphagnetorum* (50.00%), while the lowest dominance was detected in *T. tubifex* (7.41%). In Yıldız Lake, 778 individuals were examined and five

Table 2. Taxa distribution of lakes and calculated index values (*: Tolerance level evaluated at genus level)

Systematic Categories	Lakes			Tolerance	Reference
	Karagöl Lake	Çömçe Lake	Yıldız Lake		
Diptera					
Chironomidae					
Virgatanytarsus arduennensis (Goetghebuer, 1922)	+				
Chironomus tentans (Fabricius, 1805)	+		+		
Micropsectra paraecox (Wiedemann, 1818)	+	+		7*	Bode et al., 2002
Macropelopia nebulosa (Meigen, 1804)	+		+		
Procladius sp.	+		+	9	Bode et al., 1996
Psectroladius limbatellus (Brundin, 1949)		+			
Coleoptera					
Dytiscidae					
Porhydrus lineatus (Fabricius, 1775)	+				
Trichoptera					
Limnephilidae					
Limnephilus bipunctatus (Curtis, 1834)	+		+	3*	Bode et al., 1996
Haplotaksida					
Naididae					
Tubifex tubifex (Müller, 1774)		+		10	Bode et al., 1996
Uncinais uncinata (Ørsted, 1842)			+		
Enchytraeidae					
Cognettia sphagnetorum (Veydovsky, 1878)		+			
Number of Species	7	4	5		
Species Number of Individuals	465	54	778		
Calculated Indexes					
Shannon-Weaver (H) Diversity	0.867	1.178	0.830		
Shannon Evenness (EH) Density	0.340	0.812	0.458		

species were identified from three families (Chironomidae, Naididae, Limnephilidae) in three orders (Diptera, Trichoptera, Haplotaxida). Among them, the highest dominance was observed in *L. bipunctatus* (70.44%), while the lowest dominance was detected in *C. tentans* (1.54%).

In determining the diversity of an ecosystem, the number of species in that ecosystem and the number of individuals attached to each species are very important (Magurran, 1988). When the species richness in the habitats is compared, the highest diversity belongs to Karagöl Lake, with seven species, followed by Yıldız Lake, with five species. The lake with the lowest diversity was determined to be Çömçe Lake, which is represented by four species. According to the results of Shannon-Weaver diversity index (H) calculated using the abundance values of the determined macrobenthic fauna, the highest diversity was observed in Lake Çömçe with a value of 1.18, followed by Karagöl Lake and Yıldız Lake with values of 0.87 and 0.83, respectively. According to the results of Shannon Evenness density index (EH), in which the balance-equality values was calculated depending on the distribution of the species, the lake with the most balanced distribution was Çömçe Lake with a value of 0.81, followed by Yıldız Lake and Karagöl Lake with values of 0.46 and 0.34, respectively (Table 2 and Figure 2).

The differences in the calculated diversity values between stations that are the same in terms of species richness are due to the differences in the distribution characteristics of the species that are there. Likewise, the stations that have a higher ratio in terms of H value, which expresses diversity, however many lower species they contain, are also related to the distribution characteristics of the individuals belonging to the species that are found there. Therefore, although the species richness of Çömçe Lake is lower than that of Yıldız Lake and Karagöl Lake, the higher calculated diversity value (H) is explained by the more balanced distribution of the species found there. As a matter of fact, according to the Shannon Evenness index (EH) results applied to calculate the balance-equality characteristics of the species belonging to the lake ecosystems, it was observed that the EH value calculated for Çömçe Lake was 0.81, while this value was calculated 0.46 and 0.34 in Yıldız Lake and Karagöl Lake, respectively.

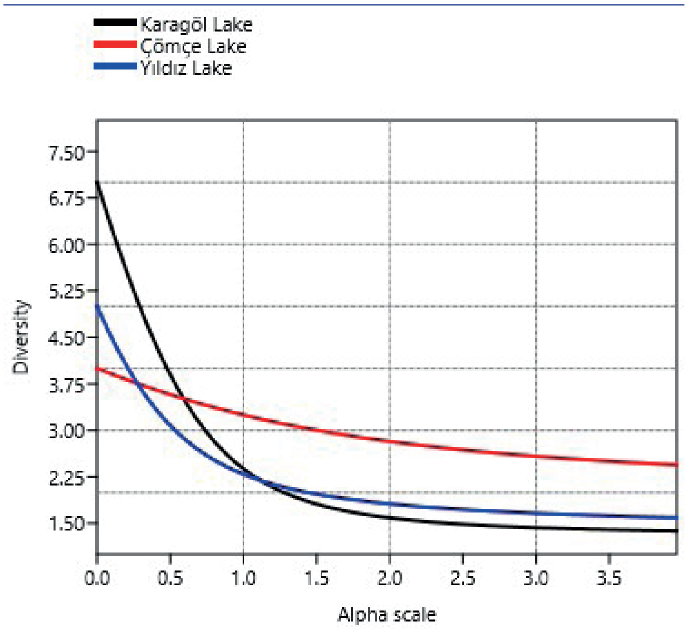

Figure 2. Diversity profile of lakes.

It is known that the diversity and density of macrobenthic fauna elements are low in limnological studies on mountain lakes (Rieradevall et al., 1999). This is in agreement with the findings in our study.

According to the results of the applied two-way cluster analysis; while the highest similarity was calculated between Karagöl Lake and Yıldız Lake with a rate of 0.65%, it was determined that there was no similarity between Yıldız Lake and Çömçe Lake (Table 3, Figure 3 and Figure 4). Regarding the distinctions among lakes, the following were determined to be distinctive taxa: for Karagöl, *V. arduennensis* and *P. lineatus*, for Çömçe Lake, *P. limbatellus*, *T. tubifex* and *C. sphagnetorum* and for Yıldız Lake, *U. uncinata*.

Table 3. Similarity rates between stations (Bray-Curtis)

	Karagöl Lake	Çömçe Lake	Yıldız Lake
Karagöl Lake	1		
Çömçe Lake	0.03	1	
Yıldız Lake	0.65	0.00	1

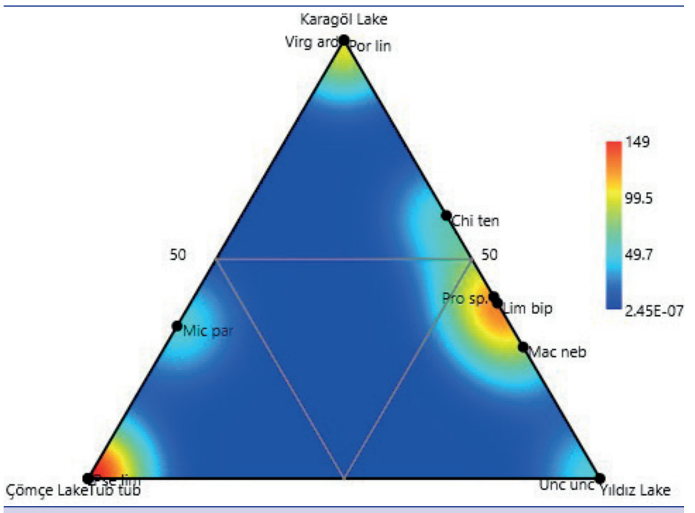

Figure 3. Distribution of detected taxa in lakes (*Virg ard: Virgatanytarsus arduennensis, Chi ten: Chironomus tentans, Mic par: Micropsectra paraecox, Mac neb: Macropelopia nebulosa, Pro sp.: Procladius sp., Pse lim: Psectroladius limbatellus, Por lin: Porhydrus lineatus, Lim bip: Limnephilus bipunctatus, Tub tub: Tubifex tubifex, Cog sph: Cognettia sphagnetorum, Unc unc: Uncinais uncinata*).

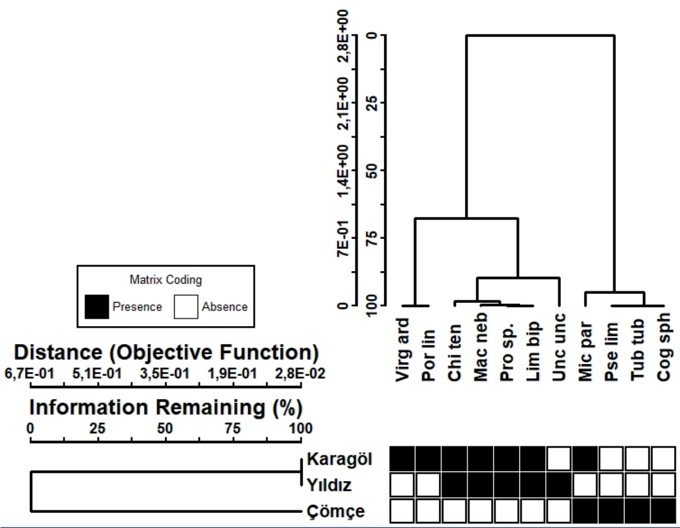

Figure 4. Two-way cluster cluster dendogram of lakes (Bray-Curtis).

Within the scope of the study, the most dominant group in terms of number of individuals is the Trichoptera order with a dominance rate of 70.47%. The members of this order are known for their high population rates in freshwater habitats and their low tolerance for organic pollution. Therefore, the members of this order are important biological indicators used in water quality determination studies (Wiggins & Mackay, 1978; Bouchard, 2004). In our study, *L. bipunctatus* (tolerance level=3) (Bode et al., 1996) species belonging to the Limnephilidae family determined in this order was observed as the dominant species in Karagöl Lake and Yıldız Lake. The species belonging to the genus *Limnephilus* are generally the species that can live in lakes and are widely distributed in Palearctic and Holarctic regions (Sipahiler, 2010). Records of *L. coenosus* and *L. lunatus* species belonging to this genus were given in some lakes on the Taurus Mountains (Topkara et al., 2009; Zeybek et al., 2012). *L. bipuctatus* larvae usually live in stagnant or slowly flowing waters such as ditches, streams and small rivers, and their feeding areas can be permanent or temporary (Wallace et al., 2003). In our study, this species was found in muddy soils with stone and rocky biotopes.

The order represented as the second dominant group in terms of the number of individuals was observed as Haplotaxida, with a dominance rate of 16.47%. The taxa belonging to this order are generally cosmopolitan species and their records have been reported from some high mountain lakes in Turkey (Brinhurst, 1969; Geldiay & Tareen, 1972; Milbrink, 1980; Ustaoğlu, 1980; Taşdemir et al., 2004; Yıldız & Ustaoğlu, 2016; Topkara et al., 2018). The species identified in this order in our study belong to the families of Nadidae and Enchytraeidae. The species belonging to the Nadidae family are known to have high ecological tolerance (Brinkhurst and Jamieson, 1971). The *T. tubifex* species, which was determined to be in the Nadidae family, was only observed with four individuals in Lake Çömçe. It is known that the *T. tubifex* species (tolerance level=10) is abundant in aquatic systems with high pollution level and organic matter input (Bode et al., 1996; Yıldız, 2003). It is thought that this species is represented by few individuals in Çömçe Lake because the lake water exhibits oxygen-rich clean water and there is no organic pollution due to the absence of any livestock activities around the lake. While many species belonging to the order Haplotaxida have been reported from some lakes on the Taurus Mountains, records of *T. montanus*, *T. nerthus*, *T. bergi* and *T. ignotus* taxa belonging to the *Tubifex* genus have also been given (Yıldız et al., 2005, 2007). *U. uncinata* species, which was determined to be in the same family, was only found in Yıldız Lake. This species is known for its high population rates in freshwater ecosystems (Baturina, 2012). The *C. sphagnetorum* species, which was determined to be in the Enchytraeidae family, was observed only in Çömçe Lake. This species, which is known to have a very high ecological tolerance, is known to spread in coastal areas in fresh water and rarely in the bottom water of oligotrophic lakes (Zeybek et al., 2016).

In our study, six species belonging to Chironomidae family were identified in the Diptera order, which is represented by the highest species richness. Chironomidae species have a cosmopolitan distribution and show a wide distribution in many environments, from clean water to very polluted water (Stribling et al., 1998). The observation of the members of this family with high density in aquatic systems allows to make an evaluation about the heavy metal accumulation in the environment. Records belonging to this family have been reported from some mountain lakes in Turkey (Ustaoğlu et al., 2008; Taşdemir & Ustaoğlu, 2016; Topkara et al., 2018). In our study, *C. tentans*, *M. nebulosa* and *Procladius* sp. species identified from this family were found in Karagöl Lake and Yıldız Lake, *M. paraecox* species were detected in Karagöl Lake and Çömçe Lake, *V. arduennensis* was only detected in Karagöl Lake and *P. limbatellus* was only detected in Çömçe Lake. It is known that *C. tentans* is generally distributed in a wide tolerance range (tolerance level=10) in eutrophic lakes (Hilsenhoff, 1987; Bode et al., 1996; Kazancı et al., 1997; Ayık, 2006). In our study, this species was found in shallow water on the shores of lakes. It is known that *M. praecox* (tolerance level=7) and *M. nebulosa* species are commonly found in lakes with moderate oligotrophic properties (Bode et al., 2002; Kökçü, 2016). It is known that *Procladius* sp. has a high tolerance range (tolerance level = 9) and is distributed in slow flowing or shallow regions of aquatic systems (Bode et al., 1996; Taşdemir et al., 2010). It is known that *P. limbatellus* can adapt to different environmental conditions and can spread in different habitats (such as stagnant or flowing waters and rackish waters) (Taşdemir et al., 2010). In some lakes on the Taurus mountain range, the taxa of *C. plumosus*, *C. thummi*, *C. tentans*, *C. viridicollis* and *C. anthracinus* belonging to the *Chironomus* genus, *M. notescens* and *M. junci* belonging to the *Micropsectra* genus and *Procladius* sp belonging to the *Procladius* genus have been reported (Yıldız et al., 2005; Taşdemir et al., 2011).

Dytiscidae members identified in the Coleoptera order are generally adapted to all aquatic habitats (Nilsson 1996). Records of many taxa belonging to this order have been reported from mountain lakes in Turkey (Ustaoğlu et al., 2008; Topkara & Ustaoğlu, 2011, 2015; Zeybek et al., 2012; Topkara et al., 2018). In our study, the *P. lineatus* detected in this family was determined to be from the regions where vegetation is abundant in Çömçe Lake. In a study on the Taurus mountains, the record of this species was reported (Topkara et al., 2009).

Although there are some studies on the determination of the macrobenthic fauna of some lakes in the Taurus mountain range, there is no study on the determination of the macrobenthic fauna in the lakes included in this study (Sipahiler, 1992; Balık et al., 2003; Ustaoğlu et al., 2004; Yıldız et al., 2005, 2007; Topkara et al., 2009; Taşdemir et al., 2011; Yence, 2019). Therefore, the taxa detected is a new record for the relevant lakes.

CONCLUSION

It is important to determine the biological diversity of high-altitude lakes, which are special ecosystems, by increasing the number of studies done on them. The results obtained from this study are a prestudy in determining the biodiversity in lakes. In a study with a longer sampling period in which all the high-altitude lakes in the Aladağlar mountains are included, it will be of great importance to evaluate the macrobenthic fauna of these lakes together with the physicochemical parameters of the lakes and to reveal them fully. In addition, it should not be forgotten that these ecosystems should be protected and the negative effects that may occur on the existing fauna and flora should be taken into account against possible future ecotourism activities.

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