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The Investigation of Bioindicator Bacteria in the Supralittoral Zone Sands and Seawater in the Sea of Marmara, İstanbul Province

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Abstract Investigation of the presence of bioindicator bacteria in the seas is important in terms of detecting bacteriological marine pollution due to human activities and as an indicator of the possible presence of pathogenic bacteria in the environment. This study definitively determined bio-indicator bacteria levels in the coastal area of the Sea of Marmara, İstanbul province, over a 1-year period from June 2021 to May 2022. The study was conducted seasonally, and samples were taken from eight heavily used swimming areas and the beach sand. The measurement of the variable environmental parameters – temperature, salinity, dissolved oxygen and pH – was conducted in situ. Sea water samples collected from the supralittoral zone under aseptic conditions were taken from the surface (0-30 cm) and sand samples were taken from 0-50 cm. In bacteriological analyses, total coliform, fecal coliform and intestinal enterococci were investigated using membrane filtration methods. Heterotrophic aerobic bacteria (HAB) levels were determined by the spread plate method using marine agar. Using descriptive statistical analysis and independent samples t-tests, the variability and significance of differences in bacterial indicator levels were assessed between beach sand and seawater samples. During the study, the higher bacteria levels were detected in beach sands than in sea waters. The annual indicator bacteria levels obtained from seawater and supralittoral zone sands were recorded at a maximum of 72.540 CFU/100 ml (the SD for total coliform was ± 0.53) at Kabakoz Beach in July 2021 and 322.400 CFU/10 g (the SD for total coliform was ±0.75) at Elmas Kum and Poyraz Beaches in July and August 2021, and a minimum of 78 CFU/100 ml (the SD for intestinal enterococci was ± 0.46) in winter season (January 2022) at Güneş Beach and spring season (May 2022) at Caddebostan Beach and 234 CFU/10 g (the SD for fecal coliform was ± 0.75) in winter season (January 2022) at Büyük Liman Beach, respectively. The annual HAB levels in seawater and supralittoral zone sands reached up to 98×10^{15} CFU/ml in May 2022 at Menekşe Beach and 59×10^{17} CFU/10g in August 2021 at Çiroz Beach, with the lowest values recorded as 1×10^{12} CFU/ml in winter 2022 at Çiroz Beach and 35×10^{13} CFU/g in July 2021 at Poyraz Beach, respectively. The obtained bacteriological data clearly reflect the extent of bacterial pollution. Moreover, statistically significant differences were observed between stations even within the same season. Dissolved oxygen levels decreased markedly during the summer months across all stations, while bacterial indicator levels showed a corresponding increase. This trend was particularly pronounced at Kabakoz, Elmas Kum, and Güneş beaches. Increases in sea water temperature, changes in pH and salinity, geographical location and human-induced terrestrial effects are thought to influence the degree of bacterial contamination of beaches. This situation show that beach sand may pose a higher biological risk of microbial contamination than seawater, as bacteria are less exposed to sunlight, retain moisture longer, and allow organic matter to accumulate. The high bacterial levels detected in this study indicate a potential bacterial risk to public and ecosystem health in coastal areas. These first regional data obtained from the supralittoral zone showed that the supralittoral zone creates suitable habitats for bacteria and that this humid zone, where seawater and land interact, has the potential for bacteriological risk, especially for children's activities. The study finding contributed first data on presence of bioindicator bacteria of supralittoral zone sands in the coastal areas of the Sea of Marmara İstanbul Province.

Keywords Indicator bacteria · heterotrophic bacteria · supralittoral zone · sea water

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INTRODUCTION

The coastal area of the Sea of Marmara İstanbul Province is under the influence of different pollution pressures such as intense population growth, domestic and industrial wastewater agricultural activities, animal breeding activities, ship transportation, construction, animal sourced feces (seagulls, dogs, etc.). In addition, there are studies showing that pollution inputs entering the İstanbul Strait (Bosphorus) with the upper current from the Black Sea reach the Sea of Marmara (Altuğ & Filik, 2002; Balkis et al., 2007; Çiçekalan & Öztürk, 2017; Güven & Süngü, 1993; Kut et al., 2000; Okus et al., 2008; Öztürk & Şeker, 2021; Topçuoğlu et al., 1990). As a result of all these effects, the mucilage seen in the Marmara Sea in 2021 shows the point where the threat posed by pollution pressures has come.

Moreover, bacteriological studies of sand samples collected from coastal areas across the globe have revealed that it poses a significant threat to ecosystems and public health. The present studies investigated the extent to which the bacteriological quality of sand in recreational coastal areas poses a threat to human health (Efstratiou, 2019; Halliday & Gast, 2011; Heaney et al., 2009; Solo-Gabriele et al., 2016; Subhan et al., 2018; Whitman et al., 2014; WHO, 2003).

It has been established that the presence of bacteria in coastal areas is attributable to a variety of factors, including precipitation, aged structures (sewage), tides, and construction (limification) (Halliday & Gast, 2011). The reasons for the increase in the number of fecal pollution indicator bacteria in the coastal sands used for swimming purposes are; the sand contains oxygen-free cavities; sea water is filled with oxygen; Most fecal bacteria from the intestine can be found in higher concentrations on beach sands due to their preference for oxygen-free environments. Bacteria form biofilms by adhering to sand particles. Wastewater bacteria can easily be embedded with biofilm in the sand that provides shelter to the bacteria. Sunlight, on the other hand, can affect light-sensitive molecules such as riboflavin and porphyrins found in aerobic heterotrophic bacteria, causing them to die. In this case, sand grains protect bacteria from the sun and allow easy access to nutrients (Zhang et al., 2015). Human skin is one of the most important targets for pathogenic bacteria found on beaches. In this regard, it is reported that about 24% of the UK civilian population suffers from a pathological skin condition every year. In 2009-2010, 900,000 patients in the UK were referred to dermatologists (Tanzer et al., 2014).

Dunes, which are open to environmental parameters, especially recreational areas frequently used by people such as beaches, come to the fore today in order to be closely examined in terms of the risk of bacterial infection, to understand the possible impact on sensitive groups and the risks in common areas, and to support the legislative studies to be carried out in the future (Adeniji et al., 2019; Gangneux et al., 2024; Halder et al., 2024). The Bathing Water Directive (BWD) (2006/7/EC) is define based on the identification and enumeration of some fecal coliforms and enterococci's as fecal indicator parameters, although BWD does not mention regulatory parameters in sand. Led to understand and monitor sand based risk keeps in the background (ANON, 2006). Similarly, in our country, in line with the Regulation on the Management of Bathing Water Quality, there are no monitoring parameters for sea sand, and it is seen that some of the marine bacteriological monitoring are considered in the bathing areas, and the risks in the dune area cannot be considered (ANON, 2015).

It is known from the literature studies that bacterial studies are used as an indicator group to understand pollution and anthropogenic effects in terms of environment and public health. In the sampling of areas that are predicted to be exposed to anthropogenic effects, the change of total coliform, fecal coliform and intestinal enterococcus on the environment with bacterial indicators is examined and the pressure on the environment is understood (Akita et al., 2021).

Studies investigating the presence of bio-indicator bacteria and the presence of pathogenic bacteria in seawater samples taken from the areas used for recreational purposes in the coastal area of the Sea of Marmara, İstanbul Province and from the stations selected from the points close to the pollution inputs show the presence of bacteria above the national and international limit values in these regions (Altuğ, Gürün, Çiftci, et al., 2016; Altuğ & Hulyar, 2020; Gürün & Kimiran-Erdem, 2013)

The studies to determine the presence of supralittoral zone bioindicator bacteria in marine areas of the Sea of Marmara İstanbul Province are limited. Additionally, there is no data documented related to bacterial load of supralittoral zone sands. In this study, with the aim of providing the first data on bacterial pollution related to land-sea interaction in terms of human and ecosystem health, the presence of bioindicator bacteria was investigated in beach sand and seawater samples belonging to the supralittoral zone of beaches where human activities are carried out for recreational purposes in the Sea of Marmara at the border of İstanbul province.

MATERIALS AND METHODS

Sampling

Seawater and supralittoral zone sand sampling was carried out monthly (in June, July, August) during the summer period of 2021. Autumn sampling was carried out in September 2021. Winter sampling was carried out in January 2022 and spring sampling was carried out in May 2022.

The samples were taken under aseptic conditions, transferred to sterile containers and delivered to the laboratory with cold chain on the same day.

Sea water samples were taken from 0-30 cm surface into sterile glass bottles, sand samples were taken with a clean stainless-steel spatula and taken into sterile containers. All samples were brought to İstanbul University Faculty of Aquatic Sciences, Marine Bacteriology Laboratory on the same day with the cold chain.

Statistical Analyses: To understand one way and two-way variance analysis, it used to open-sourced R language Companion to Applied Regression “car” library (Fox & Weisberg, 2019; R Core Team, 2024) and figures is prepared via open sourced Phyton programme (*Python (Version 3.11)*, 2023). The visualizations and graphics to figure 6 and 7 were created using Matplotlib and Seaborn (Hunter, 2007; Waskom, 2021), and statistical tests were performed using SciPy (Virtanen et al., 2020) and Stats Models (McKinney, 2010).

Variable Environmental Parameters

Temperature (°C), salinity (‰), pH and dissolved oxygen (mg/l) values of seawater at the determined 8 stations were measured with a multiparameter device (YSI 556, USA) during sampling. The stations sampled are summarized in Table 1 and shown in Figure 1.

Table 1.

The stations and coordinates where samples were taken

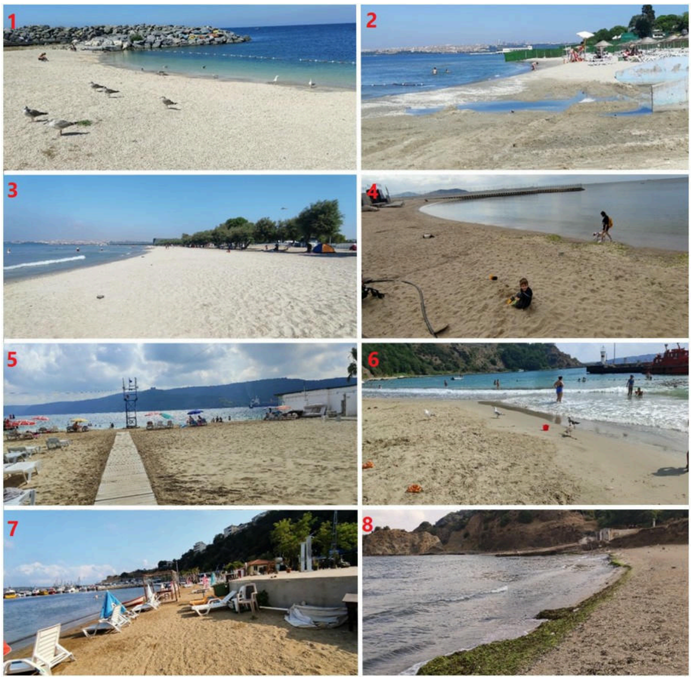
NO	STATIONS	COORDINATES
1.	Çiroz Beach, Yesilkoy	40.9592855,28.8096427,17
2.	Güneş Beach, Florya	40.9711129,28.7709726,14
3.	Menekşe Beach, Florya	40.9789618,28.7691549,16.96
4.	Caddebostan Beach, Caddebostan	40.9633347,29.0411516,14
5.	Elmas Kum Beach, Sariyer	41.1838506,29.0736102,17
6.	Büyük Liman Beach, Sariyer	41.2040217,29.0975133
7.	Poyraz Beach, Beykoz	41.2047222,29.0660265,13
8.	Kabakoz Beach, Şile	41.1590079,29.6894408,14

Figure 1.

Google Earth image of 8 stations where sea water and beach sand samples were taken from Sea of Marmara İstanbul Provincial Border, 2022

Figure 2.

*Sampling stations; **1)** Çiroz Beach, **2)** Güneş Beach, **3)** Menekşe Beach, **4)** Caddebostan Beach, **5)** Elmas Kum Beach, **6)** Büyük Liman Beach, **7)** Poyraz Beach, **8)** Kabakoz Beach*



Conditions Observed at the Stations During Sampling

Çiroz Beach 1, This beach, which is used for recreational purposes, is a public beach used by many people for recreational purposes and where they swim in the sea. There are settlements around it and a fisherman's shelter about 500 m away. It has been observed that there is a pipeline directly connected with seawater right next to this beach and it is frequented by seagulls that have the potential to carry bacteria from waste areas to the beaches.

Güneş Beach (2): It is a large beach in Florya, which is preferred by many people and has an entrance fee. There is a hotel right next to it, and it has been observed that there is a passage with a puddle of water that divides the beach into two.

Menekşe Beach (3): It is a public beach close to Küçükçekmece lake, on the road and used by people for both resting and swimming.

Caddebostan Beach (4): It is connected to the Kadıköy district on the Anatolian side of İstanbul, it was closed for swimming purposes in the summer months and for swimming purposes by the Provincial Directorate of Health in June 2021 due to the presence of bacteria above the limit values. It was put into use in the following months, and it was observed that there was a density.

Elmas Kum Beach (5), The European side is a paid beach located in the Sarıyer region and is heavily preferred by people.

Büyük Liman Beach (6): Located close to the Black Sea in the North of the Bosphorus right next to the 3rd bridge on the European side, this paid beach is a little far from the settlements compared to the other beaches and it is seen that there is a small harbor right next to it.

Poyraz Beach (7): It is a public beach on the Anatolian side, adjacent to the 3rd bridge, with a small harbor right next to it and used by people for swimming purposes.

Kabakoz Beach (8): It is a public beach located between the Bosphorus exit and the Black Sea on the Anatolian side, a little far from the settlements. There was a stream right next to the beach that merged with the sea, and smelled bad during the work.

Bacteriological Analyses

Bacterial analyses which total coliform, fecal coliform and intestinal enterococci were performed according to the membrane filtration method. The determination of heterotrophic aerobic bacteria was carried out using the spread plate method on marine agar according to the American Public Health Association (APHA, 1998).

Indicator Bacteria Analyses

In the indicator bacterial analysis, dilutions of seawater samples up to 10^{-8} were prepared with sterile phosphate buffered distilled water. For beach sand analysis, 10 g of the sand samples were weighed and added to 50 ml sterile distilled water, shaken and dilutions up to 10^{-8} were prepared with sterile phosphate buffered distilled water (Sabino et al., 2011).

The sterile filter device (Sartorius) was connected to the vacuum pump and membrane filters with a pore diameter of 0.45 µm were aseptically placed in the filter device. The sample of each station, which was filtered with membrane filters, was placed in ready-made dehydrated media (3 ml) soaked at the time of operation with sterile distilled water in the cabin.

The media used are respectively; m-ENDO-NKS (Sartorius, Germany) for total coliform, m-FC-NKS (Sartorius, Germany) for fecal coliform, Azide-NKS (Sartorius, Germany) for intestinal enterococcus.

After the seawater and beach sand samples were reconstituted with phosphate buffer, they were filtered under vacuum through sterile filters (Sartorius) with a pore diameter of 0.45 µm, two repeats for each sample. Afterwards, m-MFC-NKS and m-Endo-NKS media placed in the filters were incubated at 45.5±0.2 °C for 24 hours. The m-Azide-NKS medium placed in the filters was incubated at 37±0.2 °C for 24 hours. After incubation, the average of the two repetitions was taken of the colonies.

Heterotrophic Aerobic Bacteria Analyses

For the analysis of heterotrophic aerobic bacteria in the Sea water samples taken from the stations under sterile conditions were taken at 0.1 microliters and dilutions up to 10^{-11} were prepared with previously prepared phosphate buffered distilled water. For the analysis of heterotrophic aerobic bacteria in beach sand, 10 g of sand samples taken from stations under sterile conditions were weighed and 0.1 µl microliter was taken from the sample prepared by shaking in 50 ml sterile distilled water and dilutions were prepared in the range of 10^{-10}–10^{-12} with sterile phosphate buffered distilled water (Sabino et al., 2011).

The final dilutions of the samples were poured 0.1 ml on the surface of the Marine Agar medium, which had been left to dry in the refrigerator beforehand and smeared with a sterile glass rod.

Petri dishes were kept at 22 ±0.1 °C for at least 48 hours). The total number of heterotrophic aerobic bacteria in 1 ml of seawater was counted by multiplying the number of colonies that grew after the waiting period by the dilution factor (Rice et al., 2017). The count was multiplied by the dilution factor, and the results were recorded as a colony formed unit at CFU/ml. After incubation, the average of the two repetitions was taken of the colonies.

RESULTS

Between June 2021 and May 2022, total and fecal coliform, fecal streptococcus and total heterotrophic bacteria (HAB) determinations were made in sea water and beach sand samples taken from 8 stations determined on the Sea of Marmara İstanbul Provincial Border.

The lowest total coliform bacteria count in the sea water samples was recorded as 130 CFU/100 ml in September (autumn) 2021. The highest total coliform bacteria count in the sea water samples was recorded to be 72,540 CFU/100ml in the July 2021. The lowest fecal coliform bacteria count in sea water samples was recorded as 234 CFU/100 ml in the September 2021 (autumn). The highest fecal coliform bacteria count in seawater samples was found to be 6,470 CFU/100 ml in the August 2021. The lowest intestinal enterococci count was recorded as 78 CFU/100 ml in winter season (January 2022) and spring season (May 2022). The highest intestinal enterococci count was recorded as 5,356 CFU/100ml in the July.

While the highest total coliform counts in supralittoral sand samples was determined as 322.400 CFU/10 g in July-August 2021 sampling, the lowest total coliform count was determined as 780 CFU/gr in January 2022 sampling. While the highest fecal coliform count of beach sand was recorded as 181.350 CFU/10 g in July 2021 sampling, the lowest fecal coliform count was defined as 234 CFU/10 g in May 2022 sampling. The highest intestinal enterococci count in beach sand was determined as 64.610 CFU/10 g in May 2022 sampling, while the lowest intestinal enterococci count in beach sand was determined as 260 CFU/10 g in January-May 2022 sampling (Figure 3).

On the X-axis, measurements between numbers 1 - 6 represent seawater samples, while measurements between numbers 7 - 12 represent sand samples. The numbers are presented in the order of the sampling period (June, July, August, Autumn, Winter, Spring). In addition, the horizontal red line represents the legal or reference limit values for each variable (2.69 for fecal coliform, 3.0 for total coliform and 2.0 for intestinal enterococci).

During the study, the highest total heterotrophic aerobic bacteria (HAB) count was determined as 98x10^{15} CFU/ml in the May (spring) 2022 sampling of sea water at Menekşe Beach, while the lowest count was determined as 1x10^{12} CFU/ml in the June 2021 sampling of sea water at Caddebostan Beach (figure 4).

While the highest total heterotrophic aerobic bacteria (HAB) level was defined as 59x10^{17} CFU/g in August 2021 sampling of beach sand at Çiroz Beach, the lowest value was defined as 35x10^{13} CFU/g in June 2021 sampling of beach sand at Poyraz Beach (figure 5). With these data obtained, it was shown that supralittoral zone sand can harbor bacteria higher than seawater.

Figure 4. Presence of heterotrophic aerobic bacteria (HAB) in sea water samples taken from beaches in the Marmara Sea in the 8 stations we determined in Istanbul Province between June 2021 and May 2022.

Figure 5. Presence of heterotrophic aerobic bacteria (HAB) in sand samples taken from beaches in the Marmara Sea in the 8 stations we determined in Istanbul Province between June 2021 and May 2022.

Bacterial indicators were evaluated based on ANOVA according to seasons and stations. The results shown in Figure 6.

These F-values, which are around 3, indicate moderate but meaningful differences among the seasonal groups. Among them, IE shows the highest F-value, suggesting that its seasonal variation is slightly more pronounced compared to FK and TK. While the differences are statistically significant, the F-values are not very large (e.g., >10), indicating that the group means differ but not drastically. The accompanying boxplots visually support this: seasonal shifts in distribution can be seen, particularly when grouped by habitat type (sea water vs. supralittoral sand). Red asterisks and connecting lines highlight statistically significant differences between specific seasonal pairs, based on post-hoc Tukey HSD tests. Additionally, horizontal dashed blue lines represent regulatory or reference limit values for each variable (2.69 for fecal coliform (FK), 3.0 for total coliform (TK) and 2.0 for intestinal enterococci (IE)), allowing for immediate comparison of observed values against environmental thresholds.

Seasonal distribution of variable environmental parameters (temperature, dissolved oxygen, pH and salinity) were summarized in the Figure 7.

Figure 3.

Fecal coliform, total coliform and intestinal enterococci analyses were performed in taken from sea water and sand samples from 8 stations one year along seasonal determined in recreational areas of Istanbul Province

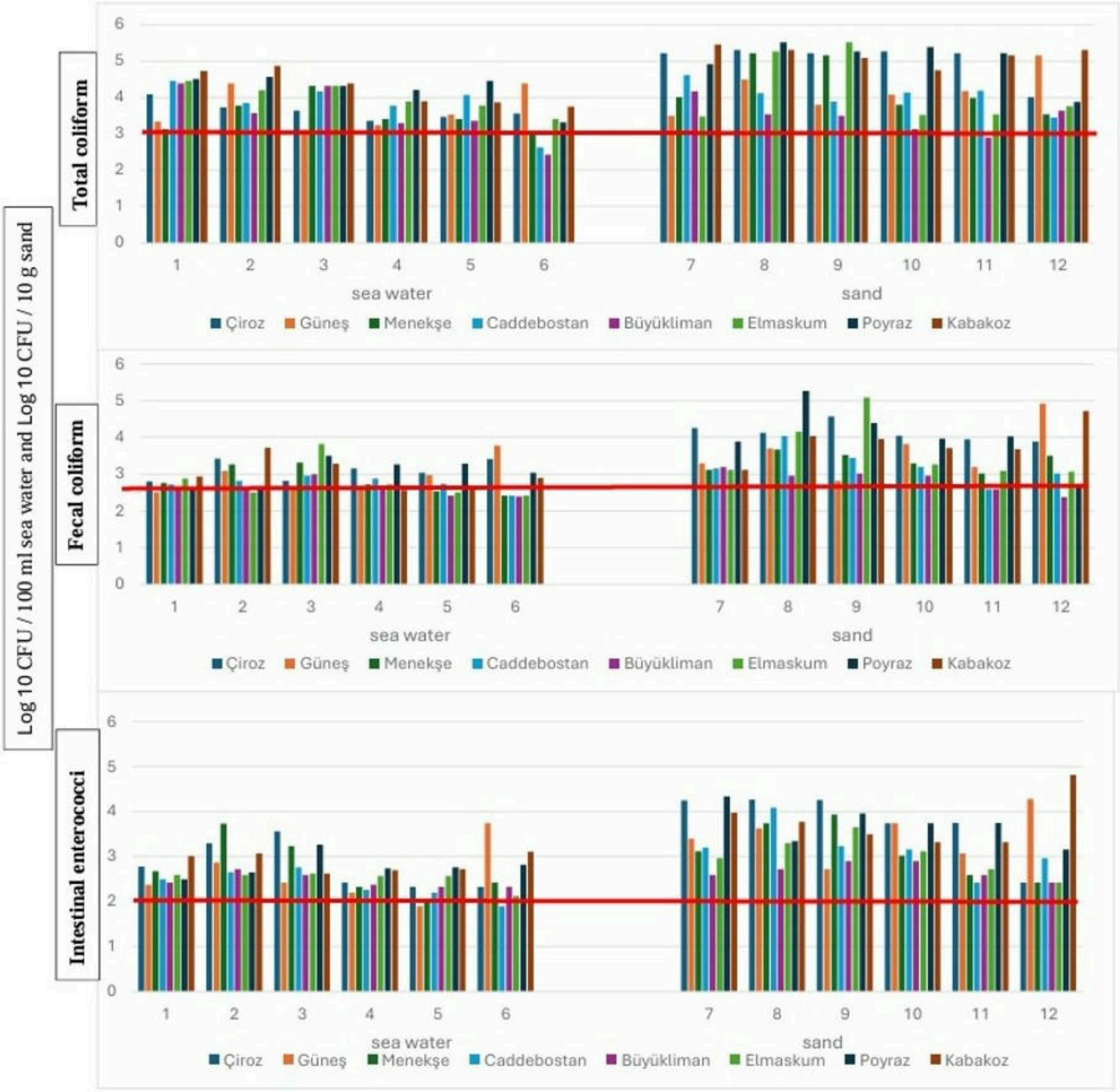


Figure 4.

Presence of heterotrophic aerobic bacteria (HAB) in sea water samples taken from beaches in the Marmara Sea in the 8 stations we determined in Istanbul Province between June 2021 and May 2022

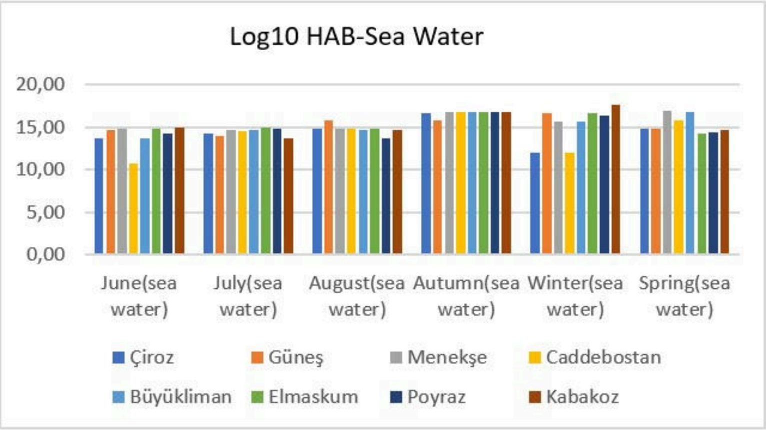


Figure 5.

Presence of heterotrophic aerobic bacteria (HAB) in sand samples taken from beaches in the Marmara Sea in the 8 stations we determined in Istanbul Province between June 2021 and May 2022

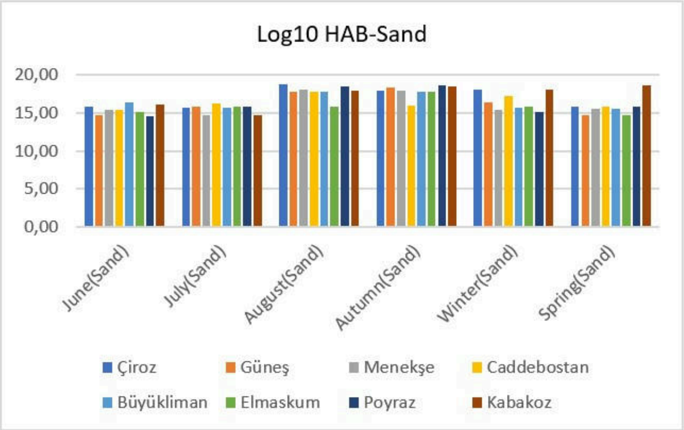
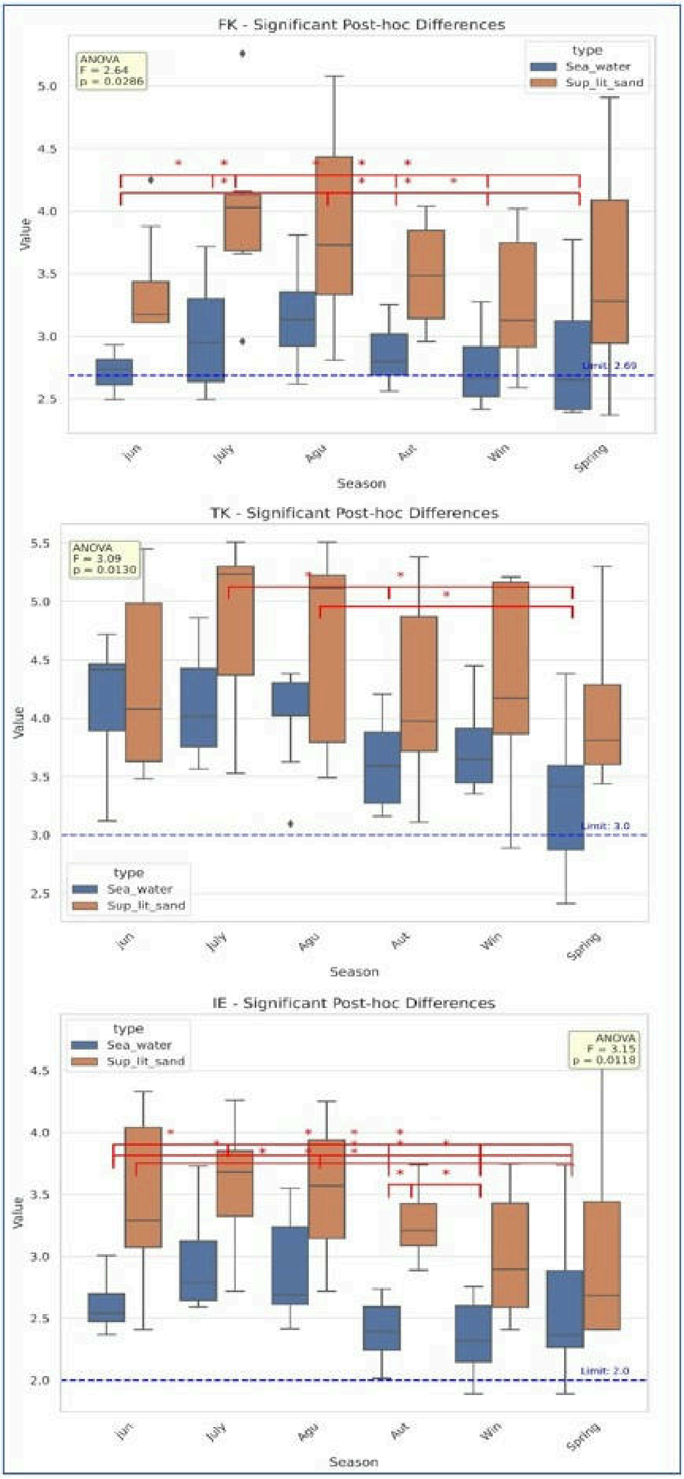


Figure 6.

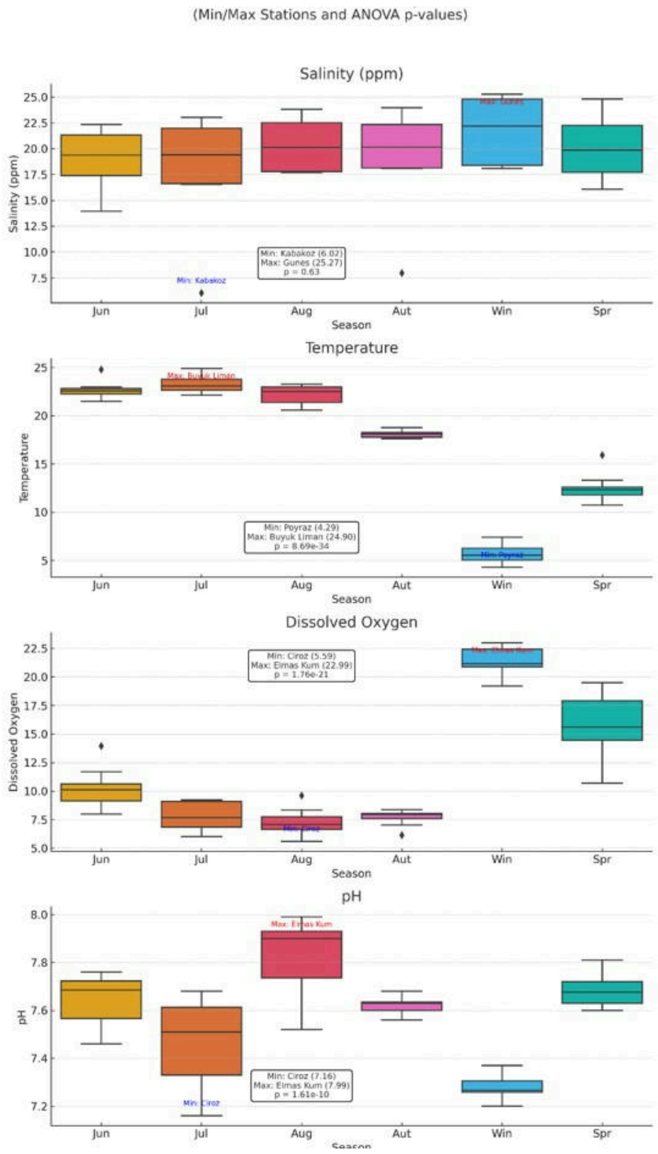
The ANOVA results reveal that seasonal effects are statistically significant across all three variables: FK (F = 2.64, p = 0.0286), TK (F = 3.09, p = 0.0130), and IE (F = 3.15, p = 0.0118)



Temperature (p<0.001), dissolved oxygen (p<0.001) and pH (p<0,05) has significant effect but salinity (p=0,63) not has significantly for ANOVA. There is limited data to compare the results obtained in the region with the previous studies by stations. However, Aydoğdu reported that the highest salinity value was at Güneş Beach in winter due to the mixing effect of the Turkish Strait System. (Aydoğdu et al., 2018). In this study the salinity values ranged between a minimum of 6.02 PSU (Kabakoz Beach, July 2021) because station so close the small freshwater which effecting the area and a maximum of 25.27 PSU (Güneş Beach, Winter 2022). Minimum temperature was shown in winter season at Poyraz station (4.29°C), which is northern part of İstanbul, also highest was Büyük Liman in July (24.9°C). It is because Büyük Liman is so shallow seaport side and water exchange is not high. Dissolved oxygen is lowest in Çiroz Beach in August (5.59 mg/L), highest was Elmas Kum in Winter (22.99 mg/L). pH values fluctuated between a minimum of 7.16 (Çiroz Beach, July 2021) and a maximum of 7.99 (Elmas Kum and Büyük Liman Beaches, August 2021). When Dissolved oxygen and pH considered together, Elmas Kum station would be high water exchange in Strait, also Çiroz station is low exchange level.

Figure 7.

Seasonal Distribution of Variable Environmental Parameters



DISCUSSION

Since indicator bacteria levels are accepted as an indicator of the presence of pathogenic bacteria in the environment all over the world (Ashbolt et al., 2001; da Costa Andrade et al., 2015; Dibra, 2024; Souza-Santos et al., 2025), it has been seen that this situation poses a potential threat to the ecosystem and public health in seawater and beach sands (Bakaj et al., 2023; Bolívar-Anillo et al., 2022; De Giglio et al., 2022; İçemer & Topaloğlu, 2020; Toubiana et al., 2021).

Data on bacterial pollution levels in seawater in coastal areas of the Sea of Marmara have been well documented (Altuğ, 2016; Altuğ et al., 2013; Altuğ, Çardak, & Çiftçi, 2010; Altuğ et al., 2007; Altuğ, Çardak, Çiftçi, et al., 2010; Altuğ, Çiftçi, et al., 2016; Altuğ et al., 2021; Altuğ et al., 2011; Altuğ, Gürün, Çiftci, et al., 2016; Altuğ, Gürün, et al., 2010; Altuğ, Gürün, Kalkan, et al., 2016; Altuğ, Gürün, Türetken, et al., 2016; Çardak & Altuğ, 2010, 2014, 2016; Çiftçi & Altuğ, 2009, 2010; Elçiçek & Çakmakcı, 2016; Gürün, 2008; Gürün et al., 2011; Gürün et al., 2013; Karaman Baş & Altuğ, 2022). However, there are no bacteriological pollution data on sand samples in the coastal area. In this study, the first regional bacteriological data for indicator bacteria (total and fecal coliform, intestinal enterococci) and heterotrophic aerobic bacteria in the Sea of Marmara for supralittoral zone sands were investigated.

When the HAB and indicator data obtained from seawater were compared with the bacteria levels detected in supralittoral sand samples, bacterial levels in sea sand were recorded at higher values. This situation shows the high level of bacterial activities in the beach sand, and when it is associated with the desire of bacteria to hold on to the particle rather than living freely, it has been shown that the bacteria adhering to the sand in the supralittoral zone can use these areas as suitable habitats for reasons such as nutrient evaluation. This situation has been defined as a risk in the international literature (Efstratiou, 2019; Halliday & Gast, 2011; Heaney et al., 2009; Solo-Gabriele et al., 2016; Subhan et al., 2018; Whitman et al., 2014; WHO, 2003).

There is no national bacteriological data to compare the findings of this study. However, the data obtained in this study indicate that the presence of bacteria lurking in the sands poses a potential threat to public health and ecosystem health.

Seagulls that visit the beaches can act as vectors by carrying fecal microorganisms that can contaminate sea water and sand to the beach (Alm et al., 2018). In this study, seagulls were observed intensively in the study areas during the sampling.

Streams are one of the important sources of point pollution for the coastal area of the Sea of Marmara. Studies reporting the bacteriological pollution load originating from streams in the region (Altuğ, Gürün, Çiftci, et al., 2016; Altuğ & Hulyar, 2020; Başer, 2006; Gürün & Kimiran-Erdem, 2013) are compared to the presence of streams close to the sampling areas, and it is understood that streams pose a threat to ecosystem and public health. The example of Menekşe Beach (Station 3), which is a public beach between the exit of both channels of Küçükçekmece Lake and is used extensively by people, also stands out as a beach under the influence of point pollution sources.

CONCLUSION

With this study, the levels of indicator bacteria (fecal and coliform, intestinal enterococcus) and HAB were determined in the sand of the supralittoral zone of the Sea of Marmara and the first regional bacteriological data were obtained in the sea sands. The results revealed significantly elevated bacterial concentrations in beach sand compared to seawater. According to the one-way ANOVA results, both seasonal variation and sampling environment differences (seawater vs. beach sand) led to statistically significant differences in the levels of FK (Fecal Coliforms), TK (Total Coliforms), and IE (Intestinal Enterococci) ($p < 0.05$). These findings indicate that bacterial concentrations vary significantly across both time and habitat. Beach sand samples consistently exhibited higher bacterial levels than seawater, with this difference becoming particularly pronounced during the summer months. In July and August, elevated temperatures and increased human activity may have contributed to the rise in bacterial concentrations (Figure 6). Besides, this elevated risk is partly attributed to factors such as biofilm formation, the oxygen-depleted structure of beach sand, reduced exposure to sunlight, and the prolonged retention of moisture (Qian Zhang ve diğ., 2015). The increase in marine pollution in coastal areas is bacteriologically important. Marine areas are also considered as reservoirs for the spread of global antibiotic resistance. The high HAB levels obtained in this study and the presence of indicator bacteria indicate that the increase in bacteria may pose a threat to marine areas in terms of the spread of potential pathogenic bacteria, the formation of plastisphere by attaching to microplastics and the potential of these bacteria to develop resistance to antibiotics. In this case, detailed bacteriological monitoring studies should be carried out in areas used for swimming purposes and in sandy areas where children have close contact for a long time. Currently, there are no national/internationally defined bacteriological standard values for sea sands. The data of this study also show the necessity of establishing bacteriological standards for indicator bacteria indicating the presence of pathogenic bacteria in the sand of the supralittoral zone used for swimming purposes. This situation is also valuable in terms of the "One Health" approach, which includes human/environmental/animal health.

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Research Article

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The Source of DPPH Radical Scavenging Activity: Insights from Freshwater Streams Isolated in Giresun

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Abstract Microalgae produce bioactive compounds, specifically antioxidants, that play a central role in fighting oxidative stress. It is a major factor in the development of aging, cancer and cardiovascular diseases. In this study five freshwater microalgal species from Giresun, Türkiye, namely *Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia*, and *Desmodesmus opoliensis* were isolated. They were stringently screened for antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was the method followed in evaluating antioxidant capacity. *Chlorococcum hypnosporum* showed higher antioxidant activity among the other species. However, the other species showed lower antioxidant activity under the experimental conditions compared to *C. hypnosporum*. The results of the our study show that microalgae are a good source of high-potential antioxidant compounds and they can be used in therapeutic and health-related fields as eco-friendly alternatives compared to the currently globally used synthetic derivatives.

Keywords Green Microalgae · Antioxidant Activity · DPPH Radical Scavenging · Health Applications

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INTRODUCTION

Antioxidants are also essential components of defense mechanisms that protect living organisms against oxidative stress, causing numerous damages to cells and molecules. Oxidative stress is a term used to describe the imbalance between the production of reactive oxygen species (ROS) and the availability of antioxidants in the body to neutralize the reactive intermediates or fix their damage (Pizzino et al.,2017). Reactive oxygen species (ROS), which include free radicals such as superoxide anion and hydroxyl radical, and non-radicals, such as hydrogen peroxide, are produced on a daily basis as an ordinary course of oxygen metabolism and contribute to cellular signaling processes and homeostasis preservation (Phaniendra et al., 2015).Even though the organism contains antioxidant defense mechanisms, excessive production of ROS can lead to oxidative damage to proteins, lipids, and DNA (Finkel and Holbrook, 2000; Kaminski et al., 2002; Sharifi-Rad et al., 2020). This damage is associated with the aging process and with the pathology of most diseases, including diabetes, cancer, cardiovascular pathologies, and neurodegenerative diseases like Parkinson's disease and Alzheimer's disease (Valko et al., 2007).Therefore, the elucidation of mechanisms of action involved in antioxidation represents one of the gravest aspects for modern medical research into both physiological senescence processes and pathological states.Enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, but also exogenous antioxidants: Vitamin C, vitamin E, carotenoids and polyphenols (Ratnam et al., 2006; André et al., 2010; Bouayed and Bohn, 2010; Biehler and Bohn, 2010).

Food antioxidants greatly contribute to increasing the coping ability of the body to oxidative stress. They are rich in good quality antioxidants that have been proved to reduce chronic diseases by providing scavengers to free radicals and are found in fruits, vegetables, nuts and some beverages (Okarter and Liu, 2010; Zhu and Sang, 2017; Wallace et al., 2020; Ponnampalam et al., 2022). These include polyphenols, flavonoids, and carotenoids which are powerful antioxidants present in plant foods that account for their beneficial health effects (Vasanthi et al., 2012; Pawase et al., 2024). Consumption of antioxidant active food on a daily basis is associated with better general health and lower disease risk (Caple et al., 2010).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay has become a validated technique for the assessment of antioxidant activity of different compounds (Sharma and Bhat, 2009). The DPPH method is based on Blois's work and later a modification of the method by Brand-Williams et al., which is the most commonly used procedure in literature today (Blois, 1958; Brand-Williams et al., 1995; Akar et al., 2017). The assay relies on direct reduction of the stable free radical DPPH, which has a deep violet color, to a yellow, diphenyl-picrylhydrazine, by an antioxidant, indicating radical-scavenging activity.

The color intensity of the colour change (being directly proportional to the antioxidant concentration) can then be quantified by measuring the 517 nm absorbance using spectrophotometer. The DPPH assay is more preferable because it is simple, quicker, and also reproducible. Except for such complex mixtures as foodstuffs and plant extracts, the test also serves to give a rapid evaluation of the pure compound's antioxidant potential (Baliyan et al., 2022). There is the need to memorize that DPPH test is in vitro and was unable to demonstrate the antioxidant activity in vivo, where metabolism and bioavailability are crucial factors.

Because of its simplicity, velocity, and replicability, DPPH test is ideal. With the exception of complex mixtures such as food and plant extracts, it is a fast antioxidant potential estimation of pure substances (Baliyan et al., 2022). It should be noted that the DPPH assay, despite its widespread use, emerges from the validation of antioxidant compounds, and in fact is an in vitro method that cannot reliably mimic the properties of in vivo, where factors such as metabolism or bioavailability can heavily influence the activity observed.

Microalgae are being considered as significant sources of natural antioxidants owing to their versatile and rich biochemical composition. For centuries, diverse communities have used these photosynthetic organisms that can exist in fresh water and marine environments for their medicinal and nutritive attributes. The antioxidant capacity of microalgae is linked to the high content of bioactive compounds such as phycobiliproteins, carotenoids, polyphenols and vitamins (Ng and Chew et al 2020; Pereira et al., 2024).

In contrast, secondary metabolites like polyphenols have strong antioxidant activity and neutralize free radicals via transfer of an electron or hydrogen atom (Hassanpour and Doroudi, 2023). Likewise, microalgae-meditated antioxidants, especially polyphenols, have exhibited anti-carcinogenic properties in a wide variety of experimental methods, both in vivo and in vitro, mainly by diminishing oxidative stress and inhibiting tumor cell proliferation. (Avila- Roman et al., 2021). In addition to imparting colour to microalgae, carotenoids such as beta-carotene, lutein and zeaxanthin protect microalgae against oxidative damage through singlet oxygen quenching, and free radical scavenging (Black et al., 2020; Gülçin, 2020; Swapnil et al., 2021). Repeating experiments consistently indicate that carotenoids cause the majority of microalgae species to possess a significant antioxidant ability (Jahnke, 1999; Takaichi, 2011). Although the most used supplemented carotenoid is beta-carotene, the red xanthophyll pigment astaxanthin comes second in place (Novoveská, et al., 2019) It has shown potent antioxidant activity (Bouissiba and Vanshak, 1991; Boussiba, 2000; Lorenz and Cysewski, 2000). Among the reasons making astaxanthin so valuable is its very high free radical absorption capacity; experiments have found that it has antioxidant activity about 10 times greater than in other frequently encountered carotenoids (Borowitzka, 1995; Hamed, 2016; Berton et al., 2017; Mourelle et al., 2017). Some microalgae species such as *Chlorella zofingiensis*, certain *Chlorococcum sp*. and *Scenedesmus sp*. are presently being used to produce astaxanthin by way of biosynthesis. (Yaakob et al., 2014, Odjadjare et al., 2017; Bhalamurugan et al., 2018; Mavrommatis, 2023).

Microalgae also have other valuable antioxidants such as vitamin E (tocopherol) and vitamin C (ascorbic acid), which contribute notably to the antioxidative power of microalgae (Goiris et al., 2015). Microalgae hold a very heterogeneous mixture of antioxidant molecules, including various carotenoids (β-carotene, lutein, and astaxanthin), tocopherols (vitamin E), and phycobiliproteins in red algae such as *Porphyridium*. (Rhodophyta). These pigments are synthesized by such species as *Dunaliella*, *Chlorella*, *Haematococcus*, *Scenedesmus*, and *Trentepohlia* (Chlorophyta), and *Euglena* (Euglenophyceae).

Researches have found that several microalgal species, such as *Chlorella vulgaris*, *Spirulina platensis* and *Haematococcus pluvialis*, boast amazing antioxidant properties with a significant activity detected against DPPH radical scavenging assays (Rodríguez-García and Guil-Guerrero, 2008; Demorois et al., 2015; Takyar et al., 2019). Research on antioxidants enhances both functional foods and nutritional supplements. It can enable us to find environmentally friendly and sustainable sources of antioxidants. 43 % of the world's arable land goes on feed for livestock. Microalgae can be culturing non-arable land to make feed for animals out of waste water. It further proves to be environmentally friendly and offers a giant ambition: that means even larger at scale production of bioactive chems (Paek et al., 2014).

Our aim in this research study is to determine, at different concentrations, the antioxidative potential of both rutin and some microalgal species that are grown with DPPH radical scavenging assays as a method for assessing antioxidant ability. Rutin is a flavonoid compound studied widely in biochemical research, and based on its known free radical scavenging ability we believe it to be a classic antioxidant. This study will also make it possible for readers to compare the antioxidant levels of different microalgal extracts with the results for rutin and to see exactly how these natural compounds stand up against an established gold standard antioxidant. The study also aimed at comparing the antioxidant potential of different microalgal species and trace which one showed the highest antioxidative activities. This comparative study will draw attention to those candidates that need more in-depth examination and may be marketed for commercial use. An investigation into the potential implications of microalgal antioxidant extracts for health and wellness is also necessary. The specific populations affected by these extracts will become clearer, along with the potential for their incorporation as core ingredients in future health-related products.The identification of effective natural antioxidants from microalgae can help to design new health products that promote human health and disease prevention.

MATERIAL AND METHOD

Chemicals and Reagents

High-quality chemicals and reagents were used to ensure the accuracy of the experimental results. 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\cdot}$) and rutin were acquired from Sigma Chemical Co. Methanol and other reagents were of analytical grade, sourced from Merck.

Microalgal Species

Water samples were collected in September 2021 from the benthic and pelagic zones of the Aksu, Batlama, and Büyük Güre Streams, located in the central district of Giresun Province, using 1 L plastic bottles and transported to the laboratory. Water temperatures and pH values of the sampling sites are provided in Table 1. Each water sample (1 mL) was inoculated onto BG11 and Allen media solidified with 1% agar (Allen, 1968; Allen and Stanier, 1968). The culture plates were incubated at 26°C in a SANYO MLR 351 incubator under a light intensity of approximately 155 μmol/m²/s with a 12:12 light-dark photoperiod.

Table 1.

Physicochemical Parameters of Water Samples

Stream Name	Water Temperature (°C)	pH
Batlama Stream	22.4	7.85
Aksu Stream	21.7	7.40
Büyükgüre Stream	23.0	7.10

After one month of incubation, distinct colonies that developed on the agar plates were carefully transferred to fresh solid media using an inoculating loop. This procedure was repeated multiple times until single-species isolates were obtained (Demiriz, 2008). The purified isolates were then transferred to liquid media and cultured under controlled incubation conditions to promote growth. Samples were aseptically collected from cultures grown in liquid media for further analysis. Species identification was performed using light microscopy and inverted microscopy, with measurements conducted using a micrometric eyepiece. Identification was based on established references, including *Freshwater Algae of North America* and the AlgaeBase database (Wehr and Sheath, 2003; Guiry and Guiry, 2023). DNA isolation and molecular identification of the algal species were carried out by BM Software Consulting and Laboratory Limited Company. The obtained sequences were analyzed and evaluated using the NCBI-BLAST program for species confirmation. The isolated species, which were identified through both morphological and molecular characterization, include *Chlorococcum hypnosporum*, *Stichococcus bacillaris*, *Chlorella vulgaris*, *Chlorolilaea pamvotia*, and *Desmodesmus opoliensis*.

This study's objective is to measure the antioxidant activity of isolated microalgae species and contrast the results with established antioxidant values.

Preparation of Microalgal Extracts

Microalgal biomass was harvested from cultures were centrifuged at 8000 rpm for 5 minutes. The biomass was washed with distilled water, dried at 65°C, and ground into a fine powder. For extraction, 1 grams of the dried biomass was mixed with 20 mL of methanol and and extracted at 50°C for 48 hours (Vehapi et al., 2018). After that, the mixture was centrifuged for 10 minutes at 4000 rpm. After centrifugation, the supernatant was filtered, and the methanol in the supernatant was evaporated. The resulting dry biomass was then dissolved in methanol (Gürlek et al., 2020).

DPPH Radical Scavenging Assay

The scavenging ability of the microalgal extracts was determined according to the method of Brand-Williams et al (Gürlek et al., 2020) using 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical. The DPPH working solution (20 µg/mL) was prepared in methanol, and 1.5 mL of this solution was added to 0.75 mL of microalgal extracts at their respective concentrations (250, 500, 750, 1000 µg/mL). Mixtures of DPPH solution and microalgal extracts were incubated for 30 min in the dark. The mixtures' absorbance was measured at 517 nm using a spectrophotometer after incubation. (Aydın, 2012). The DPPH radical scavenging activity percentage can be determined using the following equation

DPPH Scavenging Effect (%)= $[(A0-A1) / A0] \times 100$

A0: Absorbance value of the control

A1: Absorbance value of the sample or standard

DPPH· + Antioxidant → DPPH-H + Antioxidant

(purple color) (yellow color)

At varying doses (250, 500, 750, and 1000 µg/mL), the free radical scavenging activity of microalgal extracts was assessed, and activity comparisons were performed using rutin as the reference.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's test. Results were presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant Activity of Rutin

The highest antioxidant capacity in all the samples examined was seen in rutin. At the maximum of 1000 µg/mL, rutin's activity of DPPH radical scavenging was 92.0% (Figure 1, Table 2). As the concentration increased, the rutin activity also increased and justified its status as a good antioxidant. Such a high activity is a proof of rutin's high ability to scavenge free radicals, a perfect test of antioxidant potency. This is in line with previous reports (Yang et al., 2008), which supported the high antioxidant capacity of rutin.

The scavenging activity demonstrated is in line with previous work showing the capability of rutin to abate oxidative stress and its potential therapeutic implications (Butchi et al., 2011; Patil et al., 2013; Kamel et al., 2014; Ganeshpurkar and Saluja, 2016). With its higher antioxidant activity, Rutin may be greatly effective in numerous applications, such as application as the basis for supplements or functional foods intended to counteract oxidative damage.

Antioxidant Activity of Microalgal Species

Microalgal extracts showed differential antioxidant activity depending on the species and concentration. *Chlorococcum hypnosporum* was found to have the highest scavenging activity of 90% at a concentration of 1000 µg/mL (Figure 1). Antioxidant activities of *Stichococcus bacillaris, Chlorella vulgaris* and *Chlorolilaea pamvotia* (80.00%, 75.00% scavenging activity at 1000 µg/mL, respectively) were purified.The lowest antioxidant activity was observed for *Desmodesmus opoliensis* (70.00% maximum scavenging at 1000 µg/mL) (Figure 1). Table 2 summarizes the DPPH radical scavenging activities (%) of rutin and microalgal extracts at various concentrations (µg/mL).

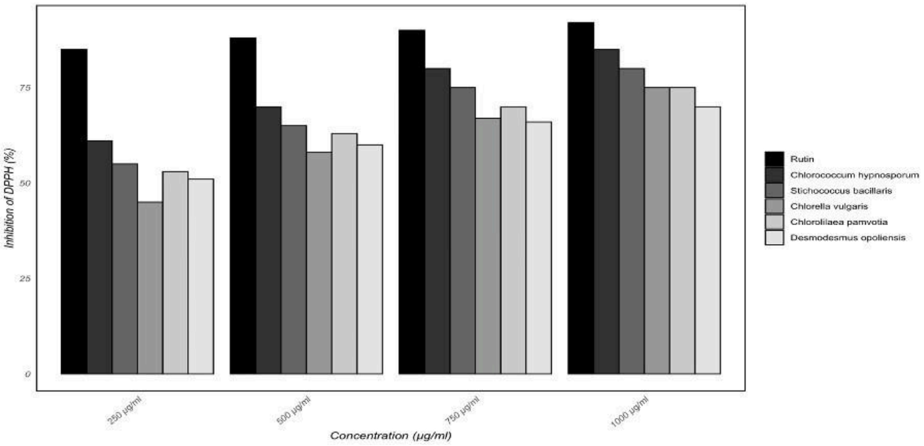
Table 2.

DPPH Radical Scavenging Activity (%) of Rutin and Microalgal Extracts (µg/mL)

Concentration (µg/mL)	***Rutin***	***C.hypnosporum***	***S. bacillaris***	***C.vulgaris***	***C. pamvotia***	***D.opoliensis***
250 µg/mL	85	61	55	58	51	51
500 µg/mL	88	70	65	67	63	60
750 µg/mL	90	80	75	72	70	66
1000 µg/mL	92	90	80	75	75	70

Figure 1.

DPPH Radical Scavenging Activity (%) of Rutin and Microalgal Extracts (µg/mL)



Among the microalgae, scavenging activity of *Chlorococcum hypnosporum* was highest, i.e., 90% at concentration of 1000 µg/mL (Figure 1). Thus, *Chlorococcum hypnosporum* is rich in antioxidant potential and can be ascribed to a high content of antioxidant compounds carotenoids, chlorophylls, and other phytochemicals with excellent radical-scavenging activity (Hajialyani et al., 2019). The *Chlorococcum hypnosporum* of the present study may have activities that can effectively perform the role of natural antioxidants as a source of and application in food, pharmaceuticals, and cosmetics. Also, research by Sassi and co-workers (2019) demonstrated that several microalgal species, *Chlorella cf. minutissima* D101Z, *Chlorococcum sp. (cf. hypnosporum) strains* D28Z, D37Z, D65Z, and D76Z, *Pediastrum tetras* D121WC, *Planktothrix isothrix* D39Z, and *Scenedesmus acuminatus* D115WC, are rich in polyunsaturated fatty acids (PUFAs), comprising ω-3, ω-6, and ω-9 types, besides carotenoids and chlorophylls that are relevant to maintaining the human physiological state. These microbes have established themselves as feasible alternative supplies of metabolites for the food industry. *Chlorococcum hypnosporum* exhibited greater antioxidant potential than other species which is primarily a result of biochemical constellations and adaptation-based environmental responses by this organism (Chlumsky et al. 2019). The organism is rich in comprising antioxidant flavonoids, polyphenols, chlorophyll, and carotenoids that are centrally involved in free radical neutralization and oxidative stress. Furthermore, the high content of PUFAs with high levels of ω-6 and ω-3 fatty acids helps in cell membrane structure maintenance and hence promote antioxidant activity.Its genomic composition supports the active synthesis of antioxidant enzymes such as superoxide dis-mutase, catalase, and peroxidase and thereby maintain oxidative homeostasis. Additionally, adaptation mechanism in *Chlorococcum hypnosporum* in response to environmental stresses leads to heightened synthesis of antioxidant compounds.Additionally, Goiris et al. (2012) screened the antioxidant capacity of 32 microalgal biomasses and estimated their phenolic and carotenoid content. Their findings showed that both carotenoid and phenolic compounds contributed to the antioxidant capacity of microalga, but the antioxidative property was species-dependent, growth condition-dependent and extraction solvent-dependent.

The antioxidant capacity of *Chlorolilaea pamvotia* has been evaluated for the first time in the literature. Similarly, *Chlorolilaea pamvotia* was demonstrated to possess strong antioxidant properties reaching a scavenging percentage of 75.0% at the same concentration. Notably, *Chlorolilaea pamvotia* showed lower activity but suggests its potential as a source of antioxidants. *Chlorolilaea pamvotia* has different compounds which may lead to antioxidant activity which makes it a potentialcandidate in this area with potential use as natural sources of antioxidant. Lortou and Gkelis (2023) isolated a high level of valuable metabolics from *Chlorolilaea pamvotia*, isolated from Greece.

A rutin, a well-known antioxidant standard, was used to compare the antioxidant activity of *Stichococcus bacillaris*. Data revealed an appropriate antioxidant capacity of *Stichococcus bacillaris*, even if not as high as that of rutin. According to Gürlek et al. (2019), they investigated the antioxidant capacity of *Stichococcus bacillaris* for the first time and presented its results in literature. The RSA value of this species was found to be 89 ±0.1% for both methanolic and hot water extracts. Such experiment explained that *Stichococcus bacillaris* has considerable application potential in the pharmaceutical, food, and cosmetic industries. These findings are similar with the findings of our study.

On the other hand, both *Chlorella vulgaris* and *Desmodesmus opoliensis* demonstrated antioxidant activities, as reflected by scavenging percentages of 75% and 70%, respectively, at 1000 µg/mL. Despite showing good antioxidant activity, these species performed less well than *Chlorococcum hypnosporum*, whereas *Chlorolilaea pamvotia* showed similar activity. These species reported moderate activities which could be attributed to their chemical compositions or to the concentration of antioxidant compounds. However, despite this, these microalgae still have potential for efforts toward antioxidant applications. Following Abdel-Karim et al. (2020) revealed that Chlorella *vulgaris* contained an extensive composition of biologically active metabolites and exhibited considerable antioxidant activity based on a cascade of assays. Acetone extract had maximum antioxidant activity with 50.81% scavenging activity at 50 mL of 2.58 mg AAE/g DW antioxidant capacity and 1.95 mg AAE/g DW of reducing power. Acetone extract had a high total phenolic content 3.17 mg GAE/g DW. High antioxidant activities of *Chlorella vulgaris* were found earlier to be present by other researchers. For instance, Yu et al. (2019) investigated antioxidant functions of *Chlorella vulgaris* polysaccharides in vitro and in vivo.Their study found that *Chlorella vulgaris* possesses high antioxidant activity and high biological activity metabolites. Altogether, these researches provide the basis for the potential application of *Chlorella vulgaris* in food, cosmetic and pharmaceutical applications. Stoica et al. (2013) *Scenedesmus opoliensis* maximum radical scavenging activity with ethanol concentrations and DPPH assays is 54.9%. The finding is in line with the outcome of our study.findings.

The results obtained exhibit concentration-dependent and species-specific antioxidant activity of microalgal extracts, with *Chlorococcum hypnosporum* and rutin showing higher potential. Percentages of DPPH inhibition by different microalgae species varied significantly (p< 0.001). The remark accounts for the heterogeneity of the antioxidant activity of microalgae species. Additionally, concentration levels exhibited a staggering effect on inhibition of DPPH (p<0.001), indicating the significance of the effect of concentration on the antioxidant activity.

Apart from analysis, by applying Tukey's test, rutin's DPPH scavenging activity was considerably higher compared to *Chlorella vulgaris* and *Chlorococcum hypnosporum* (p<0.001), which reveals that rutin is significantly more effective than the microalgal genera of *Chlorella vulgaris* and *Chlorococcum hypnosporum* in quenching DPPH radicals. Relatively significant differences also appeared between *Desmodesmus opoliensis* and *Chlorococcum hypnosporum* (p<0.01), and between *Chlorolilaea pamvotia* and *Chlorococcum hypnosporum*. All these results illustrate the variable antioxidant activities of *Chlorococcum hypnosporum* and *Chlorolilaea pamvotia*, established by the two species showing different levels of antioxidant activity. Nevertheless, statistically significant differences could not be indicated between *Desmodesmus opoliensis* and *Chlorella vulgaris* (p>0.05), but it means the difference in antioxidant activity between these two species is less pronounced as that depicted among rutin and the microalgae.

The research considered the IC50 behavior of rutin and five different microalgae species, namely *Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia,* and *Desmodesmus opoliensis*.The got information uncovered critical contrasts among the tried mixes and microalgae types, as appeared in Table 3.

Table 3.

IC50 Values (µg/mL) of Rutin and Microalgae Species for Antioxidant Activity

Sample	IC50 (µg/mL)
Rutin	54.94 ± 68.8
Chlorococcum hypnosporum	567.0 ± 124.1
Stichococcus bacillaris	780.2 ± 86.9
Chlorella vulgaris	952.0 ± 87.3
Chlorolilaea pamvotia	955.6 ± 171.5
Desmodesmus opoliensis	1211 ± 142.7

The IC50 value of the control compound in this study, rutin, was determined to be 54.94 µM, the lowest IC50 value for all algae tested in this experiment and a figure indicative of its high biological activity. The low IC50 values for rutin indicate that it is functioning as an effective bioactive compound (Ayaz Seyhan, 2019). The IC50 value of the control compound in this experiment, rutin, was 54.94 µM, the lowest IC50 value for all the algae examined in this experiment and a value that reflects its very high biological activity. The low IC50 values for rutin indicate that it is functioning as an effective bioactive compound (Ayaz Seyhan, 2019). The IC50 value of *Chlorococcum hypnosporum* was 567.0 µM. IC50 values of its extracts, as indicated by the study carried out by Olasehinde et al. (2020), ranged from 13.83 to 493.90 µg/mL, depending on the solvent applied. On the other hand, IC50 values of *Chlorococcum hypnosporum*, indicated a widely diverse range of more than 500 µg/mL for some extracts. Moreover, this difference is due to differences in the chemical composition, metabolic activities, and bioactive compounds between these two members. Variation in the percentage composition of phenolic compounds, alkaloids, and other phytochemicals, and also a difference in the efficiency of solvent extraction, can influence the various results for the cholinesterase inhibitory and antioxidant activities of the two species at various concentrations. The results show that different species, even from the same genus, may have the ability to exhibit considerably different patterns of bioactivity. Therefore, in looking for the pharmacological potential of microalgae, a relevant question is how these differences affect different types from the same genus. The IC50 value of Stichococcus bacillaris was 780.2 µM. Gürlek et al. (2019) examined the antioxidant activity of crude *Galdieria sulphuraria, Ettlia carotinosa, Neochloris texensis, Chlorella minutissima, Stichococcus bacillaris, Schizochytrium limacinum, Crypthecodinium cohnii,* and *Chlorella vulgaris* extracts (DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate radical) as well as their total phenol content (Folin-Ciocalteu). They assumed that in references of theirs Stichococcus bacillaris had an IC_{50} value of 372.5 µg/mL, whereas in our study put the figure decidedly higher at 780.2 µg/mL. The reason behind this difference may be due to various extraction methods, solvents used in the process, culture conditions, varied genetic variant lines between microalgal strains, or analysis methods used. All these would impact the quality and concentration of bioactive compounds and hence finally the antioxidant activity.

Chlorella vulgaris isolated from Giresun exhibited a worse IC50 value of 952 µg/mL compared to the 325 µg/mL reported for the Diyarbakır isolate by Çakmak et al. (2024). This could be due to the chemical structure of algae, which differs based on the conditions in the environment. Physical and biogeochemical characteristics of the water, such as nutrients and stress in their habitat, can affect the production of secondary metabolites, leading to variation in the biological activities of the compounds. *Chlorolilaea pamvotia* yielded an IC50 value of 955.6 µM. *Desmodesmus opoliensis* yielded the highest IC50 value of all the microalgae analyzed (1211 µM), approximately 22 times higher than rutin.

CONCLUSION

Comparative study of antioxidant activities of rutin and five different types microalgae (*Chlorococcum hypnosporum, Stichococcus bacillaris, Chlorella vulgaris, Chlorolilaea pamvotia, Desmodesmus opoliensis*) by DPPH scavenging method revealed. Rutin showed the highest antioxidant effects, involving a 92% scavenging ratio and IC50 value of 54.94 µg/mL. Of these microalgae species, *Chlorococcum hypnosporum* had best characteristics, exhibiting 90% radical scavenging capacity and an IC50 value 567.0 µg/mL. Amongst the five species, *Chlorolilaea pamvotia* exhibited promising potential (75% scavenging) tested for the first time in this paper: IC50 was 955.6 µg/mL. However, *Desmodesmus opoliensis* was the least active of all algae species (70% scavenging, IC50: 1211 µg/mL), in fact showing that its biological activity was about 22 times lower than that of rutin.

These results show particularly that *Chlorococcum hypnosporum* has a high antioxidant potential, with potential application in such different industrial sectors as dietetic and functional foods, cosmetics or health products of virtually any description. In the long term, antioxidants from microalgae can bring benefits to our health. They include guarding cells against oxidation stress, delaying the signs of aging, being good for the immune system or probably even help to prevent chronic diseases altogether. Thus, these algae species could be of use for potential anti-aging cosmetics, dietetic foods, functional beverages, food preservatives or pharmaceutical preparations with both anti-inflammatory and immunity-enhancing effects.

Based on these findings, conclusion can be drawn that *Chlorococcum hypnosporum* has significant potential as a natural source of antioxidants for use in the food, pharmaceutical and cosmetic industries. Additionally, *Chlorolilaea pamvotia*, evaluated for the first time, has been identified as a promising candidate for future research.

Conflict of Interest	The authors have no conflicts of interest to declare.
Ethics committee approval	Ethics committee approval is not required. Both authors declare that this study does not include any experiments with human or animal subjects.
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Data Availability Statement	The data that support the findings of this study and generated during and/or analysed during the current study are available in the Mendeley Data [https://data.mendeley.com/preview/n7s8gzyvsb?a=ec31d7e6-634c-4868-a485-3e894f0c68c0].
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Research Article

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Sex-Specific Vulnerability of Fish to Dredging in the Mediterranean Coastal Lagoon (Lake Manzala, Egypt)

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Abstract Lagoon dredging disrupts aquatic ecosystems and fish populations. A study in Lake Manzala, Egypt, assessed these impacts by analyzing 2616 fish across ten species. Results showed significant variation in the condition factor (K) between sexes, ranging from a low of 0.220 in male European eels (*Anguilla anguilla*) to a high of 1.794 in female redbelly tilapia (*Coptodon zillii*). Sex ratios were predominantly male-biased. The male-to-female ratio was approximately 3:1 in *C. zillii*, *Chelon ramada*, and *Chelon auratus*, and 2:1 in *Dicentrarchus punctatus*, with males also dominating in *Dicentrarchus labrax* and *A. anguilla*. Only three species were female-biased: *Oreochromis niloticus* (1:1.40), *Sarotherodon galilaeus* (1:1.34), and *Clarias gariepinus* (1:3.31). These imbalances suggest dredging may negatively impact female populations, potentially reducing overall fish production. Therefore, incorporating sex ratio data into conservation management for Lake Manzala is crucial. Further research on species' reproductive biology is recommended post-dredging.

Keywords Length weight relationship · Condition factor · Sex ratio · Length frequency

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INTRODUCTION

Mediterranean lagoons are vital coastal ecosystems, renowned for their rich biodiversity and the critical role they play in driving numerous ecological processes (El Mahrad et al., 2022). Lake Manzala, similar to many other lakes in the northern Egyptian Delta, has unfortunately experienced substantial environmental degradation stemming from both anthropogenic and natural factors (Abd Ellah, 2021). Over recent decades, human activities have profoundly altered the lagoon's ecological balance, resulting in noticeable environmental shifts (Elmorsi et al., 2017). A significant influx of pollutants from various drains flows into Lake Manzala, exacerbating the existing environmental challenges. These pollutants, in conjunction with other encroachments, contribute to a range of significant issues, including diminished fish productivity, alterations in the lagoon's morphology and surface area, deterioration of water quality, and increased eutrophication (Halim & Guerguess, 1978; Hegab et al., 2020).

Despite these considerable challenges, Lake Manzala remains a crucial component of Egypt's fisheries, contributing nearly 14% (59.6 thousand MT) of the nation's total annual fish production (Mostafa et al., 2019). LFRPDA (2021) reported that, total production of fish from Lake Manzala was 82541 MT during 2020. This represents 42 % of the fish production from northern lakes(197973 MT) and 35 % of the total fish production from all Egyptian lakes combined(237758 MT). The lagoon's high fertility, calm waters, and shallow depths establish it as one of Egypt's most economically significant water bodies for fisheries (Shalloof et al., 2022). Recognizing the importance of Lake Manzala, the Egyptian government launched a major initiative focused on the sustainable development of all Egyptian lakes, with the overarching goal of improving water quality for both present and future generations. Since 2017, a concerted national effort has been underway to develop and restore Lake Manzala (Abd Ellah, 2021; Shalloof et al., 2022). One key method employed in lake restoration is dredging, which currently represents the most prevalent approach for maintaining aquatic ecosystems and enhancing their protection and aesthetic value (Hadnagy et al., 2015).

Sex ratio is a fundamental stock characteristic in fisheries management, wielding direct influence over a population's reproductive potential (Jakobsen et al., 2009). This crucial parameter is incorporated into various models employed to assess spawning stock biomass and overall population fecundity (Marshall et al., 2006). Furthermore, environmental factors are recognized as having a substantial impact on sex differentiation in fish (AbouelFadl et al., 2024).

Dredging, an intensive engineering activity frequently undertaken in coastal regions, can exert detrimental effects on fish populations. The suspended sediments generated during dredging operations can negatively affect the survival and abundance of fish eggs and larvae (Yang et al., 2019). Early life stages, such as eggs and larvae, are particularly vulnerable to the fatal impacts of dredging (Wenger et al., 2017). As a result of the habitat alterations caused by dredging, existing species and organisms dependent on the original habitat structure face the risk of local extinction. Sediment removal can lead to elevated turbidity levels and the oxidation-induced mobilization of heavy metals. Therefore, dredging represents a significant re-engineering of a lake and should not be undertaken without a comprehensive understanding of its potential consequences (Gustavson et al., 2008; Abd Ellah, 2022). AbdElghany et al. (2024) concluded that levels of toxic heavy metals in the Manzala Lagoon had increased when comparing recent clean-up and dredging activities to previous investigations conducted on the same lake before dredging operations.

Understanding the drivers and consequences of sex ratio imbalances is crucial for implementing effective conservation strategies (Wearmouth & Sims, 2008). If these imbalances are detrimental to population health, conservation approaches may require adjustments, such as habitat restoration initiatives, captive breeding programs, or regulations designed to protect vulnerable species. Natural and fishing mortality differences between the sexes, along with gear selectivity related to sex-specific behavior and morphology, can contribute to variations in sex composition (Smith, 1956). Any kind of mortality should be seen as a potentially disturbing factor in studies of environmental impacts on sex determination. Sex ratio imbalances can create genotype–phenotype mismatches with impacts on population demography (Geffroy and Wedekind, 2020).

While a multitude of factors can influence sex ratios, this study concentrates specifically on the potential impact of dredging operations, particularly on substrate-spawning fish, which exhibit heightened vulnerability to habitat disturbances.

This study aims to investigate the potential sex-specific impacts of dredging activities on the population structure encompassing size distribution, growth patterns, condition, and sex ratio of key fish species inhabiting Lake Manzala, Egypt. This is in addition to the scarcity of research regarding the reproductive biology of fish in the lake and the sex ratio in particular. Furthermore, this research seeks to contribute essential data for understanding the broader ecological consequences of such anthropogenic disturbances within coastal lagoon ecosystems.

Figure 1.

Location of Lake Manzala (adapted from Abd Ellah, 2022)

MATERIALS AND METHOD

Area of Study

Lake Manzala (Fig. 1), the largest Egyptian lagoon, is situated along the Mediterranean coast on the eastern edge of the Nile Delta. Covering an area of 1071 km², it is considered a major source of fish. Lake Manzala, a shallow brackish lake, connects three governorates: Port Said, Damietta, and Dakahlia (Abd-Elghany et al., 2024; Khedr et al., 2024). Freshwater input into the lake is minimal, primarily occurring through the Enanya Canal, which links the lake to the Damietta branch of the Nile River to the west. A narrow canal (El-Qabuty) connects the lake to the Suez Canal (saline water) to the east. The northern boundary of the lake comprises several outlets connecting it to the Mediterranean Sea, with El-Gamil and the New El-Gamil being the primary outlets contributing to improved water quality within the lagoon (Elshemy, 2016). Furthermore, Lake Manzala receives domestic, industrial, and agricultural waste from numerous drains along its eastern and southern boundaries, including the Hadous, Mataria, Elserw, Faraskour, and Bahr El Baqar drains (Abd-Elghany et al., 2024).

Samples collection and Data analysis

Samples from ten different fish species were collected between 2019 and 2021 from various locations within Lake Manzala using a variety of fishing gear and methods. A total of 2,177 cichlid fish were sampled, encompassing:

- *Oreochromis niloticus:* 454 males, 633 females, 152 unsexed; size range: 4–28 cm total length (TL)
- *Oreochromis aureus:* 318 males, 241 females, 14 unsexed; size range: 5–26 cm TL
- *Coptodon zillii:* 72 males, 25 females, 5 unsexed; size range: 3–16.5 cm TL
- *Sarotherodon galilaeus:* 106 males, 142 females, 15 unsexed; size range: 5–23 cm TL

Additionally, the study included:

- *Clarias gariepinus:* 58 individuals (13 males, 43 females, 2 undetermined); size range: 15.2–59.2 cm TL
- *Chelon ramada:* 197 individuals (131 males, 42 females, 24 unsexed); size range: 11–39 cm TL
- *Chelon auratus:* 38 individuals (27 males, 9 females, 2 undetermined); size range: 11–24 cm TL
- *Dicentrarchus labrax:* 51 individuals (31 males, 18 females, 2 unsexed); size range: 10–55.8 cm TL
- *Dicentrarchus punctatus:* 42 individuals (25 males, 12 females, 5 unsexed); size range: 9–22.5 cm TL
- *Anguilla anguilla:* 53 individuals (25 males, 20 females, 8 unsexed); size range: 39–74.5 cm TL

Total length (TL) was measured to the nearest centimeter, and body weight (W) was recorded to the nearest 0.1 g. Sex determination was performed macroscopically via specimen dissection. Although macroscopic staging is faster and less expensive, there is a greater chance of bias and inaccuracy (Flores et al., 2019). Immature individuals are hard to identify, because juvenile individuals often lack any visible sex-specific features. So, their gonads are underdeveloped and can be easily misidentified or indistinguishable macroscopically.

The length-weight relationship for each fish species was estimated using the following formula (Ricker, 1975):

$W = aL^b$

where:

- *W* represents the weight of the fish (g) • *L* represents the total length of the fish (cm)
- *a* and *b* are constants.

The allometric coefficient (*b*) was used to determine the growth type: isometric ($b = 3$), positive allometric ($b > 3$), or negative allometric ($b < 3$) (Morey et al., 2003).

The condition factor (*K*) was calculated using the following formula (Pauly, 1993): $K = 100W / L^3$ where:

- *K* represents the condition factor
- *L* represents the total length (cm)
- *W* represents the total weight (g)

The overall sex ratio was calculated as the number of males: number of females (Vazzoler, 1996).

A chi-square test (Sokal and Rohlf, 1987) was performed using XLSTAT 2021 software to determine if significant differences existed between observed sex ratios and the expected 1:1 ratio. The chi-square test was also used to assess differences in sex ratios across species.

Expected frequencies were calculated as:

Expected frequency = (Species total * Sex total) / Grand total The chi-square statistic was calculated as:

$\chi^2 = \Sigma$ [(Observed frequency - Expected frequency) ^{2} / Expected frequency] Degrees of freedom were determined using:

Degrees of freedom = (Number of rows - 1) * (Number of columns - 1)

A chi-square distribution table and a significance level of $\alpha = 0.05$ were used to determine the critical value for the given degrees of freedom. The null hypothesis (that there were no significant differences in sex ratios across species) was rejected if the calculated chi-square value exceeded the critical value.

RESULTS AND DISCUSSION

Distribution of lengths of males and females

The size range of the 454 male *O. niloticus* individuals sampled was 5.0 to 26.0 cm with an average length of 15.05 cm, while the length range of the 633 females varied between 6.0 and 28.0 cm with an average length of 16.64 cm. The size ranges (and average lengths) of male *O. aureus*, *C. zillii*, and *S. galilaeus* were 7.7–24.8 cm (15.5 cm); 4.0–16.5 cm (10.42 cm); and 7.5–23.0 cm (15.0 cm), respectively. The corresponding size ranges (and average lengths) for females of these species were 7.7–26.0 cm (15.35 cm); 6.7–16.0 cm (9.88 cm); and 8.3–21.3 cm (14.5 cm), respectively.

The average lengths of males and females, respectively, for the remaining species were: *C. gariepinus* (32.7 cm, 35.2 cm); *C. ramada* (21.0 cm, 22.8 cm); *C. auratus* (16.5 cm, 17.7 cm); *D. labrax* (21.5 cm, 25.8 cm); *D. punctatus* (13.7 cm, 16.2 cm); and *A. anguilla* (50.9 cm, 42.9 cm) (Table 1). Note that females attained larger lengths than males in *O. niloticus*, *O. aureus*, *C. gariepinus*, *C. ramada*, *D. labrax*, and *D. punctatus* (Fig. 2).

Various ecological and life-history characteristics, including aquatic health, selectivity, stock conditions, and breeding seasons, can be inferred from the size distribution of the fish caught (Beyer, 1987). This study demonstrates that females of *O. niloticus*, *O. aureus*, *C. gariepinus*, *C. ramada*, *D. labrax*, and *D. punctatus* grow to larger lengths than their male counterparts. In many fish species, males and females exhibit different growth rates and achieve different sizes. Female fish often grow larger in species where natural selection and environmental factors play a significant role. Conversely, male growth can be more strongly influenced by sexual selection, where traits that enhance male attractiveness to females or improve their competitive ability for mates are favoured (Zachary & Tobler, 2017).

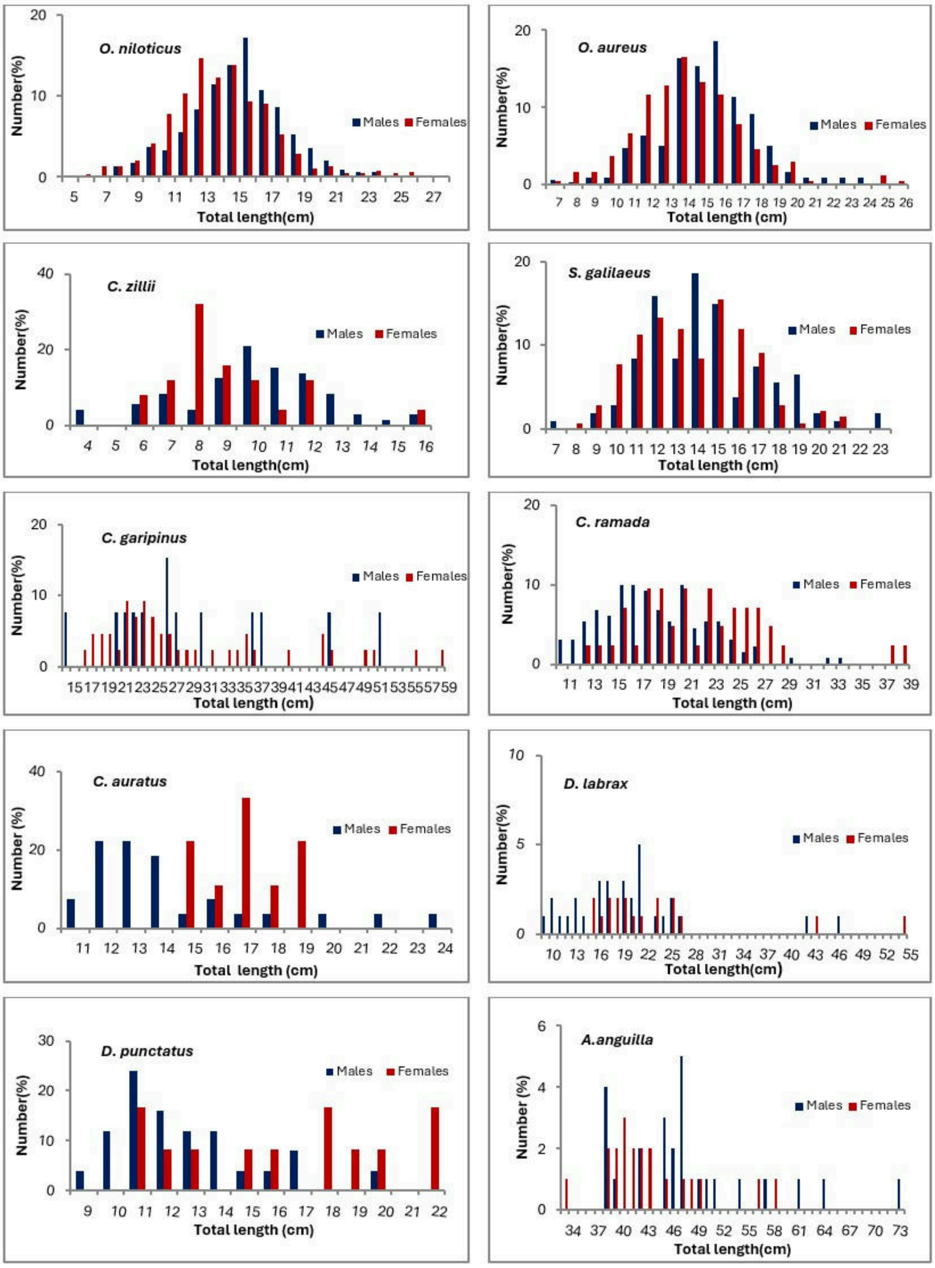
Length- weight relationship and condition factor

The current study estimated parameters *a* and *b* of the length-weight relationships and the condition factor (*K*) for each sex of the various species studied in the Manzala Lagoon (Table 1). R^2 values were greater than 0.94 for all regressions. The *b* exponent ranged from 2.72 (female *D. punctatus*) to 3.56 (male *A. anguilla*). A tendency towards isometric growth was recorded for males and females of *O. aureus*, females of *O. niloticus*, and males of *D. labrax*. Positive allometric growth was observed for males and females of *C. gariepinus* and *A. anguilla*, females of *C. zillii*, and males of *C. auratus*. The remaining sexes of the different species exhibited negative allometric growth (Table 1).

The condition factors (*K*) for both sexes of the different species studied in the Manzala Lagoon showed slight variations. *K* values ranged from 0.220 (male *A. anguilla*) to 1.794 (female *C. zillii*). Both sexes of the studied cichlid species exhibited good condition, with *K* values ranging from 1.794 (female *C. zillii*) to 1.649 (male *O. aureus*). Females of *O. niloticus*, *O. aureus*, and *S. galilaeus* had slightly higher condition factors than males, while *C. zillii* males had better condition than females. Additionally, males of *C. ramada* and *D. punctatus* displayed slightly higher condition factors than females. Males and females of *C. gariepinus* and *A. anguilla* exhibited similar, but poor, condition factors (less than 1), with values of 0.728 and 0.220, respectively (Table 1).

Figure 2.

Length Frequency Distributions of Fish Species (Males and Females) Studied in Lake Manzala



In the present study, a tendency towards isometric growth was recorded for males and females of *O. aureus*, females of *O. niloticus*, and males of *D. labrax*. Positive allometric growth was observed for males and females of *C. gariepinus* and *A. anguilla*, females of *C. zillii*, and males of *C. auratus*. The remaining sexes of the different species exhibited negative allometric growth. A negative allometric growth pattern (b=2.625) was observed for female *A. anguilla* in Bardawil Lagoon, while males exhibited isometric growth (b=2.997) (Mahmoud et al., 2024). Negative allometry indicates a relatively thinner body, whereas positive allometry indicates that the body becomes relatively broader as it increases in length (Jobling, 2002). Isometric growth signifies that the body increases in all dimensions proportionally. Shalloof et al. (2024) reported that the lowest *K* values for pooled data in the Manzala Lagoon were recorded for *A. anguilla* and *C. gariepinus* (0.21985 and 0.7287, respectively). This may be attributed to the alteration of the environment by dredged materials, significantly impacting food availability. Mean condition factors (K) in Lake Manzala before dredging were higher than recorded in the present study. Bayoumi and Khalil (1988) mentioned that K= 1.96, 1.93, 2.09 and 2.04 for *O. niloticus, O. aureus, S. galilaeus* and *C. zillii,* respectively. The condition factor of *A. anguilla* in Bardawil Lagoon also indicated poor condition, ranging from 0.178 ± 0.002 for females to 0.180 ± 0.002 for males (Mahmoud et

Table 1.

Length-Weight relationships and condition factors (k ± sd) for both sexes of ten fish species in the Manzala lagoon.

Species	Length range (cm)	Average Length (cm)	Sex	a	b	R^2	K± SD
O. niloticus	5-26	15.05	Male	0.0182	2.99	0.97	1.752 ± 0.184
	6-28	16.64	Female	0.0146	3.07	0.98	1.780 ±0.190
O. aureus	7.7- 24.8	15.5	Male	0.0143	3.05	0.96	1.649±0.176
	7.7-26	15.35	Female	0.0153	3.03	0.96	1.686±0.197
C. zillii	4- 16.5	10.42	Male	0.0224	2.90	0.98	1.794±0.250
	6.7- 16	9.88	Female	0.0085	3.33	0.98	1.775±0.185
S. galilaeus	7.5-23	15.0	Male	0.0199	2.94	0.97	1.713±0.174
	8.3- 21.3	14.5	Female	0.0191	2.97	0.97	1.763±0.178
C. gariepinus	15.2- 52.5	32.7	Male	0.0027	3.23	0.99	0.728±0.089
	17- 59.2	35.2	Female	0.0049	3.12	0.99	0.728±0.084
C. ramada	11- 34	21.0	Male	0.0101	2.95	0.97	0.882±0.117
	13- 39	22.8	Female	0.0101	2.95	0.97	0.870±0.098
C. auratus	11- 24	16.5	Male	0.0031	3.35	0.99	0.799±0.081
	15.2- 19.9	17.0	Female	0.0111	2.91	0.95	0.868±0.056
D. labrax	10- 47	21.5	Male	0.0100	3.01	0.99	1.036±0.122
	16- 55.8	25.8	Female	0.0109	2.98	0.99	1.040±0.095
D. punctatus	9- 20	13.7	Male	0.0123	2.92	0.95	1.000±0.135
	11- 22.5	16.2	Female	0.0210	2.72	0.99	0.969±0.104
A. anguilla	39- 74.5	50.9	Male	0.0002	3.56	0.96	0.220±0.033
	39- 59	42.9	Female	0.0010	3.20	0.94	0.221±0.023

Table 2.

Sex ratio variations and expected frequencies of males and females for the investigated ten fish species.

Species	Males	Females	Total	Sex ratio (M:F)	Expected Males	Expected Females
O. niloticus	454	633	1087	1: 1.40	479.61	607.39
O. aureus	318	241	559	1: 0.76	246.43	312.57
C. zillii	72	25	97	1: 0.35	42.79	54.21
S. galilaeus	106	142	248	1: 1.34	110.08	137.92
C. gariepinus	13	43	56	1: 3.31	24.39	30.61
C. ramada	131	42	173	1: 0.32	76.55	96.45
C. auratus	27	9	36	1: 0.33	15.95	20.05
D. labrax	31	18	49	1: 0.58	21.74	27.26
D. punctatus	25	12	37	1: 0.48	16.37	20.63
A. anguilla	25	20	45	1: 0.80	19.95	25.05

al., 2024). Balazik et al. (2020) reported that the migration of anadromous fish to spawning habitats is believed to be seriously threatened or impacted by dredging activities.

Sex ratios of different studied fish species

In the present study, the sex ratio distributions presented in Table 2 reveal that sex ratios were not evenly distributed among the populations of the studied species during the study period. A chi-square test was performed to assess the significance of these deviations. The calculated expected frequencies are shown in Table 2. The calculated chi-square statistic was 114.76, with 9 degrees of freedom. Using a chi-square distribution table with $\alpha = 0.05$ and 9 degrees of freedom, the critical value was approximately 16.92. Since $114.76 > 16.92$, the null hypothesis was rejected. Therefore, the chi-square test revealed a statistically significant difference in sex ratios across the ten species.

In this study, the proportion of males exceeded that of females in most of the studied species. The male proportion was approximately three times that of females in *C. zillii*, *C.*

ramada, and *C. auratus* (M: F ratios of approximately 3:1, or 1:0.35, 1:0.32, and 1:0.33, respectively), while this proportion was approximately double in *D. punctatus* (1:0.48). The proportion of males also surpassed that of females in *D. labrax* (1:0.58) and *A. anguilla* (1:0.80). Females outnumbered males only in *O. niloticus*, *S. galilaeus*, and *C. gariepinus*, constituting proportions of 1:1.40, 1:1.34, and 1:3.31 (male: female), respectively.

The sex ratio is the most significant demographic parameter influencing the structure and sustainability of fish populations (Penman & Piferrer, 2008). Information on the sex ratio is essential for understanding the complex interplay between individuals, their environment, and the overall state of the population (Vicentini & Araújo, 2003). A variety of factors can cause the sex ratio to deviate from the expected 1:1 ratio, either between species or even within the same population at different times. These factors include population adaptation, reproductive behavior, food availability, and environmental conditions (Nikolsky, 1963; Emlen & Oring, 1977; Baroiller & D'Cotta, 2001; Brykov et al., 2008; Vandeputte et al., 2012). Deviations can also arise from differential mortality rates between males and females (Arendt et al., 2014), local competition (Hamilton, 1967), and environmental pollution.

Several factors can contribute to sex ratio imbalances. Temperature, resource availability, and pollution can influence sex determination in some species (Shen & Wang, 2018). Mutations or genetic disorders can also affect sex ratios (Stelkens & Wedekind, 2010). Furthermore, anthropogenic pressures such as overfishing, habitat destruction, and pollution can disrupt natural sex ratios (Heppell et al., 2005; Islam & Tanaka, 2004; Jones & Reynolds, 1997; Rothschild et al., 1994). The observed differences in this study, particularly the increased proportion of males compared to females in most of the studied species, may be due to increased male vulnerability to fishing operations. Additionally, dredging operations can expose fish to elevated levels of suspended dust (clay or sand), potentially hindering their ability to find food, increasing stress levels, and causing gill damage that impairs growth, development, and swimming ability.

The impact of lagoon dredging on fish populations, and specifically their sex ratios, is a complex issue with significant ecological and environmental ramifications. Numerous studies have documented the impact of dredging on the life history parameters of fish species. Bellucci et al. (2002) observed that heavy metals and endocrine-disrupting chemicals can disrupt hormonal balances, affecting sexual differentiation in European sea bass (*Dicentrarchus labrax*) and leading to skewed sex ratios with a higher prevalence of intersex individuals. A study on Indian mullet (*Mugil cephalus*) in the Sundarbans lagoon revealed that pollutants disturbed by dredging had significant effects on sex ratios, favoring the development of females over males. This skewed sex ratio was attributed to elevated levels of bisphenol A (BPA) and other endocrine-disrupting chemicals released during dredging activities (Mukherjee et al., 2015; Bhattacharya et al., 2017). Research by Browne et al. (2013) on coral trout (*Plectropomus leopardus*) demonstrated that dredging-related temperature changes can alter sex ratios, resulting in a higher proportion of males.

A considerable preponderance of males in *O. niloticus*, *O. aureus*, and *C. zillii* was observed by Shalloof (1991) in studies conducted on Lake Manzala. The overall sex ratio (female: male) of *C. zillii* in the Pınarbaşı Spring Creek (Turkey) was reported as 0.7:1 (Innal & Giannetto, 2017). The overall male: female ratio of *Liza ramada* in Bardawil Lagoon was 1:1.28 (El-Aiatt & Shalloof, 2018), while in Lake Timsah, the sex ratio (male: female) was 1:1.7 (El-Halfway et al., 2007). Unequal sex ratios can reduce genetic diversity, increasing population vulnerability to environmental changes and diseases (Stelkens & Wedekind, 2010). Sex ratio imbalances may also lead to changes in mating systems or behaviors (Székely et al., 2014).

Although *C. gariepinus* is a substrate spawner (Bruton, 1979), the proportion of females in the current study was higher than that of males. This may be because the mortality rate of males is higher than that of females, or because the altered depths resulting from dredging are less suitable for male survival. Previous research has demonstrated that various factors, including dredging technique, depth, external controls, and lake characteristics, can influence the impact of dredging (Zhong et al., 2018). Lagler et al. (1977) showed that *C. gariepinus* and eels may avoid nets by burying themselves in soft, muddy bottoms. Conversely, shoals of mullet, typically found in shallow water areas, are fast-moving and timid, making them difficult to encircle with nets unless a deliberate effort is made (Ishak et al., 1988). Within-population variance in family sex ratios can be substantial due to differences in micro-ecological factors affecting eggs or larvae (Wedekind, 2012). Santi et al. (2017) reported that temperature appears to be the primary environmental factor influencing sex differentiation. Consequently, changes in global temperature may negatively impact the fitness of certain populations by altering sex ratios and influencing sex-specific survival through genetic and epigenetic mechanisms (Consuegra & Rodríguez López, 2016). Bruton (1979) also noted the importance of factors such as water level, temperature, clarity, and biological elements like flooded marginal plants, associated chemical changes, and the availability of suitable spawning areas.

Sex ratio imbalances can have profound consequences for population dynamics. A skewed sex ratio can limit reproductive potential if one sex is considerably more abundant than the other (Lowerre-Barbieri et al., 2011). Therefore, we posit that the noticeable differences in sex ratios observed in most of the studied species may contribute to the general decline in fish populations within the lagoon in recent years. The lower productivity of the lake in 2021 compared to 2020 may be attributed to dredging operations, which could have resulted in female mortality, nest destruction, and egg damage. The Lake Fisheries Resources Development Authority (LFRPDA, 2021) reported that Lake Manzala's fish production decreased by 13.38%, from 82,541 metric tons in 2020 to 71,496 metric tons in 2021. Tilapia production also decreased by 7.55%, from 41,147 metric tons in 2020 to 38,039 metric tons in 2021. Given that cichlids constitute the majority of the fish community in the study area, this decline in tilapia production may be the primary reason for the lake's reduced overall productivity. Furthermore, the noise, turbidity, and water disturbance generated by dredging activities may have negatively impacted female tolerance. The removal of most aquatic plants during lake cleaning operations is another potential factor contributing to the lower production. Stock abundance within a region or productivity zone is often correlated with the density of aquatic vegetation (i.e., stocks increase with increasing vegetation density). This aligns with findings from other African systems dominated by tilapia stocks (Lagler et al., 1977; Toews, 1977).

CONCLUSION

Dredging operations in Lake Manzala have unavoidable impacts on aquatic life, as the removal of bottom substrates disrupts established habitats and essential ecological processes. We hypothesize that the observed male predominance in most studied species, especially substrate-spawners, is linked to female intolerance of the disturbances induced by dredging activities. This sex ratio imbalance likely contributes to the recently observed decline in the lake's overall productivity. It is important to note that conclusions for species with smaller sample sizes are preliminary and have limited dependability. Further investigation into the reproductive biology of these species, particularly their fecundity and population structure, is recommended once dredging activities have ceased. Sex ratio imbalances in this study underscore the need to incorporate sex ratio data into population assessments and conservation management strategies for Lake Manzala.

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Research Article

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Isolation and Identification of Cultivable Bacteria in an Aquaponic System with Koi Carp and Parsley *(Petroselinum crispum)*: Implications for Water Quality and System Health

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Abstract Aquaponic integrates aquaculture and hydroponic plant cultivation into a sustainable food production system. Understanding the microbial profile, particularly the cultivable bacterial species in such systems, is critical for maintaining the health of both aquatic animals and plants. In this study, water samples were collected from a recirculating aquaponic system containing koi carp (*Cyprinus carpio* var. *koi*) and parsley (*Petroselinum crispum*), as well as from a separate aquaculture tank. Samples were taken from the sump (AP-S), the fish tank within the aquaponic system (AP-FT), and the standalone aquaculture tank (AC). After serial dilutions, the samples were inoculated on Tryptic Soy Agar (TSA) and incubated at 22 °C for 24–48 hours. Isolated colonies were analyzed based on their morphological, physiological, and biochemical characteristics, and further identified using the API 20E system. The bacterial species isolated from the aquaponic system included *Bacillus spp.*, *Aeromonas hydrophila, Shewanella putrefaciens,* and *Flavobacterium* spp., *Micrococcus luteus, Pseudomonas* sp., *Serratia* spp. In contrast, the aquaculture tank yielded dominantly *A. hydrophila, A. caviae,* and *A. sobria*. The absence of *A. caviae* and *A. sobria* in the aquaponic system suggests that *Bacillus* spp. may exert an inhibitory effect against opportunistic pathogens such as *A. caviae,* and *A. sobria*. These findings provide valuable insight into the microbial ecology of aquaponic systems and highlight the importance of microbial monitoring for sustainable and healthy production environments. Furthermore, the presence of additional genera such as *Pseudomonas*, *Serratia* and *Micrococcus* highlights the need for broader microbial monitoring beyond typical aquaculture pathogens.

Keywords Aquaponic · cultivable bacteria · koi carp · parsley · water quality · *Bacillus* · *Aeromonas*

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INTRODUCTION

Aquaponic is a sustainable food production method that integrates recirculating aquaculture with hydroponic plant cultivation in a closed-loop system. In this setup, nutrient-rich water from fish tanks is filtered through plant grow beds, allowing for mutual biological support between fish and plants. These systems have garnered significant attention in recent years for their efficient resource utilization, minimal environmental impact, and suitability for urban and water-limited regions (Somerville et al., 2014; Goddek et al., 2016; Schmautz et al., 2017; Tunçelli et al., 2024).

At the heart of aquaponic systems lies a diverse microbial community that bridges the gap between aquaculture and hydroponics. Microorganisms, particularly bacteria, play a central role in nutrient transformation—especially through nitrification—by converting toxic ammonia excreted by fish into plant-available nitrate via *Nitrosomonas* and *Nitrobacter* species (Eck et al., 2021; Somerville et al., 2014). The establishment of these bacteria is essential during the startup phase, as an insufficient nitrifying community can result in harmful ammonia and nitrite accumulation, leading to fish mortality or impaired plant growth (Badiola et al., 2012; Tunçelli and Memiş, 2024).

Microbial diversity and abundance in aquaponic systems are highly variable and depend on system design, water quality, and the interactions between fish, plants, and microbes. A stable microbial environment supports productivity, while imbalances may allow opportunistic pathogens to proliferate (Kasozi et al., 2019; Joyce et al., 2019). In this context, using native beneficial bacteria and optimizing system conditions is increasingly emphasized in recent research (Tunçelli et al., 2023a). Furthermore, lighting and system layout have been shown to affect both plant growth and microbial dynamics (Tunçelli et al., 2023b), highlighting the need for integrative design strategies.

Several fish pathogens, such as *Aeromonas hydrophila*, *Vibrio* spp., and *Pseudomonas aeruginosa*, have been identified in aquaponic environments (Fischer et al., 2021). However, the ecological role of other bacteria—such as *Acidovorax, Sphingobium, Flavobacterium*, and *Pseudomonas*—in plant growth, disease suppression, or microbial balance remains underexplored (Sirakov et al., 2016). On the other hand, there is growing evidence that beneficial rhizobacteria such as *Pseudomonas*, *Bacillus*, *Serratia*, and *Enterobacter* species contribute to plant development by enhancing nutrient availability, suppressing plant pathogens, and forming biofilms on plant roots (Chabot et al., 1993; Nadeem et al., 2014; Lareen et al., 2016; Bartelme et al., 2018).

Bacillus spp., in particular, are of special interest due to their dual benefits: they not only support plant growth but are also widely recognized as probiotics in aquaculture. These bacteria have been shown to improve fish immunity, inhibit the growth of harmful microorganisms, and maintain microbial balance within aquaponic systems (Gatesoupe, 1999; Shafi et al., 2017; Kasozi et al., 2021). Furthermore, *Bacillus subtilis, B. megaterium, Serratia marcescens*, and *Micrococcus luteus* have been isolated from aquaponic systems and may serve beneficial roles for both fish and plants (Sheema & Dorai, 2017). Some commercial hydroponic probiotics already include these strains, supporting their practical relevance. Studies on aquaponic lettuce production have also demonstrated that systems managed with microbial care can result in high food safety and improved product quality (Tunçelli et al., 2023a).

Although the relationship between bacteria and health outcomes in aquaponic systems requires further investigation, some studies suggest that properly functioning aquaponic may reduce the prevalence of pathogenic bacteria. For example, *A. hydrophila* was detected in catfish-based aquaponic systems without evidence of disease (Chitmanat et al., 2015), and *Flavobacterium columnare* outbreaks in yellow perch were only observed following system malfunction and nitrite accumulation (Bartelme et al., 2019). Similarly, Aeromonas levels were reported to be low in tilapia-lettuce systems with healthy fish (Schmautz et al., 2017).

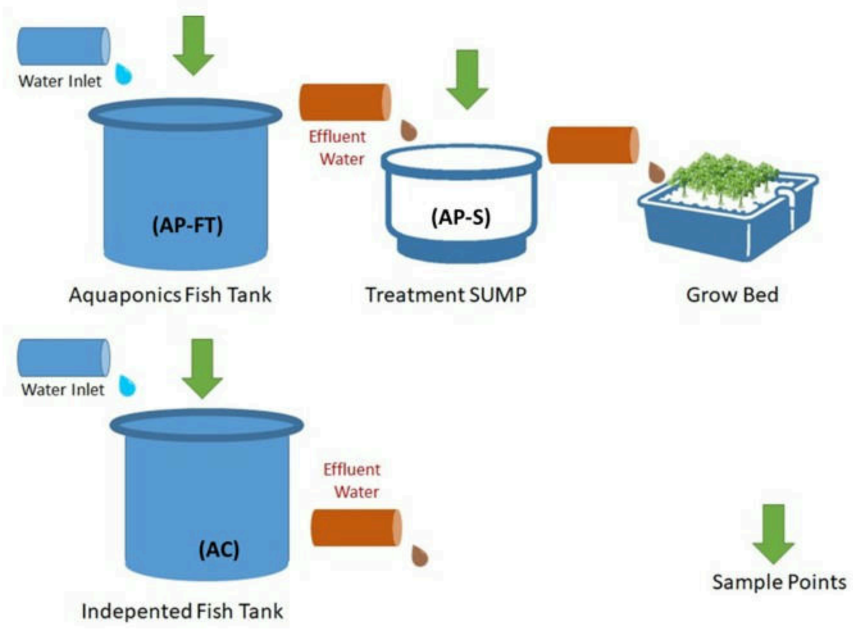
Importantly, microbial populations can vary across different compartments of aquaponic systems—including fish tanks, drum filters, biofilters, and hydroponic beds—due to differences in oxygen levels, organic matter, and nutrient flow (Schmautz et al., 2017). As such, compartment-specific microbial monitoring is crucial for optimizing system performance and preventing pathogen outbreaks.

Given these complexities, a deeper understanding of cultivable bacteria in aquaponic systems is essential. Culture-based methods, despite the popularity of molecular tools, remain indispensable for isolating and identifying metabolically active bacterial species that directly influence system dynamics and organism health.

The aim of this study was to isolate and identify cultivable bacteria present in the water of an aquaponic system containing koi carp (*Cyprinus carpio* var. *koi*) and parsley (*Petroselinum crispum*), and to compare them with those found in a separate aquaculture tank system. The findings contribute to our understanding of microbial ecology in aquaponic and inform strategies for improving system stability and health management.

Figure 1.

The schematized representation of the locations where water samples are collected



MATERIALS AND METHODS

Fish, Aquaponic and Aquaculture System Setup

The experiment was conducted using a recirculating aquaponic system that incorporated parsley (*Petroselinum crispum*) and koi carp (*Cyprinus carpio* var. *koi*). The plant cultivation was carried out in three rectangular fiberglass grow beds (dimensions: 220 × 50 × 25 cm; volume: 200 L each). A separate aquaculture tank (AC) was also included to serve as a comparative system without plants (Figure1).

The aquaponic system used in this study was designed as a Deep Water Culture (DWC) system with three interconnected fiberglass grow beds (220 × 50 × 25 cm; 200 L each). Water circulation was maintained using a CATPOWER 662 submersible pump (flow rate: 1500 L/h) and continuous aeration was provided by an Aquaticlife PG-370 blower (airflow: 60 m^3/h). Lighting was supplied by two 200W full-spectrum COB LED grow lights per tank, delivering an estimated PPFD of ~200–250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level. Water temperature was maintained at 22 ± 1 °C, pH around 7.0, and dissolved oxygen between 6.5–7.5 mg/L. No external fertilizers or mineral supplements were added; nutrients were solely derived from fish waste. Parsley was not harvested during the experimental period.

The aquaponic fish tank (AP-FT), sump (AP-S), and aquaculture tank (AC) were all operated under similar environmental conditions. Throughout the 45-day experimental period, koi carp were fed once daily at 2% of their estimated body weight using commercial juvenile trout pellets (Ozpekler Yem, Türkiye), containing 45% crude protein, 20% crude fat, and 9% ash on a dry matter basis. Feed was administered manually and uneaten feed was removed after 15 minutes to avoid excess nutrient accumulation in the system. No fish were handled, measured, or subjected to invasive procedures during the study. Fish were not disturbed or manipulated in any way, and no biometric data (e.g., weight, length) were recorded.

Following a 45-day system operation period, water samples were collected from AP-FT, AP-S, and AC. Samples were taken in sterile, dark 500 mL glass bottles and immediately transported under refrigerated conditions for microbiological analysis.

All experiments in this study were carried out in accordance with the "Regulation on the Welfare and Protection of Aquatic Vertebrates Used for Scientific Purpose" from the Republic of Turkey Ministry of Agriculture and Forestry (30751).

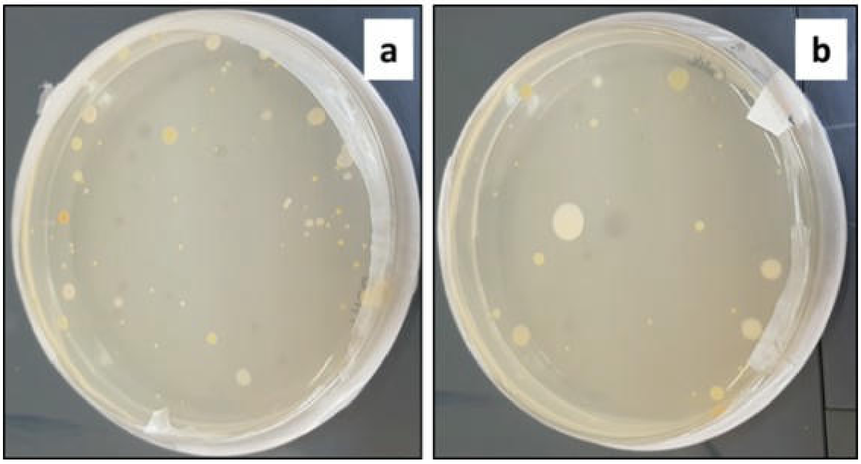
Microbiological Analysis of Water Samples

The collected water samples (AP-S, AP-FT, and AC) were subjected to serial dilution (1:9, 1:99, and 1:999) using sterile distilled water. From each dilution, 200 µL was spread-plated onto Tryptic Soy Agar (TSA) using sterile drigalski spatulas in three repetitions. Plates were incubated at 22 °C for 24–48 hours.

After incubation, colonies were examined based on morphology (color, shape, margin) and subsequently sub-cultured for pure isolation. Each isolate was subjected to a series of conventional bacteriological identification tests including:

Figure 2.

Bacterial colonies from AP-FT (a) and AP-S (b) sample on TSA after 48 h incubation (1:99 dilution)



-Morphological and physiological characterization: Gram staining, motility (hanging drop method), cytochrome oxidase, and catalase tests.

-Biochemical tests: Oxidation/fermentation (O/F), indole production, Methyl Red (MR), Voges-Proskauer (VP), nitrate reduction, citrate utilization, esculin hydrolysis, gelatin liquefaction, amylase production, urease, β-galactosidase (ONPG), and sugar fermentation tests including arabinose, maltose, mannitol, mannose, xylose, sucrose, inositol, rhamnose, galactose, lactose, sorbitol and usage amino acids such as arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase (Koneman et al., 1992; Holt et al., 1994; Austin and Austin, 2012).

Identification of bacterial isolates was also confirmed using the API 20E rapid identification system for Gram negative bacteria (bioMérieux, France), and results were interpreted via the APIweb™ software platform (Buller, 2004)

RESULTS AND DISCUSSION

Across all samples, a total of ten cultivable bacterial genera were identified. These included *Bacillus* spp., *Aeromonas hydrophila*, *A. caviae*, *A. sobria*, *Shewanella putrefaciens*, *Flavobacterium* sp., *Pseudomonas* spp., *Micrococcus luteus*, Vibrio sp, *Staphylococcus* sp. and *Serratia* spp. This broad microbial diversity reflects the complex microbiome dynamics within aquaponic and aquaculture systems.

General Observations

Water samples collected from the aquaponic system—specifically from the sump (AP-S) and the fish tank (AP-FT)—as well as from the separate aquaculture tank (AC), yielde bacterial colonies with diverse morphologies after incubation on TSA medium. The samples from the aquaponic system (AP-S and AP-FT) exhibited nine distinct colony morphologies, while samples from the aquaculture tank displayed seven unique colony structure.

Colonies from each sample were characterized based on morphology, Gram staining, and biochemical profiling. 38 representative isolates from AP-S and AP-FT, and 30 isolates from AC, were subjected to detailed identification. The results indicated the presence of both Gram-positive and Gram-negative bacteria, with variability in biochemical traits across sampling points.

Bacterial Identification from Aquaponic System (AP-S and AP-FT)

After incubation, the bacterial colonies were divided into different types according to the colony characteristics of shape, size and color, and total 38 bacteria (n=38) were isolated. It was determined that there were no differences in bacterial species between AP-S and AP-FT. These isolates showed consistent colony morphologies between sump and tank samples, suggesting similar bacterial populations in different zones of the aquaponic system (Figure 2a, 2b).

Based on morphological, physiological, and biochemical analyses, *Bacillus* sp. (n=5), *Aeromonas hydrophila* (n=8), *Shewanella putrefaciens* (n=6), *Flavobacterium* sp. (n=4), *Serratia* spp. (n= 5), *Pseudomonas* spp. (n=4) and *Micrococcus luteus* (n=6) were identified in samples from the aquaponic system (AP-FT and AP-S). Table 1 and 2 summarizes the detailed biochemical profiles and differential traits used for identification.

It has been reported that the dominant bacterial species in aquaponic system water are *Aeromonas* spp., *Pseudomonas* spp. and *Staphylococcus* spp. (Chitmanat et al., 2015). In this study, it was noted that the dominant species among cultivable bacteria was *Aeromonas*, and *Staphylococcus* species detected in aquaculture water was not detected in

Table 1.

Biochemical and morphological characteristics of bacterial isolates from aquaponics system water (AP-FT, S) and aquaculture water (AC)

Characteristic	***Bacillus* sp.**		***Aeromonas hydrophila***			***Shewanella putrefaciens***			***Flavobacterium* sp.**	
Sample origin	AP-FT (n=3)	AP-S (n=2)	AP-FT (n=4)	AP-S (n=4)	AC (n=5)	AP-FT (n=3)	AP-S (n=3)	AC (n=4)	AP-FT (n=2)	AP-S (n=2)
Gram stain	+	+	-	-	-	-	-	-	-	-
Motility	-	-	+	+	+	+	+	+	K	K
Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
O/F test	F	F	F	F	F	-	-	-	O	O
Methyl Red (MR)	+	+	-	-	-	-	-	-	-	-
Voges-Proskauer (VP)	+	+	+	+	+	-	-	-	-	-
Indole production	-	-	+	+	+	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	-	-	-	-	-
ONPG	-	-	+	+	+	-	-	-	-	-
O/129-150	ND	ND	R	R	R	ND	ND	ND	ND	ND
Citrate utilization	-	-	+	+	+	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-
Triple Sugar Iron (TSI)	-	-	-	-	-	+	+	+	-	-
Gelatinase	+	+	+	+	+	+	+	+	-	-
Esculin hydrolysis	-	-	+	+	+	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	+	+	+	+	+	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	+	+	+	-	-
Lysine decarboxylase	-	-	+	+	+	-	-	-	-	-
Lactose fermentation	-	-	-	-	-	-	-	-	-	-
Sucrose fermentation	-	-	+	+	+	-	-	-	-	-
Xylose fermentation	-	-	-	-	-	-	-	-	-	-
Rhamnose fermentation	-	-	-	-	-	-	-	-	-	-
Galactose fermentation	-	-	+	+	+	-	-	-	-	-
Mannitol fermentation	-	-	-	-	-	-	-	-	-	-
Mannose fermentation	-	-	+	+	+	-	-	-	-	-
Maltose fermentation	+	+	+	+	+	-	-	-	-	-
Arabinose fermentation	-	-	-	-	-	-	-	-	-	-
Sorbitol fermentation	-	-	-	-	-	-	-	-	-	-
Inositol fermentation	-	-	-	-	-	-	-	-	-	-
API 20E	200700416		724712457			050200441			000000402	

+: Positive reaction, −: Negative reaction, AP-FT: Fish tank water, AP-S: Sump water, K: Swarming motility, O/F: Oxidation/Fermentation test, R: Resistance, ND; Not detected

Table 2.
Biochemical and morphological characteristics of additional isolates from aquaponics system water (AP-FT, S) and aquaculture water (AC).

Characteristic	***M. luteus***		***Pseudomonas spp***			***Serratia* spp.**		***Vibrio* sp.**	***Staphylococcus* sp.**
Sample origin	AP-FT (n=3)	AP-S (n=3)	AP-FT (n=2)	AP-S (n=2)	AC (n=4)	AP-FT (n=3)	AP-S (n=2)	AC (n=4)	AC (n=3)
Gram stain	+	+	-	-	-	-	-	-	+
Motility	-	-	+	+	+	+	+	+	-
Cytochrome oxidase	+	+	+	+	+	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+
O/F test	O	O	O	O	O	F	F	F	F
Methyl Red (MR)	+	+	-	-	-	V	V	-	ND
Voges-Proskauer (VP)	-	-	-	+	-	-	-	-	ND
Indole production	-	-	-	-	-	-	-	+	ND
Nitrate reduction	-	-	-	+	V	+	+	+	ND
β-galactosidase (ONPG)	-	-	-	-	-	+	+	+	ND
O/129-150	ND	ND	ND	ND	ND	ND	ND	S	ND
Citrate utilization	-	-	+	+	+	+	+	V	ND
Urease	+	+	-	-	-	v	v	-	ND
Triple Sugar Iron (TSI)	-	-	-	-	-	-	-	-	ND
Gelatinase	ND	ND	+	-	V	+	+	-	ND
Esculin hydrolysis	ND	ND	-	-	-	V	V	-	ND
Starch hydrolysis	-	-	-	-	-	-	-	+	ND
Arginine dihydrolase	-	+	+	+	+	-	-	-	ND
Ornithine decarboxylase	-	-	-	-	-	+	+	+	ND
Lysine decarboxylase	-	-	-	-	-	+	+	+	ND
Lactose fermentation	ND	ND	-	-	-	-	-	-	ND
Sucrose fermentation	+	-	-	+	-	+	+	+	ND
Xylose fermentation	ND	ND	+	+	+	v	v	-	ND
Rhamnose fermentation	-	-	-	-	-	v	v	-	ND
Galactose fermentation	-	-	ND	ND	+	+	+	ND	ND
Mannitol fermentation	-	-	+	+	+	+	+	+	ND
Mannose fermentation	-	-	+	+	+	+	+	+	ND
Maltose fermentation	-	-	+	+	+	+	+	+	ND
Arabinose fermentation	-	-	+	+	+	+	+	-	ND
Sorbitol fermentation	-	-	-	+	+	+	+	-	ND
Inositol fermentation	-	-	-	-	-	+	+	-	ND
API 20E	001402402		220706643			531676257		534412757	-

+: Positive reaction, −: Negative reaction, AP-FT: Fish tank water, AP-S : Sump water, O/F: Oxidation/Fermentation test, V: variable reaction, t: tetrads, S : sensitive, nd: not detected aquaponic water. This suggests that a situation has developed within the system that will inhibit the existence of *Staphylococcus* species such as *S. aureus*, *S. epidermidis*, and *S. saprophyticus* that causes infection in humans and fish.

Micrococcus spp. are widely distributed and are a common bacterial species in terrestrial and aquatic ecosystems (Dinev et al., 2023). *Micrococcus luteus*, belonging to the *Micrococcus* genus, has been reported to cause infection as an opportunistic pathogen (Pękala et al., 2018; Akayli et al., 2020) and to be used in fish health as a probiotic (Abd El-Rhman et al., 2009; Akaylı et al., 2016). The isolation of this bacterium in the aquaponic system has been reported in previous studies (Chitmanat et al., 2015). In this study, it was similarly isolated from the aquaponic system water, but not from the aquaculture water.

The identification of *Shewanella putrefaciens* and *Flavobacterium* spp. in the aquaponic system aligns with previous literature. *S. putrefaciens* is a facultative anaerobic bacterium frequently isolated from aquatic environments and has been implicated in disease outbreaks in rainbow trout and European sea bass (Korun et al., 2009). While *Flavobacterium* species is a component of normal aquatic microbiota, certain species within this genus may act as opportunistic pathogens under stress conditions.

Interestingly, tanks where *Bacillus* sp. were detected exhibited enhanced parsley growth and healthy root development. While direct causal relationships remain to be confirmed, previous studies suggest that *Bacillus* species can improve nutrient uptake, promote root elongation, and enhance overall plant vigor—potentially linking microbial balance to improved plant performance (Tsotetsi et al., 2022).

Water quality parameters—particularly neutral pH (~7.0), sufficient dissolved oxygen, and increased nitrate levels observed at the end of the grow-out period—likely supported a stable microbial community. These conditions are optimal for nitrifying bacteria as well as beneficial taxa such as *Bacillus* spp., potentially suppressing the proliferation of opportunistic pathogens like *A. sobria* and *A. caviae*.

Beneficial bacteria for plants form biofilms on the roots. The dominance of *Pseudomonas* species in aquaponic system water indicates that it is an important species in terms of biocontrol (Sirakov et al., 2016). However, for aquaponic systems, these types of bacteria need to be evaluated in terms of plant, human and fish health. In particular, there are reports that many *Pseudomonas* species cause diseases in both fish (Urku, 2021; Ürkü et al., 2024b) and plants (Höfte and Vos, 2006).

As a result of the development of the microflora of an aquaponic system, an environment that suppresses microbial pathogens is formed thanks to natural antibiotic compounds secreted by beneficial microorganisms-probiotics (Dinev et al., 2023). Therefore, application of biological control with antagonistic microorganisms against pathogens is a promising alternative. It has been reported that the genera *Pseudomonas* and *Bacillus*, which are among the most important groups of Rhizobacteria, are used as plant biocontrol agents for different crops (Suslow et al. 1982; Schippers et al., 1987). It has been reported that using a strain isolated from the system that provides the characteristics of being a biological agent will contribute to increasing fish growth and plant benefit and increase the amount of production (Sirakov et al., 2016; Yousuf et al., 2023).

Bacterial Identification from Aquaculture Tank (AC)

Gram-negative rod-shaped bacteria dominated in aquaculture tank (AC). Five cultivable bacterial genera were identified from AC water sample (Table 1,2). *A. hydrophila* (n=5), *A. caviae* (n=5), *A. sobria* (n=5), *Shewanella putrefaciens* (n=4), *Vibrio* sp. (n=4), *Staphylococcus* sp. (n=3) and *Pseudomonas* spp.(n=4) were the common bacteria with *Aeromonas* species being the most dominant.

The differentiation between *A. hydrophila, A. caviae,* and *A. sobria* was confirmed through biochemical tests according to Buller (2004) and API-complementary tests. Additionally, API 20E profile numbers of the isolates are given as detailed in Table 3. Notably, *A. caviae* and *A. sobria* were not detected in the aquaponic system, but were exclusive to the aquaculture tank.

A. hydrophila is facultatively anaerobic bacterium that distressed fish with septicemia and causes a disease known as Motile Aeromonas Septicemia (MAS), which affects the aquatic environment. *Aeromonas* species, bacterial species of zoonotic importance, can be commonly found in a variety of estuaries, seaweed, sea grass, used water, drinking water and also food (Austin & Austin, 2012; Semwal et al., 2023).

Aquatic microorganisms not only affect the water quality but are also associated with the fish physiological status, diseases and postharvest quality (Al-Harbi and Uddin, 2005). Therefore, the bacterial flora reflects the aquatic environment (Shewan and Hobbs 1967). It has been reported that the dominant bacterial species in carp aquaculture water are *Aeromonas* spp. and *Shewanella putrefaciens* (Al-Harbi and Uddin, 2008). This composition is considered to be consistent with both the natural intestinal flora of the fish and the surrounding water environment. In this study, *Aeromonas* spp. (*A. hydrophila, A. caviae*, and *A. sobria*) detected with high prevalence were accepted as the dominant species in standalone aquaculture tank.

Table 3.

Biochemical traits used for distinguishing A. hydrophila, A. caviae, and A. sobria from aquaculture tank (AC) water samples

Characteristic	***A. hydrophila* (n=5)**	***A. caviae* (n=5)**	***A. sobria* (n=5)**
Lysine decarboxylase	+	-	+
Voges-Proskauer (VP)	+	-	-
Arabinose fermentation	+	+	-
Triple Sugar Iron (TSI)	-	-	+
Esculin hydrolysis	+	+	-
API profil number	724712457	324612657	764612457

Notably, *A. caviae* and *A. sobria*—despite being present in the aquaculture tank—were absent from the aquaponic system. Instead, only the aquaponic water samples yielded *Bacillus sp.*, which are Gram-positive, spore-forming bacteria known for their antimicrobial metabolite production and potential probiotic roles. Several strains of *Bacillus* have been reported to inhibit pathogenic bacteria and enhance host immunity, supporting their value in biological disease control (Keysami et al., 2007; Aly et al., 2008). This suggests a possible competitive exclusion or inhibitory mechanism mediated by *Bacillus* sp.

API 20E

Although API 20E test results provided identifications for most isolates (e.g., *A. hydrophila* – 98.8%, *S. putrefaciens* – 99.9%), the API system yielded conflicting results for some Gram-positive isolates. Specifically, *Bacillus* sp., identified as Gram-positive rods morphologically, were misclassified as *A. salmonicida* (93.9%), a known Gram-negative species. In addition, according to API 20E results, it was determined that the bacteria causing gliding movement (n=4) was *Chryseobacterium indologenes* (37%). Additionally, *Vibrio* sp. isolated from aquaculture water was identified as *Vibrio parahaemolyticus* (77.4%) with API profile number 534412757. These inconsistencies underscore the limitations of using API kits—designed primarily for clinical samples—in aquatic environmental studies.

The traditional bacteriological method, which includes morphological, physiological and biochemical tests, is known to be the gold standard for identification of bacterial species. However, it has negative features in terms of time, cost and workload (Austin, 2019). API kits are the most used for the identification of fish pathogenic bacteria (Urku et al., 2024a). A limitation was identified in the accurate identification of environmental isolates of the API kit used in this study. Similarly, Chitmanat et al. (2015) reported that the API 20E strip kit was inadequate in identifying bacteria such as *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Plesiomonas shigelloides*, *Escherichia coli*, *Acinetobacter baumannii*, *Salmonella* sp., *Staphylococcus* sp., and *Micrococcus* sp. isolated from aquaponic systems.

Overall, the microbial diversity observed in the aquaponic system was different from that in the isolated aquaculture tank. This highlights the ecological complexity of integrated systems and suggests that the combined biological activity of plants, fish, and microbial communities may contribute to a more balanced and possibly protective microbial environment.

CONCLUSION

This study demonstrates the presence and diversity of cultivable bacteria in aquaponic and aquaculture water systems and underlines their potential impact on system health and sustainability. The identification of opportunistic pathogens such as *A. hydrophila, A. caviae,* and *A. sobria* in the aquaculture tank emphasizes the risk of bacterial infections in fish-only systems. In contrast, the aquaponic system, which included both fish and plants, showed a distinct bacterial profile that notably included *Bacillus* sp.- a genus widely associated with probiotic properties in aquaculture.

The absence of *A. caviae* and *A. sobria* in the aquaponic system, along with the presence of *Bacillus* sp., suggests a possible antagonistic or inhibitory effect that may contribute to pathogen suppression. These findings support the hypothesis that aquaponic systems may offer inherent microbial benefits through ecological balance and biological competition. In our next study, we plan to investigate the probiotic properties of *Bacillus* sp. strains as well as its effects against *A. caviae* and *A. sobria.*

In conclusion, monitoring and managing the bacterial communities within aquaponic systems is essential for promoting the health of aquatic animals and plants. Further research is recommended to explore the functional roles of beneficial bacteria and their potential use as natural biocontrol agents in integrated aquaponic environments.

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Research Article

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Reproductive Biology of Bogue, *Boops boops* Linneaus, 1758 in the Aegean Sea and Sea of Marmara, Türkiye

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Abstract Bogue (*Boops boops* Linnaeus, 1758) is one of the most economic and common species in Türkiye. The bogue individuals were obtained monthly from the commercial catches of gillnet fisheries from May to August 2024 and purse seine fisheries from September 2024 to April 2024 around the Northern Aegean Sea and Southwest Sea of Marmara, Türkiye. The 378 total individuals consisted of 176 females, 201 males and 1 unspecified in the Aegean Sea. The 370 total individuals consisted of 214 females, 154 males and 2 individuals unspecified in the Sea of Marmara. The female:male ratio was calculated as 0.9:1.0 in the Aegean Sea and 1.4:1.0 in the Sea of Marmara. The total length and weight of individuals varied from 10.6 to 28.6 cm (17.52±0.15) and 8.1 to 236.86 g (54.20±2.43) in the Aegean Sea, respectively, and the total length was 10.8 to 28.5 cm (17.92±0.18) and the weight was 13.08 to 399.88 g (76.54±3.14) in the Sea of Marmara. The positive allometric growth was found for females, males and all sexes in both seas. The first maturity length was determined as 12.2 cm TL for females, 15.8 cm TL for males in the Aegean Sea, whereas it was 12.8 cm TL for females, 15.1 cm TL for males in the Sea of Marmara. The reproductive period is estimated between January and May in the Aegean Sea, while February to May in the Sea of Marmara. The total fecundity varied between 2770-85112 (mean:20021±2027) in the Sea of Marmara, while it was 2740-309457 (mean:42834±9194) in the Aegean Sea. As results of this study, some differences were determined in sex ratio, spawning period, and fecundity from the reproductive characteristics between the Sea of Marmara and Aegean Sea. Due to the limited scientific data on the reproductive biology of *B.boops* in Türkiye, the results of the present study should contribute to the strategic assessment, management, and sustainability of *B.boops*.

Keywords Length-weight relationship · First maturity length · Gonadosomatic index · Fecundity · Reproductive period

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INTRODUCTION

Many fish stocks are either fully exploited or overexploited in the world (Vuorinen et al., 2021; Gaillet et al., 2022). The reproductive biology assessment of a species is important for the protection, sustainability, and management of exploited fisheries resources. The reproductive traits, such as sex composition, first maturity length, spawning season, fecundity, gonadosomatic index, condition factor and length-weight relationship must be determined and monitored for stock management (Gebremedhin et al., 2021). These characteristics also vary depending on the abiotic and biotic factors from region to region and over time (İnnal, 2019). The human impacts such as fishing pressure, pollution, and climate change can affect the reproductive characteristics of a species over a lifetime (Vinagre & Cabral, 2015). Specific to a species, understanding reproductive characteristics will contribute to the continuity of the species. Without these valuable data, the implementations for fisheries management become impossible.

Bogue *Boops boops* (Linneaus, 1758) is a teleost fish species belonging to the Sparidae family, widely found in the Mediterranean Sea, and distributed in the eastern Atlantic from Norway to Angola, oceanic islands, from Biscay Bay to Gibraltar, the western Atlantic in Mexico and the Caribbean Sea. Bogue lives in the bottom and middle waters of coastal areas with muddy, rocky, sandy bottom structures or seagrass areas. *B.boops* can be found at depths of up to 200 m in the Mediterranean Sea and 300 m in the Atlantic Sea (Bauchot and Hureau, 1986). So, this species can catch with different fishing gears such as gillnets, trawls and purse seines in the world seas. Bogue, shown in shoals, has economic and commercial value. The fluctuations in the catch amounts of *B.boops* are observed from year to year. The mean annual catch rates of the bouge between 2000 and 2023 were reported as 50 tonnes and 2027 tonnes from the Sea of Marmara and Aegean Sea, respectively (TUIK, 2024). The maximum length of *B.boops* can reach up to 36 cm, usually 15-20 cm total length (Bauchot & Hureau, 1986).

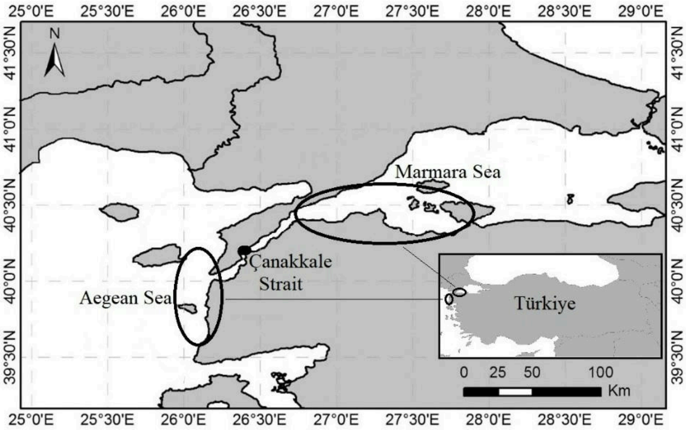
Although the reproductive biology of *B.boops* was investigated in the Aegean Sea (Bilge, 2008; Kara & Bayhan, 2015; Andsoy, 2015; Soykan et al., 2015; Cengiz et al., 2019, Cengiz, 2022; Koca, 2023), no study has investigated in the Sea of Marmara, yet. Therefore, this study is important as it presents the first findings of reproduction parameters of *B.boops* in the Sea of Marmara, and also provides important and current findings in terms of monitoring *B.boops* stocks in the Aegean Sea. Also, this study will enable us to compare *B.boops* stocks between adjacent seas and contribute to the sustainability and management of these stocks.

MATERIAL AND METHODS

A random sampling of minimum 30 individuals of *B.boops* were monthly collected from commercial catches of gillnet fisheries using 18, 20, 22, 23 mm mixed mesh sizes and purse seine fisheries using 8-10 mm mesh sizes in the Sea of Marmara and Aegean Sea. Due to the fisheries ban for purse seine between 15th April and 1st September in the Türkiye seas, the samples were collected from the gillnets fisheries between May 2024 and August 2024. After the end of the fisheries ban closure for purse seine fisheries, individuals were obtained from purse seine fisheries from September 2024 to April 2024. A total of 370 and 378 individuals were obtained in the Southwest of Sea of Marmara and the North of Aegean Sea, respectively (Figure 1). These samples were transferred to the laboratory immediately.

Figure 1.

Sampling areas in the Sea of Marmara and Aegean Sea



The total length (TL) was measured to the nearest 1 mm interval measuring board and total weight (TW) to the nearest 0.01 g precision scale. All individuals were dissected and then sex determination was assessed by macroscopic observation from the gonads. The female to male (F:M) ratio was calculated. The Chi-square (χ^2) test used with the IBM SPSS 25 (IBM Corp 2017) program to assess statistically difference between the female to male ratio. Gonads weight (GW) was recorded to the nearest 0.01 g precision scale. The sexual maturity stages from the gonads were determined by macroscopic examination according to Holden & Raitt (1974) with five maturity stages as; Stage I (Immature), Stage II (Maturing), Stage III (Ripening), Stage IV (Ripe), Stage V (Spent).

The length-weight relationship was estimated by the exponential regression equation:

$TW=a*TL^b$

where a and b were constants that were estimated by least square linear regression after log transformation (Ricker, 1975). Growth type was determined by the one-sample t-test of the "b" value for comparison of the slope (Sokal & Rohlf, 1987; Zar, 1996),

$Ts=(b-3)/SE(b)$

The gonadosomatic index (GSI) value was calculated using the formula by Gibson and Ezzi (1980):

$GSI=(GW/(TW-GW))\times 100$

The spawning period of *B.boops* was estimated by evaluating the highest GSI values coinciding the highest percentage of sexually mature individuals. The condition factor (CF) was determined from the equation by Htun-Han (1978):

$CF=TW/TL^3$

The length at first maturity (L_{50}) was estimated as the length at which 50% of males and females become mature during the months of maximum reproductive activity. The percentage mature by length class and sex by fitting a logistic function using the Newton algorithm, which is defined as:

$P(1)=1/1+e^{-(a+b1)}$

where P(l) is the proportion of mature specimens at length 1, and a and b are the parameters of the logistic equation (Piñeiro & Saínza, 2003).

The fecundity (F) was determined by using the gravimetric method. 57 mature female individuals from the Sea of Marmara and 61 mature female individuals from the Aegean Sea were used to estimate fecundity. A subsample of 0.05 g from 3 parts (anterior, middle and posterior) of each ovary was taken and weighed. The number of oocytes was counted from each ovary and their diameters were measured under stereomicroscope from 181 oocytes of 3 individuals in the Sea of Marmara and 209 oocytes of 8 individuals in the Aegean Sea. The absolute fecundity was calculated with Bagenal's formula (Bagenal 1978);

$F=Nx(GW/TW)$

where N is the mean number of oocytes ((anterior+middle+posterior oocytes)/3) in each gonad. The total length-fecundity and the total weight-fecundity relationships were estimated for females using linear and and exponential regression.

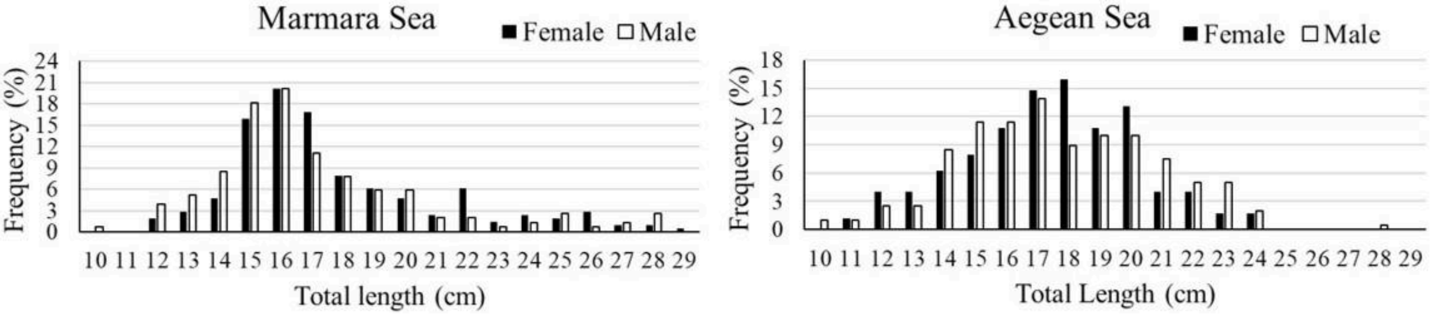
RESULTS AND DISCUSSION

In the present study, a total of 378 and 370 individuals of Bogue were examined from the Aegean Sea and from the Sea of Marmara, respectively. 176 of 378 individuals (46.6%) were detected as females, 201 individuals as males (53.2%) and 1 individual was not sexed (0.3%) in the Aegean Sea. In the Sea of Marmara, 2 individuals were not sexed (0.6%). Besides, 214 of 370 individuals were detected as female (57.8%) and 154 individuals as males (41.6%) in the Sea of Marmara. The sex ratio (female:male) was determined as 1.39:1 in the Sea of Marmara and 1:0.88 in the Aegean Sea. According to the results of Chi-square tests, a statistically important variation was detected in the sex ratios of the individuals obtained by Sea of Marmara (χ^2calc $9.783>\chi^2$table 3.841, $p:0.002<0.05$), whereas statistically important variation was not detected from the individuals obtained by Aegean Sea (χ^2calc $1.658<\chi^2$table 3.841, $p:0.198>0.05$). The sex ratio of species can generally vary from 1:1 to 1:3 in natural populations. However, this rate varies over time, even within the same population due to the temporary behavior such as migration observed in the population over time, and may even exceed this rate from time to time. So, it is necessary to determine the sex ratio in populations by sampling throughout the year (Avşar, 2016). In previous studies stated that the males are dominant in the Aegean Sea, Kara and Bayhan (2015), 1.17:1; Soykan et al. (2015), 3.57:1; Cengiz et al. (2019), 1.55:1; Seyhan Öztürk (2024), 1.67:1; Andsoy (2014), 1:0.26 the parallel in the Aegean Sea in our study. Also, El Samman et al. (2022) reported that the male:female ratio of *B.boops* was 1:0.49 on the Egyptian coast. On the contrary, Kara and Bayhan (2008) determined that females were dominant in the Aegean Sea. Koca (2023) stated that females were dominant (1:0.97) in the Gulf of Antalya in the Mediterranean Sea. The females were more dominant than males, Kasalica et al. (2011) in the Adriatic Sea (1:0.4); Massaro & Pajuelo (2018) in Canary islands of Spain (1:0.852); Dobroslavić et al. (2017) in the Adriatic Sea (1:0.85); Azab et al. (2019) coast of Alexandria, Egypt (1.03:1); Farah & Mavruk (2024) in the Mediterranean Sea (1.04:1) the parallel in the Sea of Marmara in our study.

In the Aegean Sea, the total lengths ranged between 10.6 cm and 28.6 cm (mean:17.19±0.22 cm TL) for females and distributed from 11.1 cm to 24.9 cm (mean:17.91±0.21 cm) for males. The total weights varied from 8.1 g to 236.86 g (mean:54.2±2.43 g) for females, and ranged between 10.55 g and 210.26 g (mean:63.51±2.47 g) for males. In the Sea of Marmara, the total length ranged between 10.8 cm and 28.5 cm (mean:17.52±0.29 cm TL) for females and distributed from 12.6 cm to 29.1 cm (mean:18.21±0.24 cm TL) for males. The total weight varied from 13.08 g to 399.88 g (mean:68.9±4.69 g) for females, and ranged between 19.04 g to 339.11 g (mean:81.52±4.22 g) for males. A significant difference with the Independent Samples T-test was found in the Aegean Sea between male and female individuals for total length (F:3.726, df:375, $P<0.05$) and weight (F:0.808, df:375, $P<0.05$), whereas a significant difference was not found in the Sea of Marmara for total length (F:0.160, df:366, $P>0.05$) and weight (F:2.13, df:366, $P>0.05$). The majority length groups were 16 cm TL for females (20.13%) and males (20.09%) in the Sea of Marmara, while the

Figure 2.

Sampling areas in the Sea of Marmara and Aegean Sea



18 cm TL for females (15.1%) and 17 cm TL males (13.93%) in the Aegean Sea (Figure 2).

Although the total length of *B.boops* can be up to 36 cm (Bauchot & Hureau, 1986), the largest individual reported that the total length of 40.2 cm and weight of 986 g was caught from Güllük Bay in the Mediterranean Sea (Türkiye) at a depth of 55 meters with gillnet. The proximity of the catching area to fish farms and the consequent good feeding may explain this size (Ceyhan et al., 2018). Bauchot and Hureau (1986) reported that *B.boops* is generally observed 15-20 cm in length. When previous studies are analyzed in Table 1, the mean total length of *B.boops* is close to the stated values. Babaoglu et al. (2021) reported that the mean total length and weight 10.22 cm and 11.39 g of *B.boops* in 18 mm trammel nets on the Bakırçay River in the Çandarlı Bay of the Aegean Sea. El Samman et al. (2022), determined the mean total length and weight as 13.98 cm and 28.51 g of 880 individuals *B.boops* using gillnets from 14 mm to 18 mm mesh sizes on the coast of Alexandria, Egypt. In this study, the mean total length and weight of *B.boops* was determined to be 17.52 cm, 58.46 g and 17.92 cm, 76.54 g in the Aegean Sea and the Sea of Marmara, respectively, sampled with purse seine and gillnet. The total length and weight of *B.boops* were larger in the Sea of Marmara than in the Aegean Sea. It is thought that these differences in total length and weight due to the sampling method, number of individuals and study areas.

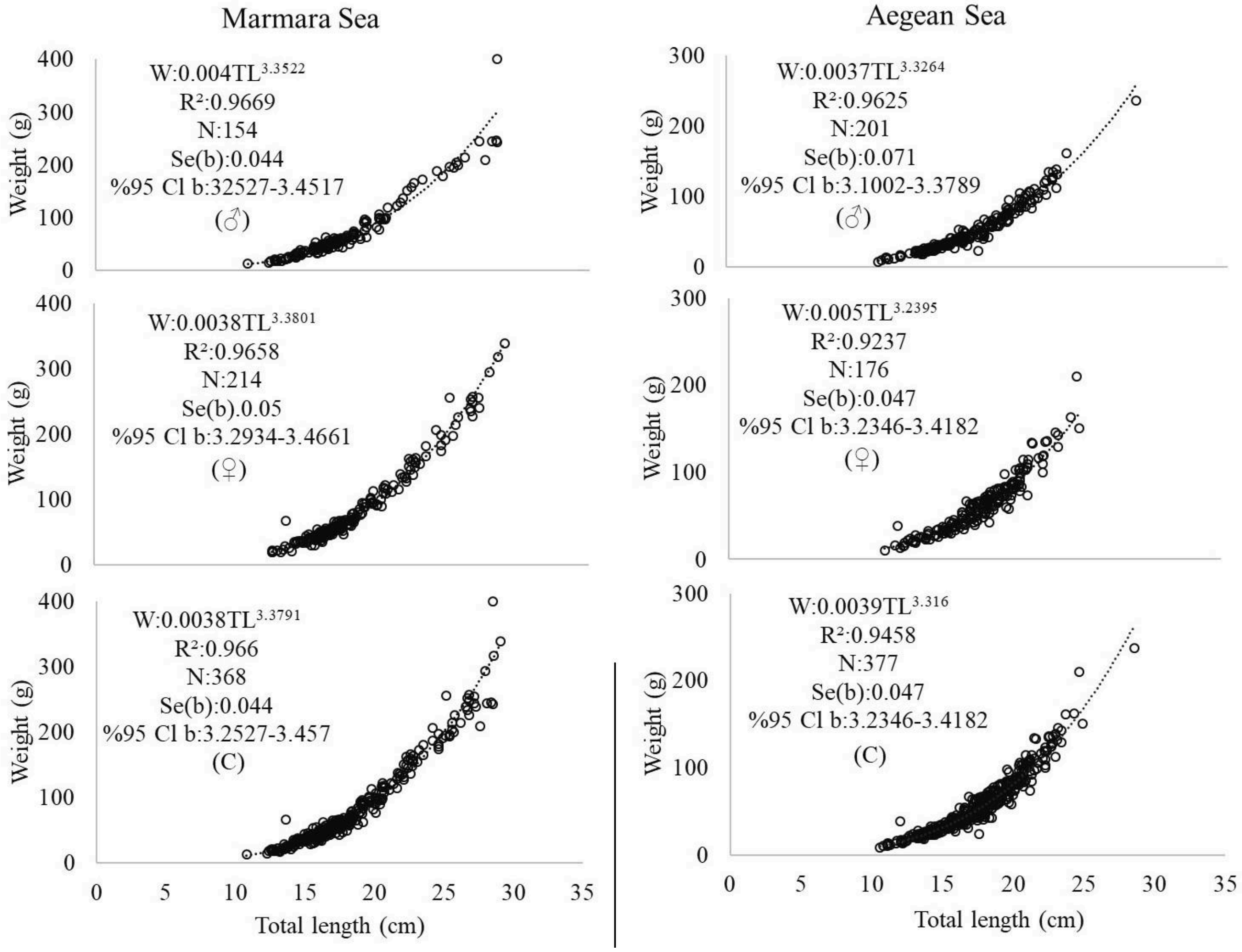
The total length-weight relationship parameters of female, male and all *B.boops* individuals for the Sea of Marmara and Aegean Sea were given in Figure 3. The exponent of the b value represented positive allometric growth in females, males and all sexes. The comparison of length-weight relationships and regression parameters of *B.boops* in this study and previous studies is given in Table 1. The "a" constant value is used as an indicator to determine the fattening degree, that is, the condition of the fish (Le Cren, 1991). The "a" values of *B.boops* showed close to each other in this study and previous studies. The "b" value is used to explain the growth type of the species according to its conditions. The "b" value of males is higher than females, indicating that males are in better condition in the Sea of Marmara and the Aegean Sea. While generally positive allometric growth from the "b" value was determined for *B.boops* in both areas, and previous studies in different areas of Türkiye. These differences in "b" values may be related to environmental conditions and sampling areas (Andreu-Soler et al., 2006; Olim & Borges, 2006). The sampling time, habitat, maturity, sex, feeding and stomach fullness may be effective in differences in the "b" value (Tesch, 1971; Hossain et al., 2006). The R^2 value of *B.boops* appears to have a high correlation value regardless of the sampling area and number of individuals. This shows that its condition is good. The differences between previous studies and this study are mainly related to the changes that may be seen in the populations due to temporal and spatial differences (Table 1).

The gonadosomatic index (GSI) of *B.boops* were recorded maximum in March for females and February for males and minimum in August for both sexes in the Sea of Marmara, whereas maximum in April for both sexes and minimum in July for both sexes in the Aegean. Sea (Figure 4). No significant difference for GSI with the Independent Samples T-test was detected between females and males in the Sea of Marmara (F:0.002, df:22, P>0.05) and Aegean Sea (F:0.046, df:22, P>0.05). Also, no significant difference for GSI was detected between the Sea of Marmara and Aegean Sea for females (F:0.034, df:22, P>0.05) and males (F:0.003, df:22, P>0.05).

The condition factor (CF) of *B.boops* was recorded maximum in October for both sexes and minimum in March for both sexes in the Sea of Marmara, whereas maximum in May for both sexes and minimum in March for both sexes in the Aegean Sea (Figure 5). No significant difference for CF with the Independent Samples T-test was detected between females and males in the Sea of Marmara (F:0.079, df:22, P>0.05), whereas significant difference was detected in the Aegean Sea (F:4.563, df:22, P<0.05). Also, no significant difference for CF was detected between the Sea of Marmara and Aegean Sea for females (F:0.578, df:22, P>0.05) and males (F:0.212, df:22, P>0.05).

Figure 3.

Total length-weight relationship parameters of B.boops in the Sea of Marmara and Aegean Sea (♂: Male, ♀: Female, C: All individuals).



The monthly sexual maturity stages were provided in Figure 6. The sexual maturity stages, GSI and CF values were evaluated together, the spawning period of *B.boops* was determined from January to May in the Aegean Sea and February to May in the Sea of Marmara. Also, the resting time of *B.boops* was determined from June to December in the Aegean Sea from June to January in the Sea of Marmara.

This study showed that the reproductive period of *B.boops* starts in January and ends in May in the Aegean Sea, while it starts in February and ends in May in the Sea of Marmara. The spawning period was generally concentrated in the spring and winter periods, when GSI is highest and CF is lowest, in the previous studies conducted in different areas (Table 2). The main reason for this temporal difference observed during the spawning period is sea temperature. Otero et al. (2013) emphasized that sea temperature has an effect on the reproductive period. Kalıpçı et al. (2021) stated that although sea temperature varies by month, it is on average 3.06 °C higher in the Aegean Sea than in the Sea of Marmara between 1970 and 2019. Some differences were observed in the spawning period in Bottari et al. (2014) in the Tyrrhenian Sea, Mediterranean Sea, Kara & Bayhan (2015) in the Izmir Bay, Aegean Sea, Layachi et al. (2015) in the Nador-Saidia, Morocco, Azab et al. (2019) in Alexandria, Egypt, Koca (2023) in the Antalya Bay, Mediterranean Sea studies. The main differences in these studies are due to the study areas and sampling time (Table 2).

The first maturity lengths (L_{50}) of *B.boops* were calculated as 12.5 cm TL in females and 15.8 cm TL in males in the Aegean Sea and 12.8 cm TL in females and 15.1 cm TL in males in the Sea of Marmara. The first gonad formation was observed at 11.1 cm TL for females and 12.6 cm TL for males in the Aegean Sea and 11.6 cm TL for females and 12.9 cm TL for males in the Sea of Marmara. The first sexual maturity length of *B.boops* was determined to be lower in female individuals than in male individuals. While the first maturity length was found to be higher in female individuals in the Sea of Marmara than Aegean Sea, while it was higher in male individuals in the Aegean Sea than Sea of Marmara. Also, the first maturity length of females was larger than males in the both seas.

Table 1.

Comparison of total length-weight relationship parameters of B.boops with previous studies.

Area	N	Sex	TL (cm) mean (min-max)	TW (g) mean (min-max)	a	b	R²	Growth type	References
Türkiye, Mediterranean Sea	-	♀	8.2-19.5	-	0.008	3.046	0.93	A (+)	Manaşarlı et al (2006)
	-	♂	8.9-18.85	-	0.010	2.978	0.96	A (-)	
	314	C	8.2-19.5	3.52-75.9	0.008	3.03	0.95	-	
Türkiye, Saros Bay	189	C	10.5-22.0	11-91	0.005	3.241	0.96	-	Ismen et al (2007)
Türkiye, Izmir Bay	640	♀	19.14 (11.3-27.6)	75.62 (12.1-261.8)	0.004	3.272	0.95	A (+)	Kara & Bayhan (2008)
	514	♂	20.28 (13.6-27.0)	91.41 (17.7-281.7)	0.002	3.522	0.93	A (+)	
	1190	C	19.55 (9.2-27.6)	81.74 (7.18-281.8)	0.004	3.419	0.95	A (+)	
Egypt, Alexandria	920	C	-	-	0.025	2.660	0.97	I	El-Okda (2008)
Montenegrin, Adriatic Sea	496	♀	12.7-22.4	-	0.015	2.828	0.89	A (-)	Kasalica et al (2011)
	325	♂	13.3-26.6	-	0.011	2.889	0.88	A (-)	
Algeria, Mediterranean Sea	1372	C	9.0-29.0	22.8–80.18	0.016	2.798	0.99	I	Rachid et al (2014)
Türkiye, Edremit Bay	297	♀	7.9–23.9	3.52–154.4	0.004	3.295	0.97	A (+)	Andsoy (2015)
	78	♂	7.7–20.9	3.81–110.7	0.004	3.304	0.98	A (+)	
	389	C	7.7–23.9	3.52–154.4	0.004	3.299	0.97	A (+)	
Türkiye, Izmir Bay	429	♀	11.3-27.9	12.2-261.7	0.007	3.128	0.95	-	Kara & Bayhan (2015)
	503	♂	12.2-27.0	17.7-241.6	0.003	3.42	0.96	-	
	932	C	19.6 (11.3-27.9)	82.7 (12.2-261.7)	0.005	3.237	0.96	A (+)	
Türkiye-Izmir Bay	82	♀	17.1 (12.8-21.3)	-	0.004	3.358	-	A (+)	Soykan et al (2015)
	293	♂	14.9 (11.2-23.8)	-	0.006	3.138	-	A (+)	
	421	C	15.5 (11.0-23.8)	37.9	0.005	3.25	0.97	A (+)	
Türkiye, Izmir Bay	51	C	10.5 (8.5-13.8)	13.13 (5.9-25.7)	0.009	3.021	0.99	I	Kara et al (2015)
Adriatic Sea	311	♀	18.41 (13.3-29.3)	-	0.013	2.884	0.91	A (-)	Dobroslavić et al (2017)
	365	♂	(18.29) 15.1-26.3	-	0.011	2.963	0.88	A (-)	
	676	C	13.3-29.3	-	0.011	2.911	0.90	A (-)	
Spain, Canary Islands	1069	♀	10.0-30.5	-	0.009	3.047	0.99	A (+)	Massaro & Pajuelo (2018)
	902	♂	8.5-34.5	-	0.008	3.079	0.99	A (+)	
	2893	C	4.0-34.5	-	0.007	3.153	0.99	A (+)	
Egypt, Alexandria	684	♀	13.81 (10.0-23.1)	27.12 (7.4-133.1)	0.012	2.912	0.91	I	Azab et al (2019)
	683	♂	13.4 (9.3-21.6)	24.4 (7.7-116.9)	0.010	2.978	0.92	I	
	1367	C	13.6 (9.3-23.1)	25.8 (7.4-133.1)	0.011	2.960	0.92	I	
Türkiye, Saros Bay	374	♀	19.8 (13.4-27.)6	97.43 (24.13-259.6)	0.009	3.11	0.96	A (+)	Cengiz et al (2019)
	564	♂	20.5 (13.7-25.6)	105.93 (25.93-20.4)	0.010	3.07	0.95	I	
Türkiye, Izmir Bay	30	C	15.9 (13.0-19.0)	39.6 (21.4-65.0)	0.005	3.209	0.97	I	Bayhan & Uncumusaoğlu (2022)
Algeria, Mediterranean Sea	1434	C	10.1-30.9	13.47-268.2	0.016	2.815	0.93	A (-)	Dahel et al (2019)
Türkiye, Çandarlı Bay	65	C	10.22 (6.8-13.8)	11.39 (3.05-25.7)	0.006	3.015	0.99	A (+)	Babaoglu et al (2021)
Türkiye-Saros Bay	363	C	16.9 (11.3-24.1)	60.4 (14.61-165.3)	0.008	3.13	0.98	A (+)	Cengiz. (2022)
Egypt, Alexandria	880	C	13.98 (13-15)	28.51(14-88)	0.012	2.945	0.99	I	El samman et al (2022)
Algeria, Mediterranean Sea	2068	C	9.7-27.4	5-212	0.004	3.28	0.92	A (+)	Handjar et al (2022)
Türkiye, Antalya Bay	316	♀	10.8-24.9	12.99-141.1	0.013	2.897	-	A (-)	Koca (2023)
	325	♂	10.9-23.0	13.47-119.6	0.014	2.867	-	A (-)	
	641	C	10.8-24.9	12.99-141.1	0.013	2.882	0.95	A (-)	
Türkiye, Aegean Sea	226	♀	18.57 (10.7-25.2)	60.74 (10.65-141.0)	0.011	2.930	0.97	A (-)	Seyhan Öztürk (2024)
	135	♂	17.63 (11.6-23.5)	60.5 (14.66-137.5)	0.010	3.005	0.99	I	
	361	C	10.5-25.2	10.65-141.0	0.011	2.933	0.97	A (-)	
Türkiye, Mediterranean Sea	488	♀	17.3 (11.9-27.8)	14.1-210.0	0.002	3.52	0.95	A (+)	Farah & Mavruk (2024)

Area	N	Sex	TL (cm) mean (min-max)	TW (g) mean (min-max)	a	b	R²	Growth type	References
	466	♂	16.9 (11.6-28.4)	14.8-281.0	0.004	3.28	0.91	A (+)	
	967	C	15.9 (11.1-28.4)	12.1-28.1	0.003	3.38	0.94	A (+)	
Egypt, Mediterranean Sea	868	C	13.57 (8.4-23.0)	29.84 (6.1-138.2)	0.010	3.045	0.96	I	Mokbel et al (2024)
Türkiye, Marmara Sea	51	C	19.17 (15.4-25.8)	84.66 (37.12-186.6)	0.009	3.066	0.96	-	Şen et al (2024)
Türkiye, Aegean Sea	176	♀	17.91 (11.1-24.9)	63.51 (10.55-210.3)	0.005	3.240	0.92	A (+)	This study
	201	♂	17.19 (10.6-28.6)	54.2 (8.1-236.86)	0.004	3.326	0.96	A (+)	
	377	C	17.52 (10.6-28.6)	58.46 (8.1-236.86)	0.004	3.316	0.95	A (+)	
Türkiye, Marmara Sea	214	♀	18.21 (12.6-29.1)	81.52 (19.04-339.1)	0.004	3.380	0.97	A (+)	
	201	♂	17.52 (10.8-28.5)	68.9 (13.08-399.9)	0.004	3.352	0.97	A (+)	
	368	C	17.92 (10.8-28.5)	76.54 (13.08-399.9)	0.004	3.379	0.97	A (+)	

N: Number of individuals, TL: Total length, TW: Total weight, Min: Minimum, Max: Maximum, C: All individuals, ♀: Female, ♂: Male, A: Allometric growth, I: Isometric growth

Figure 4.

Comparison of monthly gonadosomatic index (GSİ) values of B.boops in the Sea of Marmara and Aegean Sea.

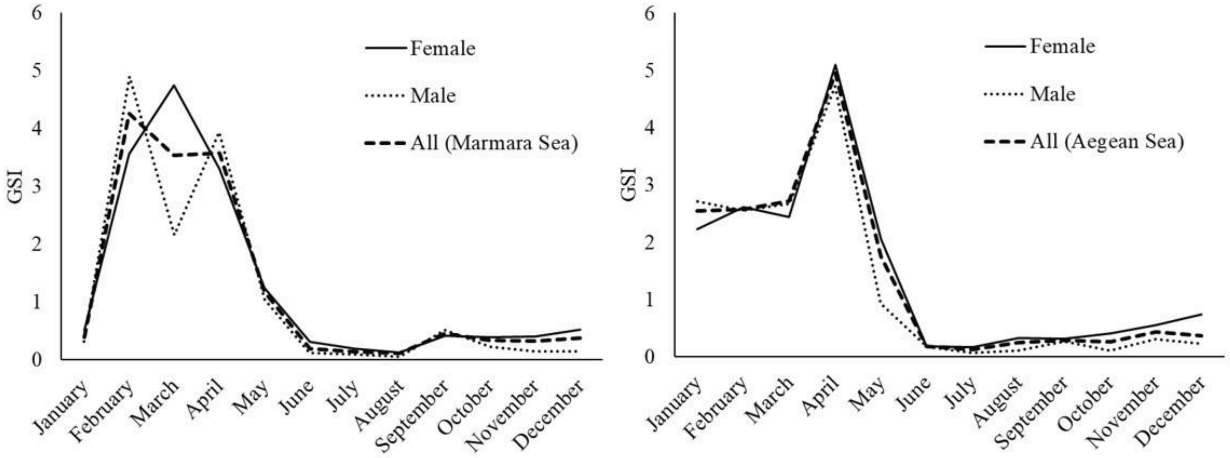


Figure 5.

Comparison of monthly condition factor (CF) values of B.boops in the Sea of Marmara and Aegean Sea.

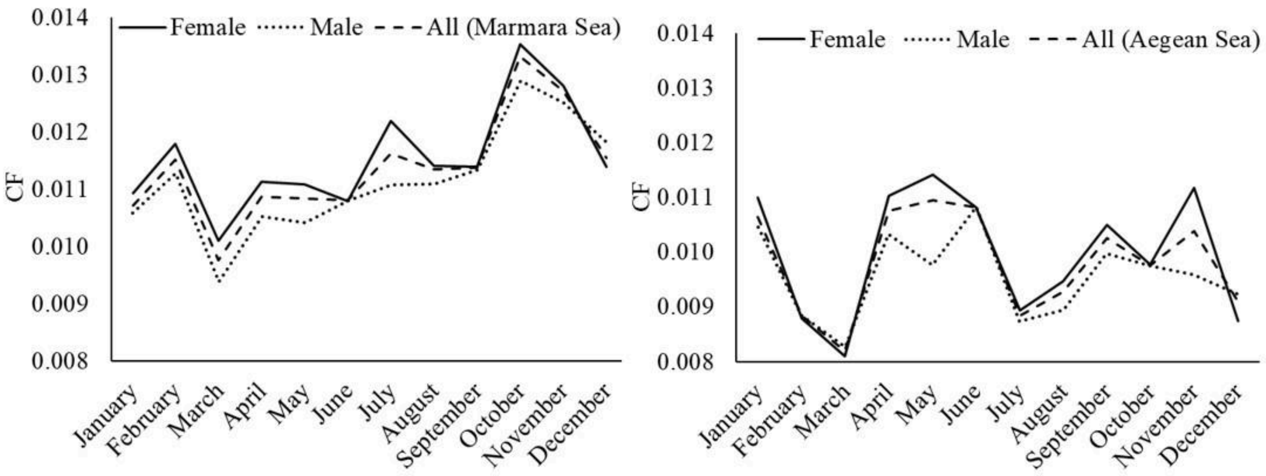
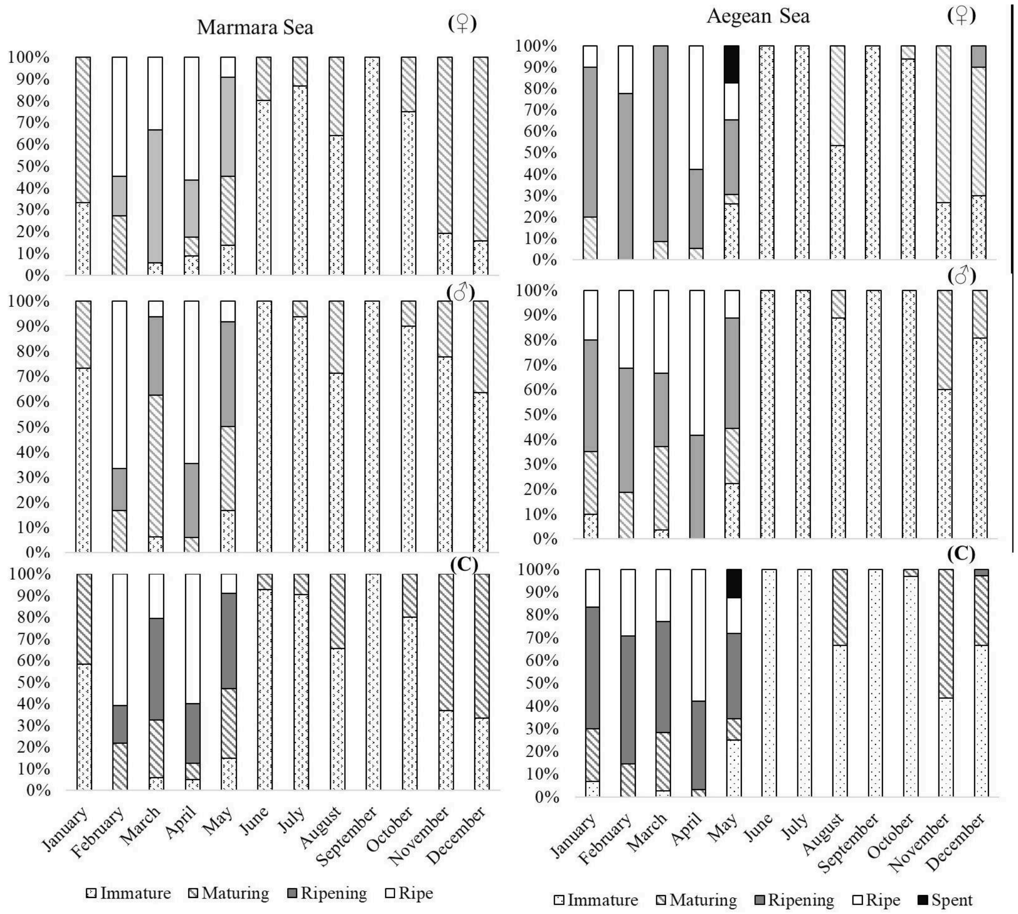


Figure 6.

Comparison of sexual maturity stages of B.boops in the Sea of Marmara and Aegean Sea (♂: Male, ♀: Female, C: All individuals).



This may be related to the decreasing population growth rate resulting from high exploitation in the Aegean Sea, which might lead to females individuals maturing earlier and younger age with small size (De Roos et al., 2006). The first maturity length of *B.boops* varies between 9.35 and 16.49 cm in previous studies, while it is generally similar to other studies (Table 2). The first maturity length of females was similar in in Soykan et al. (2015) in the Izmir Bay, Aegean Sea, whereas smaller in male individuals compared to this study. Cengiz (2022) calculated the first maturity length of *B.boops* in the Saros Gulf, Aegean Sea to be higher in all individuals compared to this study. Also, the first gonad formation was observed to be lower in males and females in the Aegean Sea than Sea of Marmara, and lower in females than males. These differences in this study with previous studies may be due to the structure of the sampled population, sampling methods, sampling areas and sampling times. The fishing pressure and stress on a species can make that species tend to mature earlier or cause the reproductive period to shift (Trippel, 1995; Aramayo, 2015). There are no regulations or restrictions regarding *B.boops* fishing in the commercial fisheries regulations in Türkiye (GDFA, 2024). The first maturity lengths calculated regarding *B.boops* in this study can be used as legal catch lengths in the Sea of Marmara and Aegean Sea (Table 2).

The oocyte counts of *B.boops* were obtained from the individuals which ranged from 12.6 cm TL to 29.1 cm TL (mean:18.66±0.66 cm TL) in length and from 10.6 g to 339.1 g (mean:95.2±11.8 g) in weight in the Sea of Marmara, while 11.1 cm TL to 24.8 cm TL (mean:18.55±2.38 cm TL) and these weights ranged from 19.04 g to 210.3 g (mean:72.77±9.32 g) in the Aegean Sea. The total fecundity varied between 2770-85112 (mean:20021±2027) in the Sea of Marmara, while 2740–309457 (mean:42834±9194) in the Aegean Sea. A statistically significant difference with the Independent Samples T-test for fecundity was detected between the Sea of Marmara and Aegean Sea (F:41.511, df:117, $P<0.05$). The oocyte diameters were measured from 3 mature females (181 oocytes) as 0.281–0.65 mm (mean:0.476±0.007 mm) in the Sea of Marmara, while from 8 individuals (209 oocytes) between 0.281 mm and 0.667 mm (mean:0.474±0.007 mm) in the Aegean Sea. No significant difference with the Independent Samples T-test for oocyte diameters (F:1.135, df:388, $P>0.05$) was detected between the Sea of Marmara and Aegean Sea. Also, fecundity–total length and fecundity–weight relationships were given in Figure 7.

Tablo 2.

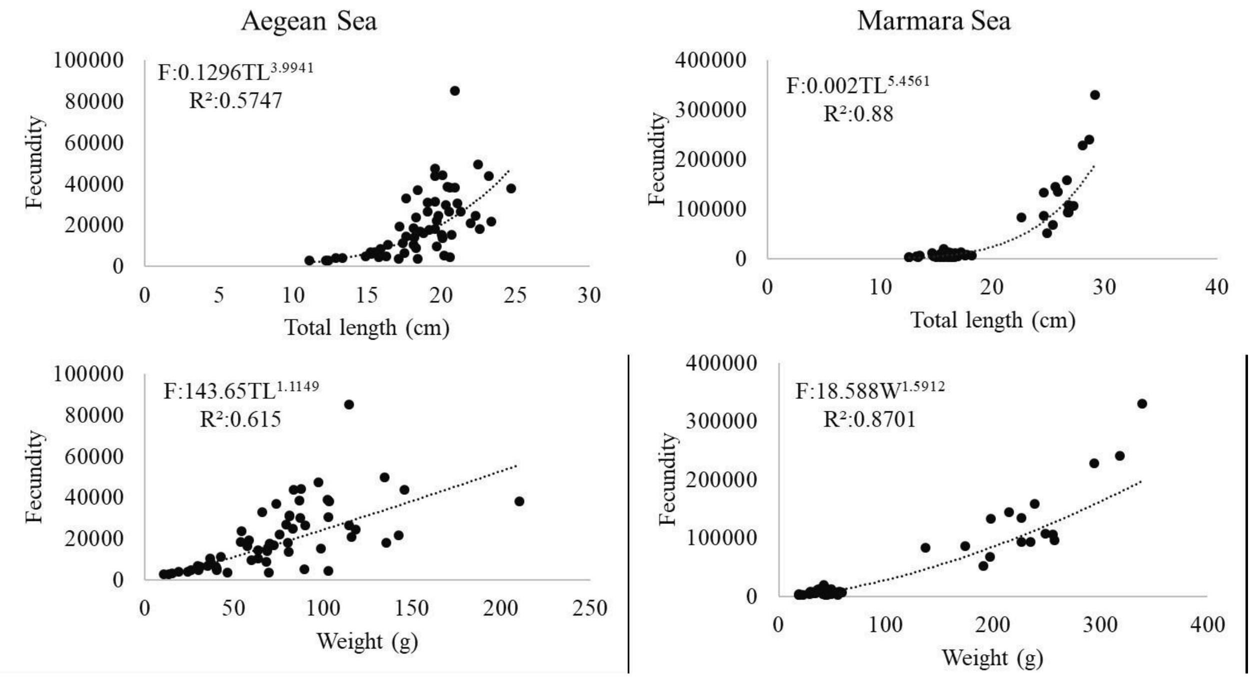
Comparison of spawning period and first maturity length of B.boops *with previous studies.*

Area	Spawning Period	First Gonad Formation (cm)	First Maturity Length (cm)	References
Egypt, Alexandria	January-May	-	13 (♀), 12 (♂)	El-Agamy et al (2004)
Portugal, Algarve	February-May	15.22 (C)	15.7 (♀), 15.3 (♂)	Monteiro et al (2006)
Türkiye, Edremit Bay	January-May	12.5	13.92	Bilge (2008)
Türkiye, Izmir Bay	January-May	12.1	13.47	
Montenegrin, Adriatic Sea	February	-	11.49 (♀), 11.9 (♂), 13.02 (C)	Kasalica et al (2011)
Tyrrhenian Sea	February-April	-	13.1 (♀), 14.2 (♂), 13.8 (C)	Bottari et al (2014)
Türkiye, Izmir Bay	February-September	-	-	Kara & Bayhan (2015)
Türkiye, Edremit Bay	January-May	-	-	Andsoy (2015)
Morocco, Nador-Saidia	February- October	-	14.3 (♀), 13.3 (♂)	Layachi et al (2015)
Türkiye, Izmir Bay	January-March	11.2	12.96 (♀), 9.35 (♂)	Soykan et al (2015)
Adriatic Sea	January-May	-	-	Dobroslavić et al (2017)
Spain, Canary Islands	January-May	13.0 (♀), 12.0 (♂)	16.02 (♀), 16.49 (♂)	Massaro & Pajuelo (2018)
Algeria coast	February-June	10.5	12.65 (♀), 15.19 (♂), 14.75 (C)	Amira et al (2019)
Egypt, Alexandria	January-April	-	13.2	Azab et al (2019)
Türkiye, Saros Bay	March-May	-	-	Cengiz et al (2019)
Türkiye, Saros Bay	-	-	16.6	Cengiz (2022)
Algeria, Mediterranean Sea	-	-	9.7	Handjar et al (2022)
Türkiye, Antalya Bay	January-September	-	-	Koca (2023)
Türkiye, Aegean Sea	January-May	11.1 (♀), 12.6 (♂)	12.5 (♀), 15.8 (♂)	This study
Türkiye, Marmara Sea	February-May	12.6 (♀), 12.9 (♂)	12.8 (♀),15.1 (♂)	

C: All individuals, ♀: Female, ♂: Male

Figure 7.

The fecundity–total length and fecundity–weight relationships of B.boops in the Sea of Marmara and Aegean Sea.



The total fecundity of *B.boops* in the Aegean Sea was found to be almost twice as high in the Sea of Marmara. This situation may be related to the physical condition factor and gonadosomatic index values of *B.boops* and environmental parameters ofthese areas. Because, fish species can shift their life-history traits in order to facilitate populations to successfully continue against environmental changes (Riesch et al., 2020). In addition, fish species can migrate to reproduction (Jørgensen et al., 2008). If *B.boops* individuals were migrating from the Aegean Sea to the Sea of Marmara, they would have used up the energy. So, they consume energy for reproduction during the migration process, and this may cause them to produce fewer eggs. Also, ages of *B.boops* individuals in these areas may be affected by egg numbers due to the relationship of fish species between age and fecundity (Avşar, 2016). Taylan and Bayhan (2015) determined that the fecundity was 33072–66123 (mean:49008) eggs per female for 23.5 cm mean length of *B.boops* in the Aegean Sea. The high number of eggs in their study can be explained by the higher mean length of the individuals compared to this study. The fecundity was 5185 to 52208 eggs for 13.0-22.0 cm total length in Egypt (Hassan, 1990). El-Agamy et al. (2004) reported that 462 individuals with 10.6-20.8 cm total length in Egypt varied between 1296 and 51528 eggs. Gordo (1996) stated that number of eggs varied between 11550 and 357800 (14-36 cm total length) in the Portuguese coast. Bauchot and Hureau (1986) stated that the egg capacity was approximately 395000 eggs for 32 cm mean length. The fecundity varied 36600 to 445200 eggs (mean 133456) in Algeria (Amira et al., 2019). Dobroslavić et al. (2017) determined the number of eggs from 125802 to 251502 (15.8-27.8 cm total length) in the Adriatic coast. These differences in fecundity for the previous studies may be related to regional differences.

The oocyte diameters of *B.boops* were found to be almost similar in the Sea of Marmara (0.476 mm) and the Aegean Sea (0.474 mm). Taylan & Bayhan (2015) determined that the oocyte diameters were 0.60 mm mean (0.53-0.74 mm) in the Aegean Sea. The oocyte diameters were 0.51-0.68 mm in Egypt (El-Agamy et al., 2004) and 0.16-0.59 mm in the Adriatic coast (Dobroslavić et al., 2017). The oocyte diameters of *B.boops* were similar, except for the study by Amira et al. (2019). They found to be 0.11-0.185 mm the oocyte diameters in Algeria.

Length and weight differences affect fecundity (Amira et al., 2019). While partially low exponential relationships of *B.boops* were determined between fecundity and length (R^2:0.57), fecundity and weight (R^2:0.615) in the Aegean Sea, whereas high exponential relationship was determined between fecundity and length (R^2:0.88), fecundity and weight (R^2:0.87) in the Sea of Marmara. El-Agamy et al. (2004) calculated that total length-fecundity, and weight-fecundity relationships in Egypt as $F{:}61688+TL^{5037}$, and $F{:}12398+W^{730.33}$. Taylan and Bayhan (2015) stated that the fecundity-total length, fecundity-weight relationships in the Aegean Sea as $F{:}8207.6e^{0.075TL}$ (R^2:0.79), $F{:}30297e^{0.003TW}$ (R^2:0.71), respectively. They reported that there was a higher correlation between fecundity and length-weight in the Aegean Sea compared to our study.

CONCLUSION

As a result of this study, the reproductive biology of *B.boops* was determined for the first time in the Sea of Marmara. Also, current data on the reproductive biology of this species in the Aegean Sea were presented, and with these data from the Sea of Marmara adjacent to the Aegean Sea. The differences were determined in sex ratio, spawning period, fecundity from reproductive characteristics between the Sea of Marmara and Aegean Sea. Also, some differences in reproductive characteristics were also stated in the Aegean Sea compared with previous studies. The main reasons for these differences are thought to be not only the structure of the population, sampling methods, sampling areas and sampling times, but also biotic and abiotic factors and human impacts as fishing pressure. Additionally, effects of climate change and fishing exploitation may cause their biological characteristics of *B.boops* to change over time. This scientific knowledge of reproductive biology contributes to the regional management of *B.boops* stocks in both seas.

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Research Article

Dietary Thymol Supplementation to Improve Growth in Common Carp (*Cyprinus carpio*) and Tambaqui (*Colossoma macropomum*)

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Abstract Common carp (*Cyprinus carpio*) and tambaqui (*Colossoma macropomum*) are the fish that are commonly farmed in Indonesia. The advancement of improved feeding practices is crucial for the sustainable production of both common carp and tambaqui, leading to better growth performance. This research was conducted to enhance growth performance and improve feed utilization efficiency by using thymol as a feed additive. For common carp, thymol was supplemented at a dose of 0, 0.1, 0.2, 0.4, or 0.8 g/kg commercial feed. While thymol was added to the tambaqui feed formulation at doses of 0.0, 0.2, 0.4, or 0.6 g/kg. Common carp with an initial weight of 3.23±0.00 g were reared in net enclosures for 60 days, while tambaqui weighing 8.22±0.01 g were kept in aquariums for 70 days. The study found that supplementing carp feed with 0.4 g/kg thymol led to the highest growth performance. Meanwhile, feeding tambaqui with thymol at 0.6 g/kg resulted in significant improvements in growth-related parameters compared to other treatments, including final total biomass, final average weight, protein efficiency ratio, specific growth rate, and antioxidant status. In conclusion, the optimal thymol supplementation levels were found to be 0.4 g/kg for carp and 0.6 g/kg for tambaqui.

Keywords Thymol · Feed additive · *Cyprinus carpio* · *Colossoma macropomum* · Growth Performance

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INTRODUCTION

Aquaculture has become one of the fastest—growing sectors in animal protein production in recent decades. Common carp (*Cyprinus carpio*) and tambaqui (*Colossoma macropomum*) are among the most commonly farmed fish in Indonesia. These species are highly valuable due to their desirable traits, such as fast growth rates, efficient feed conversion ratio for both natural and supplementary feed, and relative resistance to adverse environmental conditions and diseases (Ljubojević et al., 2013). Common carp, belonging to the Cyprinidae family, the largest family of freshwater fish, is widely farmed in Indonesia. This species is an excellent source of valuable nutrients and plays a significant role in promoting healthy human nutrition. Common carp typically inhabit freshwater environments such as ponds, lakes and rivers (Barus et al., 2001; Rahman, 2015). Tambaqui, originally from the Amazon River, is another important aquaculture commodity. Advancing feeding practices is essential for the sustainable production of both common carp and tambaqui, as it enhances growth performance. Continuous efforts to optimize feed utilization efficiency are crucial for reducing operational costs and enhancing overall aquaculture productivity. Enhancing feed efficiency directly contributes to better growth performance, including increased growth rates and improved feed conversion ratios.

Using common carp and tambaqui as models for fish growth can provide valuable insights into growth rates and feed efficiency. Studying these species helps in understanding how different feeds affect growth performance, feed conversion ratios, and overall health. Additionally, both species exhibit strong adaptability to various farming conditions, making them ideal for evaluating the performance of different feed formulations across diverse environments. This can contribute to the development of more universally applicable feeds or enhance understanding of species-specific nutritional requirements. The relevance of common carp and tambaqui in growth studies, along with the differences in their natural diets and feeding behaviors, highlights the importance of considering these factors when formulating effective fish feeds.

Feeds formulated with both animal- and plant-based ingredients have demonstrated numerous benefits for fish health, overall condition, production performance, and meat quality (Ljubojević et al., 2013 & Ljubojević et al., 2014). Thymol, a natural essential oil, has the ability to stimulate fish growth (Amer et al., 2018). Thymol, also known as 2-isopropyl-5-methylphenol, is a natural monoterpene present in specific essential oils derived from plants (Alagawany et al., 2020). The primary compounds in thymol are the terpenoid thymol itself and the phenol isomer, carvacrol (Putra, 2016). Thymol content is found in thyme (*Thymus vulgaris*), oregano, and *Carum copticum* plants. The thyme plant is an aromatic compound with an essential oil chemical compound of 0.32–4.9%. In comparison, the essential oil component has several contents, such as thymol 61.6%, carvacrol 3.33%, pinene, eugenol, and others. Thymol essential oil has properties as an antioxidant, antibacterial, immunomodulator, expectorant, and carminative and functions as an antifungal, antiseptic, carminative, expectorant, sedative, antioxidant, and antimicrobial (Queiroz et al., 2012; Rahardjo et al., 2014). Thymol is a feed additive that improves animal performance and feed digestibility by influencing the structure and function of the digestive system, improving nutrient absorption, stimulating metabolic activity, exerting antimicrobial effects within the intestines, and reducing oxidative stress. By promoting digestive enzyme activity, thymol is believed to facilitate greater nutrient uptake in fish (Platel and Srinivasan, 2004; Jang et al., 2007). Thymol in feed can activate the structure and the role of the digestive system and increase absorption and metabolism in the bodies of animals, fish, and poultry. Thymol has been reported to exhibit several beneficial physiological effects, including the stimulation of digestive enzyme secretion, enhancement of salivary amylase activity in humans, and the promotion of bile acid, gastric, and pancreatic enzyme production (El-Hack et al., 2016).

Earlier studies have shown that supplementing tilapia (*Oreochromis niloticus*) feed with 0.1 g/kg of thymol can enhance growth performance, improve the growth performance of catfish (*Clarias sp.*) at a dose of 0.2 g/kg and with a dose of 0.3 g/kg can improve the growth performance of catfish (*Pangasius hypopthalmus*) (Amer et al., 2018; Casandra, 2021; Octaviana, 2021). Therefore, research is needed on the addition of thymol to common carp and tambaqui feed using different feed processing methods, such as coating and additives in feed formulations, with varying doses of thymol in each type of feed method as a way to increase the efficiency of feed utilization and growth performance.

MATERIAL AND METHODS

Experimental diet

Thymol supplementation in common carp and tambaqui feed followed a Completely Randomized Design (CRD). The common carp experiment included five treatments (0, 0.1, 0.2, 0.4, and 0.8 g/kg) with three replications, while the tambaqui experiment had four treatments (0.0, 0.2, 0.4, and 0.6 g/kg) with three replications.

In this study, the diet for common carp consisted of a commercial feed supplemented with thymol through a coating technique. For 1 kg of feed, 100 mL of water, one chicken egg, and thymol were added to each feed treatment in amounts of 0 g, 0.1 g, and 0.2 g. 0.4 g and 0.8 g. All coating materials were stirred to form a homogeneous suspension using a hand mixer and mixed with commercial feed using a mixer. The homogenized feed is dried in an oven at 40°C for 4 hours, after which it undergoes proximate analysis using the method outlined by the Association of Official Analytical Chemistry (AOAC, 2012).

On the other hand, the experimental diet for tambaqui was prepared using carefully selected raw materials. These ingredients were ground into fine flour using a grinder and then sieved to ensure uniform particle size. The sieved materials were subsequently weighed according to Table 2 and thoroughly mixed using a mechanical mixer to achieve a consistent blend. During the final stage of the feed preparation, liquid raw materials were gradually incorporated at a ratio of 250 mL per kilogram of the dry mixture. The ingredients were continuously stirred until a homogeneous consistency was obtained. The thoroughly mixed feed was shaped into floating pellets using an extruder machine, producing pellets with a uniform diameter of 2 mm. Following pellet formation, the feed was dried in an oven at a controlled temperature of 50°C to reduce moisture content and enhance shelf stability. Once adequately dried, the feed was allowed to cool to room temperature before being transferred into an airtight container to prevent moisture absorption and nutrient degradation. The container was kept at room temperature in a dry, well-ventilated space to ensure proper preservation. The composition of the formulated diet is provided in Table 2, along with the proximate composition of each feed formulation, including moisture, protein, lipid, fiber, ash, and nitrogen-free extract contents, which were determined and are presented in Table 3.

Table 1.

Proximate Composition (%) of Common Carp Feed with Thymol Addition in Different Dose

Composition	Thymol Dose (g/kg)				
	0.0	**0.1**	**0.2**	**0.4**	**0.8**
Moisture (%)	9.25	9.31	9.46	9.74	9.35
Fat (%)	5.22	5.60	5.45	5.90	5.40
Protein (%)	31.07	30.51	31.14	31.20	30.77
Fiber (%)	3.68	3.58	3.74	3.45	3.80
Ash (%)	9.29	9.25	9.45	9.48	9.25
NFE* (%)	41.29	41.75	40.75	40.22	41.43

*NFE = nitrogen free extract

Table 2.

Feed Formulation for the Tambaqui Experiment

Raw Material	Thymol Dose (g/kg)			
	0.0	**0.2**	**0.4**	**0.6**
Fish Meal	40.0	40.0	40.0	40.0
Poultry Meal	50.0	50.0	50.0	50.0
Meat Bone Meal	97.0	97.0	97.0	97.0
Soybean Meal	130	130	130	130
Corn Gluten Meal	50.0	50.0	50.0	50.0
Cassava Chips	50.0	50.0	50.0	50.0
Wheat Pollard	328	328	328	328
Wheat Flour	200	199	199	199
Fish Oil	12.7	12.7	12.7	12.7
Cruide Palm Oil	24.4	24.4	24.4	24.4
Lysine	4.0	4.0	4.0	4.0
Vitamin C	0.5	0.5	0.5	0.5
NaCl	5.0	5.0	5.0	5.0
Methionine	2.0	2.0	2.0	2.0
Vitamin Mix	1.5	1.5	1.5	1.5
Mineral Mix	1.5	1.5	1.5	1.5
Polymethylolcarbamide	2.0	2.0	2.0	2.0
Anti-mold	0.15	0.15	0.15	0.15
Thymol	0.0	0.2	0.4	0.6
Total	1000.0	1000.0	1000.0	1000.0

Table 3.

Tambaqui's Feed Proximate Analysis with Thymol Additive in Different Dose

Composition	Thymol dose (g/kg)			
	0.0	**0.2**	**0.4**	**0.6**
Moisture (%)	7.75	7.50	7.75	7.50
Protein (%)	28.67	29.15	29.01	29.42
Fat (%)	3.50	3.50	3.83	4.00
Ash (%)	7.00	7.25	7.00	7.25
Fiber (%)	2.87	2.67	2.38	2.11
NFE* (%)	50.21	49.23	50.03	49.72
GE (kcal kg^{-1})**	4122	4129	4144	4160

*Nitrogen Free Extract = 100% - (moisture + protein + fat + ash + fiber)

**Gross Energy Composition (GE) was calculated based on protein = 5.64 kcal/g protein, fat = 9.44 kcal/g fat, and carbohydrate/NTEC = 4.11 kcal/g carbohydrate (Watanabe, 1998).

Fish maintenance

The research on common carp and tambaqui was conducted using two different schemes. In common carp, research was conducted in the experimental ponds of the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. The maintenance container used in this study was a pond lined with HDPE (high-density polyethylene) and equipped with 15 nets measuring (2×1×1) m with a water height of 70 cm. The nets used were brushed and then rinsed with water until clean, after which they were dried for 24 hours and then installed in the pond.

Common carp obtained from cultivators in Parung (Bogor District, Indonesia) with an average weight of 3.23 ± 0.00 g, were distributed into each hapa at a density of 50 fish per cubic meter, resulting in a total of 750 fish for this study. The fish were maintained for 60 days. Throughout the maintenance period, the common carp were fed based on their respective treatments, with feeding occurring three times a day at 8:00, 12:00, and 16:00 at satiation. The amount of feed provided and the number of fish that died were recorded to calculate the individual feed consumption for each carp. The final sampling was carried out at the end of maintenance by calculating the total weight of the entire fish population.

In tambaqui, the study used tambaqui from Rancabungur breeders (Bogor, Indonesia). The fish used were divided into 4 group of treatments with an initial weight of 8.22 ± 0.01 g, which were kept in 16 aquariums measuring 90×45×45 cm with a water height of 30 cm. Each aquarium contained 15 fish, with a net placed above to prevent them from jumping out. The water used as a maintenance medium was collected in a reservoir that had been deposited. One-point aeration was added to all aquariums, and they were equipped with a thermostat to maintain a water temperature of 29.3–29.8°C. Water changes of 60–70% were performed daily to replace the old water with new. The fish were maintained for 70 days and were fed three times a day at satiation throughout the period.

Water quality measurement

The water quality parameters measured during maintenance include temperature, dissolved oxygen (DO), pH, and total ammonia nitrogen (TAN). Temperature measurements were carried out three times a day, namely morning, afternoon, and evening, with values of 26.2-29.1ºC. Dissolved oxygen values are between 6.2 and 8.5 mg/L, pH ranges from 7.4 to 9, and TAN ranges from 0.07 mg/L to 0.21 mg/L. DO, pH, and TAN were measured weekly to meet quality standards (SNI 7550:2009). Water quality parameters were measured at the Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, also IPB University.

Parameters and data analysis

Based on Stickney and Gatlin (2022) growth performance in aquatic organisms is often evaluated using parameters that reflect changes over the life cycle, which supports the focus on early growth phases when assessing feed efficiency and overall development. This foundational understanding guides the selection of growth metrics in the present study. The parameters used in the study were Survival Rate (SR), Specific Growth Rate (SGR), Feed Intake (FI), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), malondialdehyde (MDA) in tambaqui and Hepatosomatic Index (HSI) in common carp (Huisman, 1987; Goddard, 1996; Tacon, 1987; Fan et al. 2019; Tsaknis, 1999). Data were analyzed using variance analysis in the Independent Sample t-Test SPPS with a 95% confidence interval using the SPSS Statistics 26.0 application (IBM, USA).

RESULT AND DISCUSSION

Growth performance

Adding thymol to the feed with different doses affected the growth performance of common carp consuming the feed. The higher the dose of thymol added to the common carp's feed, the higher the growth performance, including fish weight, SGR, PER, and FCR, but at a dose of thymol 0.8 g/kg feed experienced a decrease in growth performance compared to the thymol treatment of 0.4 g/kg.

The growth performance of common carp fed with thymol showed a positive effect (Table 4), with the addition of thymol to the feed resulting in improved growth performance values when compared to the treatment without thymol; this is characterized by better individual weight values, individual FCR, SGR, and PER. As is well known, the SGR value shows the ability of fish to store feed nutrients in the body, which are converted into energy and growth because it is determined by the nutrient content in the feed that can be digested and absorbed and provides an increase in the length and weight of the fish (Putra, 2016). The SGR of common carp in the 0.8 g/kg treatment was lower compared to the 0.4 g/kg treatment. This is considered an adverse effect of excess thymol doses, where thymol is also antimicrobial, so the microbes in the digestive tract are thought to be reduced or killed. The statement highlights that thymol exhibits antibacterial effects against *Escherichia coli* and *Salmonella typhimurium* (Gianneanas et al., 2012). One of the contents of thymol is phenol, which destroys bacterial membranes and causes the release of membranes from cells to the external media. Essential oils containing thymol function as antimicrobials, antifungals, and antioxidants and contain molecules that have intrinsic bioactivity in fish metabolism and physiology (Ahmadifar et al., 2011). However, it should be noted that the present study did not directly measure the intestinal microbial load; therefore, the proposed link between thymol's antimicrobial effects and changes in SGR remains speculative. Further studies are needed to investigate the intestinal microbiota to confirm these potential effects.

Table 4.

Growth Performance of Common Carp with the Addition of Thymol to Feed

Test Parameters	Thymol Dose (g/kg)				
	0.0	0.1	0.2	0.4	0.8
W0 (g)	3.23±0.00^{ab}	3.23±0.00^{b}	3.23±0.00^{ab}	3.24±0.00^{a}	3.23±0.00^{ab}
Wt (g)	14.70±0.55^{c}	16.22±0.37^{b}	17.43±0.32^{b}	19.30±0.68^{a}	17.43±0.32^{b}
Bt (g)	602.3±8.0^{d}	665.0±9.5^{c}	738.7±12.9^{b}	843.3±52.9^{a}	807.3±11.7^{a}
FI (g)	1097±34^{b}	1133±2^{b}	1223±53^{ab}	1293±21^{a}	1303±44^{ab}
SGR (%)	2.52±0.06^{c}	2.69±0.04^{b}	2.77±0.11^{b}	2.98±0.06^{a}	2.81±0.03^{b}
FCR	2.34±0.05^{d}	2.13±0.06^{c}	2.04±0.08^{bc}	1.85±0.06^{a}	1.98±0.03^{b}
SR (%)	82.0±2.0^{b}	82.0±2.0^{b}	86.7±4.2^{b}	87.3±3.1^{ab}	92.7±3.1^{a}
HSI	0.41±0.03^{a}	0.36±0.14^{a}	0.37±0.07^{a}	0.31±0.05^{a}	0.32±0.04^{a}
PER	1.38±0.03^{d}	1.54±0.04^{c}	1.58±0.06^{bc}	1.74±0.05^{a}	1.64±0.03^{b}

The values shown are the average ± standard deviation. Different letters in the same row indicate significant differences (p<0.05). The values shown are the average and standard deviation, W0 = initial weight, Wt = final weight, Bt = final biomass, FI = Feed Intake, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, HSI = Hepatosomatic Index, PER = Protein Efficiency Ratio.

Meanwhile, the FI of common carp treated with thymol increased. SGR and FI are related to the fish's response to the feed given, commonly referred to as palatability. Al-Souti et al. (2019) stated that fish have palatability properties, which means the level of preference for feed to be accepted by the body based on aroma, which is chemical feed factors. The level of palatability can affect FI; if the FI is high, it indicates a decent level of palatability, and vice versa. If the FI is low, the level of fish palatability is unacceptable. Moreover, adding thymol to the feed of tilapia enhanced its palatability. They stated that feed additives will affect the palatability of fish in receiving feed (Aanyu et al., 2019); Eriegha et al., 2017; Platel & Srinivasan, 2004).

The FI of common carp in the 0.2, 0.4, and 0.8 g/kg treatments did not differ significantly, but the feed conversion ratio (FCR) in the 0.4 g/kg treatment was better than in the other treatments. Low FCR values indicate more efficient feed utilization. Amer et al. (2018) demonstrated that the addition of thymol at doses of 1 ml/kg and 2 ml/kg in tilapia feed can reduce FCR values. This improvement is suggested to be linked to enhanced nutrient absorption also some nutrients are consumed by microbes, while some microbes produce secondary metabolites, potentially due to the reduction of anaerobic bacteria, making nutrients more available and decreasing bacterial metabolites, which are toxic during digestion (Giannenas et al., 2012). In the present study, the 0.4 g/kg thymol treatment for common carp resulted in better nutrient utilization compared to other treatments, as indicated by the highest SGR and lowest FCR. However, since digestive enzyme activity was not measured, this mechanism remains speculative and warrants further investigation.

Table 5 presents the growth performance of tambaqui maintained for 70 days, with different doses of thymol added to the feed. The results of the analysis indicated that supplementing the feed with thymol had a significant impact (p<0.05) on the growth performance of tambaqui. Increasing the thymol dose treatment increased individual weight and final biomass, with the highest weight in the thymol treatment of 0.6 g/kg feed. The 0.6 g/kg thymol treatment also had the highest SGR value. Adding thymol 0.6 g/kg feed increased the efficiency of feed utilization; the PER value increased, and the FCR value was low. Thymol also acts as an antioxidant because the administration of thymol reduces the malondialdehyde value. The treatment of adding thymol at various doses resulted in the same fish survival (p>0.05).

Table 5.

Growth Performance of Tambaqui with the Addition of Thymol to Feed

Test Parameters	Thymol Dose (g/kg)			
	0.0	0.2	0.4	0.6
W0 (g)	8.22±0.01	8.23±0.01	8.23±0.01	8.22±0.00
Wt (g)	39.84±2.80^{b}	41.18±1.17^{b}	42.29±0.90^{ab}	44.67±1.11^{a}
B0 (g)	123.3±0.2	123.5±0.2	123.5±0.2	123.3±0.1
Bt (g)	597.2±42.0^{b}	617.3±17.5^{b}	634.4±13.5^{ab}	670.1±16.6^{a}
FI (g)	581±11.4^{a}	587±4.8^{a}	561±4.2^{a}	585±13.4^{a}
SR (%)	96.8±6.7^{a}	98.3±3.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}
SGR (%)	2.25±0.10^{b}	2.30±0.04^{b}	2.34±0.03^{ab}	2.41±0.04^{a}
FCR	1.23±0.11^{c}	1.18±0.05^{bc}	1.09±0.02^{ab}	1.07±0.04a
PER	2.84±0.24^{b}	2.88±0.12^{b}	3.13±0.06^{a}	3.17±0.11^{a}
MDA (μM)	0.80±0.04^{b}	0.74±0.01^{a}	0.73±0.01^{a}	0.72±0.01^{a}

W0 = initial weight, Wt = final weight, B0 = Initial biomass, Bt = final biomass, FI = Feed Intake, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, PER = Protein Efficiency Ratio, MDA = Malondialdehyde. Different letters in the same row indicate significant differences in each treatment (p<0.05)

Adding thymol to tambaqui feed shows better growth performance values than the feed treatment without thymol, which can be seen from the final biomass value, PER, FCR, and SGR. The final weight and biomass values in feed with the addition of 0.6 g/kg have more optimal results, which were 44.67±1.11 g and 670.1±16.6 g (Table 5). This follows the statement that thymol can increase growth in rainbow trout, on grass carp and tilapia (Ahmadifar et al., 2011; Amer et al., 2018; Morselli et al., 2019).

In tambaqui, the most optimal SGR was found in the treatment of adding thymol 0.6 g/kg of 2.41±0.04 %; this treatment was significantly different from the addition of thymol 0.0 g/kg and 0.2 g/kg of 2.25±0.10 g and 2.30±0.04 g. This is supported by the PER and FCR value of 0.6 g/kg thymol addition, which was better than the others. The addition of 0.6 g/kg of thymol to tambaqui feed showed a low FCR value of 1.07±0.04, while PER gave more optimal results at this dose, namely 3.17±0.11. The high protein efficiency ratio (PER) and low feed conversion ratio (FCR) indicate that tambaqui can utilize the feed given optimally, leading to optimal absorption and conversion into muscle mass. A lower FCR indicates greater feeding efficiency, as the fish are able to convert the feed they consume into growth, resulting in increased body weight (Shofura et al., 2017). Research conducted by Jang et al. (2007) reported that supplementing broiler chicken feed with 0.05 g/kg of thymol can enhance the activity of digestive enzymes, namely trypsin enzymes. In addition, it can also affect the feed conversion ratio value. The trypsin enzyme can convert feed protein into amino acids more easily absorbed by the body.

Hepatosomatic Index (HSI) in common carp and Malondialdehyde (MDA) in tambaqui

Further testing on common carp is the HSI value. Adding thymol to the feed showed results that did not significantly affect the HSI value of common carp. Based on the statement of the previous study, the increasing amount of nutrients absorbed by the body will increase the HSI value of gourami seeds (Yandes et al., 2003). In this study, it is suspected that there is no accumulation of fat in the liver, and it is suspected that fat is stored in the form of glycogen in the body tissue of the fish. These results were also obtained in the malondialdehyde (MDA) value of tambaqui, where the treatment of adding thymol to the feed of 0.2 g/kg, 0.4 g/kg and 0.6 g/kg of feed showed results that were not significantly different. The efficient value was found in the treatment of adding thymol 0.6 g/kg of feed of 0.72±0.01 μM and the treatment of adding thymol 0.0 g/kg of feed of 0.80±0.04 μM. These values indicate that the antioxidant system contained in thymol works well so that the fish are not easily stressed. This follows the study's results above, which indicate that the SR in the treatment with thymol at a dose of 0.6 g/kg of feed was higher than in the other treatments. The SR value for the 0.6 g/kg thymol treatment reached 100%, whereas the SR in the treatment with 0.0 g/kg thymol was only 96.75±6.67% (Alagawany et al., 2020). Adding thymol to feed can increase antioxidant status, immune function, and meat quality and reduce animal mortality rates.

CONCLUSION

The findings of this study demonstrate that supplementing common carp feed with thymol at a concentration of 0.4 g/kg significantly enhances their growth performance. Similarly, incorporating 0.6 g/kg of thymol into tambaqui feed promotes better growth and improves the fish's antioxidant status, potentially strengthening their overall health and resilience against oxidative stress. These results suggest that thymol supplementation may have beneficial effects on certain growth and health parameters in fish, including improvements in survival rate, growth performance, and some indicators of metabolic and oxidative status. However, further comprehensive analyses of oxidative stress markers are needed to fully understand thymol's potential as a natural feed additive in aquaculture.

Ethical Approval	This study did not require ethical clearance as all procedures adhered to standard aquaculture practices and were conducted in accordance with applicable guidelines for the care and use of fish in research.
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Research Article

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Seasonal Algal Diversity and Environmental Parameters of Streams of Samur-Yalama National Park, Azerbaijan

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Abstract This study provides the first comprehensive assessment of freshwater algal diversity and ecological status in the streams of Samur-Yalama National Park, Azerbaijan—a region of high biodiversity but limited baseline data. Seasonal surveys in 2023 recorded 46 algal taxa, including 37 first regional records, across ten rivers spanning contrasting hydrochemical environments. Heterokontophyta dominated (69.6% of taxa), with epilithic and benthic habitats supporting the highest diversity. Simpson's Dominance Index (0.120–0.131) and saprobic index (0.69–0.89) indicated high diversity and predominantly oligosaprobic to β-mesosaprobic conditions, reflecting mostly unpolluted, well-oxygenated waters. Principal Component Analysis identified mineralization (sulfate, calcium, TDS) and nutrient gradients as key drivers of algal community structure. Sensitive diatoms such as *Fragilaria capucina* and *Achnanthidium minutissimum* dominated pristine sites, while tolerant taxa like *Gomphonema parvulum* and *Nitzschia palea* increased under moderate enrichment. These findings establish a regional reference for algal-based biomonitoring, highlight the vulnerability of semi-arid stream ecosystems to environmental change, and provide essential baseline data for conservation planning, future monitoring, and ecosystem management in the South Caucasus and comparable dryland regions.

Keywords Biodiversity · Water quality · Freshwater ecosystems · Benthic algae · Macro algae · Phytoplankton

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INTRODUCTION

Freshwater algae—including planktonic microalgae (phytoplankton), benthic microalgae, and macroalgae—play a fundamental role in aquatic food webs and serve as sensitive bioindicators of water quality (Dubey et al., 2022; Stancheva & Sheath, 2016). Phytoplankton alone contribute up to 50% of global primary production, while benthic forms help regulate nutrient dynamics, especially in littoral zones (Catalanotti et al., 2013).

Seasonal variations in light availability, temperature, and nutrient concentrations often drive shifts in algal community structure, favoring opportunistic and bloom-forming taxa—particularly cyanobacteria—under warm, eutrophic conditions (Paerl & Huisman, 2009; Paerl et al., 2011). In addition, water's physicochemical parameters, such as pH, turbidity, conductivity, and dissolved inorganic carbon, exert direct physiological constraints on algal growth and composition. For example, high turbidity reduces light penetration and limits diatom productivity, while silicate enrichment promotes diatom dominance (Smith, 1983; Beardall & Raven, 2016).

Many freshwater algal taxa have evolved stress-resilient life history traits, such as the ability to form dormant cysts or spores under unfavorable conditions like nutrient depletion or thermal extremes, enabling their persistence in fluctuating environments and making them robust ecological indicators (Flores-Moya et al., 2002; Graham et al., 2009).

The Samur–Davachi Lowland of northeastern Azerbaijan, which includes the Samur–Yalama National Park, is projected to experience a 1.5–3.5 °C rise in average temperature and a 10–20% decrease in precipitation by the end of this century (Abbasov et al., 2022). These climatic shifts, coupled with increased water extraction and agricultural runoff, are likely to impact streamflow regimes and nutrient loading—posing new challenges to aquatic biodiversity.

Despite the region's ecological significance, data on freshwater algal communities within these stream systems remain scarce. Previous studies have focused predominantly on vascular flora, leaving a major gap in our understanding of aquatic primary producers and their response to environmental change.

This study aims to fill that gap by integrating algal surveys with environmental measurements to:

- Document the taxonomic diversity and abundance of freshwater algae in selected streams of the Samur–Yalama National Park;
- Quantify key physicochemical parameters—such as nutrient concentrations (N, P, Si), pH, turbidity, and conductivity—and analyze their seasonal variation;
- Use principal component analysis (PCA) to explore relationships between environmental conditions and algal assemblages;

By combining species-level taxonomic identification with multivariate analysis of water quality, this study provides foundational knowledge to support biomonitoring and conservation efforts in Azerbaijan's freshwater ecosystems.

MATERIALS AND METHODS

Study Area

The Samur-Yalama National Park (SYNP; 41°37′33″–41°50′33″N, 48°33′31″–48°47′18″E) spans ca. 200 km² in the Khachmaz District of northeastern Azerbaijan, ~200 km NE of Baku (Abiyev et al., 2025) (Figure 1). The region experiences a semi-arid, temperate-warm climate, with mean annual temperature of 13 °C (range 1–17.5 °C) and annual precipitation of 300–400 mm, resulting in dry summers and mild winters. Major tributaries drain from the Caucasus Mountains into coastal plains, creating diverse lotic habitats.

Sampling Design

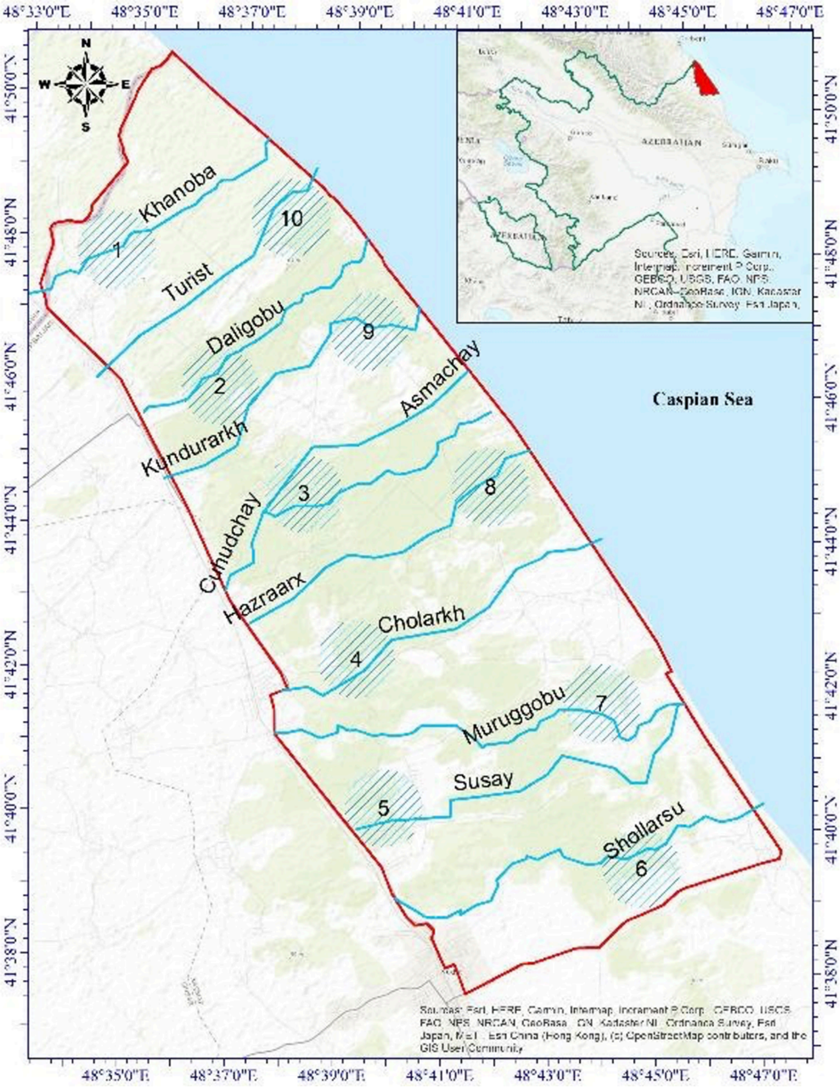
Seasonal sampling (January, April, July, October 2023) was conducted at 10 stream sites: Daligobu, Cuhudchay-Asmachay, Hazraarkh, Muruggobu, Shollarsu, Kundurarkh, Khanoba, Susay, Turist, and Cholarkh. At each site, three replicates of water and algal samples were collected along a 100 m reach to account for spatial heterogeneity.

In situ Measurements

Water temperature (°C) was measured in situ using a mercury thermometer. pH (model pH-200) and dissolved oxygen (DO, mg L^{-1}; MW 600) were determined with portable analyzers. Electrical conductivity (EC, µS cm^{-1}) and total dissolved solids (TDS, mg L^{-1}) were measured using a YK-22CT conductivity meter. Turbidity (NTU) was assessed using a TU2106 turbidimeter. Concentrations of biogenic elements (N, P, Si) were quantified by standard photometric methods as described in APHA (2017). Total suspended solids (TSS, mg L^{-1}) were determined according to Standard Method 2540D (APHA, 2005). Geographic coordinates and stream depth were recorded with a Garmin GPSMAP 64. All measurements and sampling procedures followed established quality control protocols.

Figure 1.

Study area



Water Chemistry Analysis

The samples for nutrient analyses were filtered through 0.45 µm membrane filters and stored at 4 °C until analysis. Concentrations of nitrate (NO_3–N), nitrite (NO_2–N), and ammonium (NH_4–N) were determined colorimetrically using Spectroquant® test kits (Merck) with detection ranges of 0.4–110.7 mg L^{-1} for NO_3–N, 0.02–3.0 mg L^{-1} for NO_2–N, and 0.01–3.0 mg L^{-1} for NH_4–N. Soluble reactive phosphate (SRP) was analyzed according to EPA Method 365.2. Silicate (SiO_2) was measured using the Spectroquant® Silicate Test (detection range: 0.005–5.0 mg L^{-1}). Sulfate (SO_4^{2-}) was determined in accordance with Standard Method 4500-SO_4^{2-} E, while calcium (Ca^{2+}) concentrations were measured by inductively coupled plasma–atomic emission spectrometry (ICP-AES) following EPA Method 6010D.

Algal Sampling and Identification

Phytoplankton samples were collected by vertical net hauls (10–64 µm mesh; flowmeter-calibrated) and preserved with Lugol's solution. Benthic algae were sampled by scraping defined 5 cm² areas of stones and macrophytes; epilithic diatoms via core tube (8 mm × 1 m). Biofilms were suspended and filtered through 20 µm mesh. Macroalgae (e.g., *Chara* spp.) were hand-harvested, rinsed, and weighed. Samples were stored on ice and processed within 24 h.

In the laboratory, algal taxa were identified under an optical microscope (Nikon E100) and SEM (JSM-35) following standard protocols (Van der Werff, 1999; Williams, 1985). Quantitative counts employed the Utermöhl method. Taxonomic references included Afanasyev et al. (2016, 2020) and Nuriyeva (2019).

Diversity and Saprobic Indices

Species dominance was quantified using Simpson's Dominance Index (D), calculated as:

$$D = \sum(\frac{n_i}{N})^2 \qquad (1)$$

where *ni* is the number of individuals of taxon *i*, and *N* is the total number of individuals in the sample (Magurran, 1988; Odum & Barrett, 2005). Values of *D* range from 0 (infinite diversity) to 1 (no diversity).

Organic pollution was assessed via the Saprobic Index (SI), defined as:

$$SI = \frac{\sum s.h}{\sum h} \qquad (2)$$

where *si* is the saprobic value assigned to species *i* (scale 1–4: oligio-, β-meso-, α-meso-, polysaprobic), and *hi* is the frequency of species *i* in samples (Persoone & De Pauw, 1979). Higher *SI* reflects increased organic load.

Classification of saprobic categories follows Zahradkova & Soldan (2008).

Statistical Analysis

Seasonal field data on physical parameters and hydrochemical composition of water collected in 2023 were analyzed using Principal Component Analysis (PCA) in PAST software (v4.13). For accurate results, only variables with normal or log-normal distribution were included in the analysis (Nong et al., 2024).

RESULTS AND DISCUSSION

Taxonomic Composition and Seasonal Distribution

A total of 46 algal species were identified, representing 5 phyla, 8 classes, 20 orders, 26 families, and 30 genera. The taxonomic structure of the studied flora is characterized by the overwhelming dominance of diatoms (Heterokontophyta: *Bacillariophyceae*), accompanied by relevant contributions from green algae (Charophyta, Chlorophyta) and cyanobacteria.

At the phylum level, Heterokontophyta accounted for 69.6% (32 species) of all taxa, forming the dominant group within the community. Charophyta (13.0%) and Cyanobacteria (10.9%) represented the next most abundant phyla, while Chlorophyta (4.3%) and Dinoflagellata (2.2%) were present in low proportions.

Within the class spectrum, *Bacillariophyceae* was strongly predominant (67.4%), reflecting the general pattern observed in freshwater habitats worldwide. *Cyanophyceae* and *Zygnematophyceae* were the leading secondary classes (each comprising 10.9% of the species pool), whereas other classes, including *Xanthophyceae, Charophyceae, Trebouxiophyceae*, and *Dinophyceae*, each accounted for only a single species (2.2%).

At the order and family levels, the highest species richness was recorded for Naviculales (21.7%) and *Naviculaceae* (17.4%), with Bacillariales, Zygnematales, and Oscillatoriales (each 8.7–10.9%) acting as additional key contributors. The most diverse families after *Naviculaceae* included *Stauroneidaceae, Oscillatoriaceae, Cymbellaceae*, and *Spirogyraceae* (each 6.5%).

Regarding the genus spectrum, *Navicula* was the most species-rich genus, comprising 15.2% of all recorded taxa, followed by *Spirogyra* and *Nitzschia* (each 6.5%). Although the total genus richness was relatively high (30 genera), the majority of species belonged to a small number of dominant and subdominant genera.

This taxonomic spectrum demonstrates a markedly polarized community structure, with diatoms clearly dominating both in species richness and ecological role, while green algae and cyanobacteria provide substantial secondary contributions.

A total of 37 algal species marked with an asterisk (*) were newly recorded for the Samur–Yalama region during this study. The majority belonged to Heterokontophyta (73%), followed by Cyanobacteria (14%) and Chlorophyta (11%), while Dinoflagellata was represented by a single species (2%). The dominance of diatoms among the new records reflects the overall taxonomic structure of the studied algal flora. These species were associated with a broad range of habitats, including benthic (*Navicula lanceolata*, *Spirogyra crassa*), epilithic (*Cocconeis placentula* var. *placentula*, *Staurosirella pinnata*), and epiphytic (*Achnanthidium affine*, *Tribonema affine*) environments. Some taxa, such as *Nitzschia palea* and *Chlorella vulgaris*, showed ecological plasticity by occurring in both planktonic and attached communities. This diversity of habitats reflects the substantial microenvironmental heterogeneity in the region's streams. Many of these species are important bioindicators, contributing to water quality assessment and ecological monitoring (Table 1, Figure 2).

Among the identified taxa, epilithic forms were most frequently encountered, followed by benthic and epiphytic taxa; planktonic, epipelic, and periphytic species were less common. Heterokontophyta exhibited the broadest habitat range and dominated both epilithic and benthic communities, reflecting their ecological plasticity. Charophytes were mostly confined to benthic habitats, while cyanobacteria were abundant in epilithic and planktonic niches (Table 1).

Seasonally, Heterokontophyta were present throughout the year, whereas other groups displayed distinct seasonal patterns: Chlorophyta were absent in winter, while Dinoflagellata occurred only in cold seasons. This pattern illustrates that environmental factors, particularly temperature and light, drive algal community succession in the region.

Seven taxa documented by Muxtarova and Muradova (2021) (*Frustulia vulgaris, Navicula cryptotenella, N. oblonga, Pinnularia viridis, Oscillatoria margaritifera, Phormidium ambiguum, Microspora palustris*) were confirmed in this study, indicating community persistence. Earlier charophyte records included *Chara vulgaris* f. *vulgaris* and *C. vulgaris* f. *longibracteata* (Romanov et al., 2025), but only *C. vulgaris* was observed here, suggesting local environmental shifts.

Diversity and Pollution Indices

Simpson's Dominance Index (D) values ranged from 0.120 to 0.131, confirming a high level of algal diversity with no single taxon dominating the assemblages throughout the studied streams. Saprobic Index (SI) values (0.69–0.89) classified the sites as oligosaprobic to β-mesosaprobic, indicating clean to only slightly polluted waters, typically associated with low organic enrichment from minor local inputs (Table 2).

Bioindication based on algal communities further supports these findings: upper reaches of the streams harbored predominantly oligosaprobic diatom species such as *Fragilaria capucina* and *Achnanthidium minutissimum*, well-known for their intolerance to organic pollution and their prevalence in oligotrophic, well-oxygenated environments. In contrast, downstream sites exhibited a subtle increase in organic matter, as reflected by the presence and greater relative abundance of more tolerant taxa such as *Gomphonema parvulum* and *Nitzschia palea*—both classic β-mesosaprobic indicators that flourish under mild organic enrichment and elevated nutrient levels.

The co-occurrence of both sensitive (*Fragilaria*, *Achnanthidium*, *Encyonema*) and tolerant (*Gomphonema*, *Nitzschia*) taxa in some samples highlights the mixed influence of pristine and mildly enriched conditions—a pattern consistent with ecological gradients described in similar Caucasus mountain streams (Neplyukhina et al., 2022). This is further corroborated by the occasional presence of *Chara vulgaris* (Charophyta), a macrophyte known to thrive in clear, calcareous, low-nutrient environments and to disappear under significant eutrophication. Although *Chara* is not typically included in saprobic indices, its persistence provides additional evidence that these streams have not crossed into heavily polluted or eutrophic states (Bellino and Baldantoni, 2023).

Comparative data from regional studies (Barinova and Kukhaleishvili, 2017) show similar upstream–downstream transitions: headwaters classified as oligosaprobic, with lower reaches becoming oligo- to β-mesosaprobic due to slight anthropogenic inputs, but without substantial loss of sensitive taxa or a shift toward full eutrophication. The dominance of Heterokontophyta (over 50% of identified taxa), rapid response of these groups to changes in water quality, and the consistent performance of algal-based indices (including saprobic and trophic Heterokontophyta indices) together provide a robust, evidence-based assessment of ecological condition.

In summary, the integration of structural (Simpson index), functional (Saprobic index), and taxonomic bioindication confirms that the streams of Samur-Yalama National Park are predominantly characterized by low nutrient loads, high oxygenation, and mineral-rich waters. Only minor spatial and seasonal signs of organic enrichment are observed, and the overall ecosystem remains far from critical pollution thresholds.

Hydrochemical Parameters and Seasonal Variation

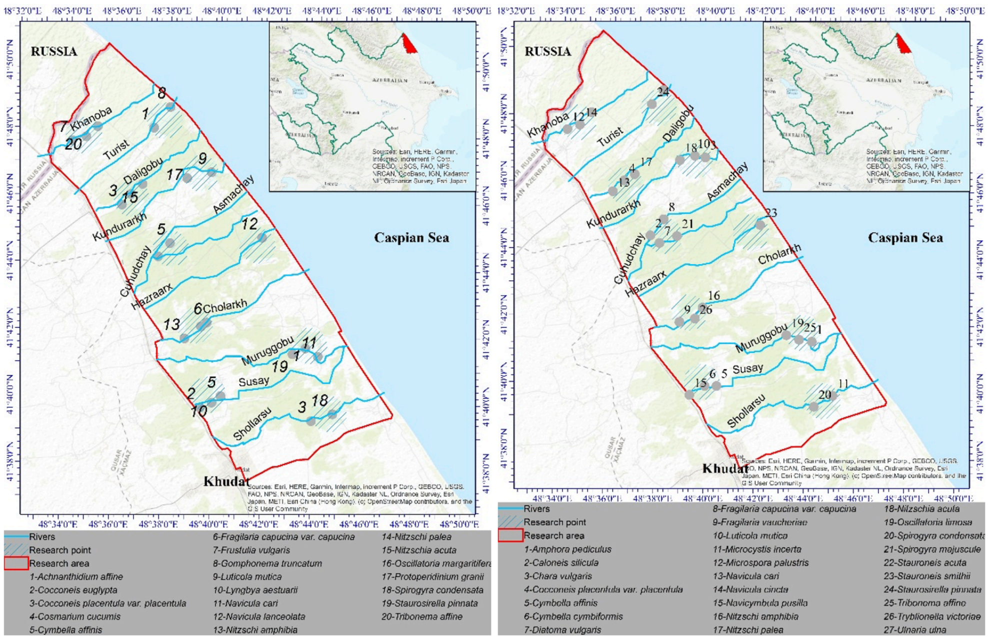
The ecological status of streams in Samur-Yalama National Park was assessed through the analysis of fourteen hydrochemical parameters and their relationships with algal community structure. Water temperature exhibited pronounced seasonal and spatial variation, ranging from 1.4 °C to 21.6 °C (mean: 11.6 °C), with the majority of samples falling within the 7.2–16.8 °C interval. Seasonal averages were 2.6 °C in winter, 13.1 °C in spring, 18.4 °C in summer, and 10.8 °C in autumn.

The first principal component (PC1) accounted for approximately 50% of variance during spring, summer, and autumn, increasing to about 70% in winter. PC1 was strongly and positively correlated with sulfate (SO_4^{2-}), calcium (Ca^{2+}), and total dissolved solids (TDS), and negatively correlated with dissolved oxygen (DO) and turbidity. This axis reflected a mineralization gradient governed primarily by lithological and hydrological characteristics of the catchments (Figure 8).

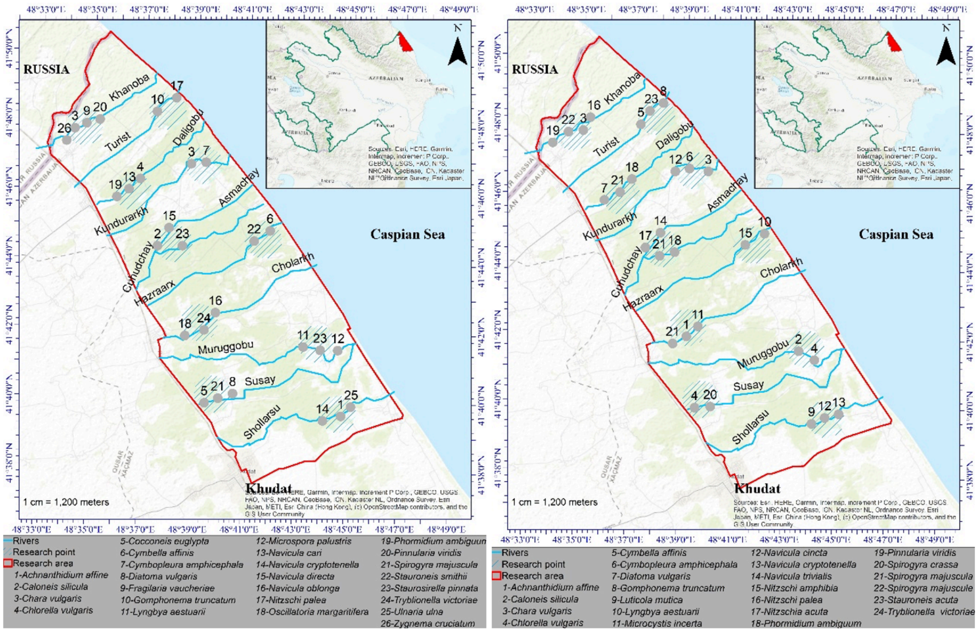
The second principal component (PC2) exhibited season-dependent variation: in winter and summer, PC2 was strongly associated with biogenic nutrients—phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), and silicate (Si)—capturing a nutrient enrichment gradient. Conversely, in spring and autumn, PC2 shifted towards physical parameters such as pH, temperature, and DO, which gained prominence during transitional periods.

Figure 2.

(a-d). Distribution maps of algae due to seasons



a) winter *b) spring*



c) summer *d) autumn*

Thus, the nutrient (biogenic) component became the third most important variance factor in these seasons (Figure 8).

Ordination plots derived from PCA revealed three main river groups based on hydrochemical similarity:

- **Group 1:** Rivers Susay, Muruggobu, and Colarkh exhibited relatively stable water chemistry year-round, characterized by moderate mineralization (350–520 mg L^{-1} TDS), slightly acidic pH, and consistently low nutrient concentrations. These conditions supported diverse and sensitive algal communities typical of oligotrophic to mesotrophic systems (Wetzel, 2001).

These fluctuations directly influenced algal succession: Heterokontophyta such as *Fragilaria capucina* and *Achnanthidium minutissimum* dominated during colder periods, reflecting the well-documented preference of psychrophilic diatoms for low temperatures (Butterwick et al., 2005; Lürling et al., 2018). Under warmer conditions (above 18 °C) led to increased occurrence of filamentous green algae (e.g., *Ulothrix*, *Spirogyra*) and cyanobacteria (*Oscillatoria margaritifera*, *Microcystis incerta*), although diatoms continued to prevail. This pattern is consistent with observations from Caucasus mountain streams and

Table 1.

*Algae species distributed in Samur-Yalama National Park (*it was found for the first time in this research for the region.)*

Family	Species	Habitat	Saprobe
***Heterokontophyta* Moestrup, R.A. Andersen&Guiry**			
Catenulaceae Mereschkowsky	*Amphora pediculus* (Kützing) Grunow*	Benthic	-
Achnanthidiaceae D.G. Mann	*Achnanthidium affine* (Grunov) Czarn*	Epiphytic	o-β
Cocconeidaceae Kützing	*Cocconeis euglypta* Ehrenberg*	Epilithic	-
	Cocconeis placentula var. *placentula* Ehrenberg*	Epilithic	o-β
Cymbellaceae Kützing	*Cymbopleura amphicephala* (Nägeli ex Kützing) Krammer*	Periphytic	o-β
	Cymbella cymbiformis C. Agardh*	Benthic	-
	Cymbella affinis Kütz*	Epilithic	β- o
Diadesmidaceae D.G. Mann	*Luticola mutica* (Kütz.) D.G. Mann in Round, Crawford et Mann	Epipelic	o
Fragilariaceae Kützing	*Fragilaria capucina* Desmazières *	Epilithic, benthic	o
	Fragilaria vaucheriae (Kütz.) J.B.Petersen*	Epiphytic	-
Gomphonemataceae Kützing	*Gomphonema truncatum* Ehrenberg*	Epilithic, benthic	o-x
	Gomphonema parvulum Kütz*		x
Tabellariaceae Kützing	*Diatoma vulgaris* Bory*	Epiphytic	x
Naviculaceae Kützing	*Caloneis silicule* (Ehrenberg) Cleve*	Benthic	x
	Navicula cryptotenella Lange- Bert. In Krammeret Lange-Bert	Epilithic	o-β
	Navicula oblonga (Kütz.) Kütz	Epilithic	β
	Navicula lanceolata (C. Agardh) Ehrenb*	Benthic	x- β
	Navicula cari Ehrenb*	Epilithic, benthic	-
	Navicula directa (W. Smith) Brébisson*	Benthic	o-β
	Navicula trivialis Lange-Bertalot *	Benthic	-
	Navicula cincta (Ehrenb.) Rafls*	Epiphytic, epilithic	-
Bacillariaceae Ehrenberg	*Nitzschia acuta* Hantzsch*	Epiphytic	-
	Nitzschia amphibian Grunov*	Epiphytic	-
	Nitzschia palea (Kütz) W.Sm. *	Planktonic, epiphytic, epilithic	-
	Tryblionella victoriae Grunov*	Epiphytic, epilithic	o-x
Amphipleuraceae Grunow	*Frustulia vulgaris* (Thwaites) De Toni	Epilithic	x- β
Pinnulariaceae D.G. Mann	*Pinnularia viridis* (Nitz.) Ehrenb	Benthic	o-x
Stauroneidaceae D.G.Mann	*Stauroneis acuta* W. Smith*	Benthic	o-x
	Staurosirella pinnata (Ehrenb.) D.M.Williams & Round*	Epilithic	β
	Stauroneis smithii Grunow	Epilithic	-
Ulnariaceae E.J.Cox	*Ulnaria ulna* (Nitzsch) P. Compere*	Planktonic, epilithic	o-α
Tribonemataceae G.S.West	*Tribonema affine* (Kütz.) G. S. West*	Epiphytic	-
***Cyanobacteria* Stanier ex Cavalier-Smith**			
Microcoleaceae O.Strunecky, J.R.Johansen & J.Komárek	*Lyngbya aestuarii* Liebman ex Gomont*	Planktonic, benthic	o
Oscillatoriaceae Engler	*Oscillatoria limosa* Agardh ex Gomont*	Planktonic, epilithic	β
	Oscillatoria margaritifera Kützing ex Gomont*	Benthic	-
	Phormidium ambiguum Gomont ex Gomont*	Epilithic, benthic	β
Microcystaceae Elenkin	*Microcystis incerta* Lemmerm*	Epilithic	β
***Chlorophyta* Reichenbach**			
Microsporaceae Bohlin	*Microspora palustris* Wichmann	Benthic	-
Chlorellaceae Brunnthaler	*Chlorella vulgaris* Beij*	Planktonic	α
***Charophyta* Migula**			
Characeae S.F.Gray	*Chara vulgaris* Linnaeus	Benthic	-
Desmidiaceae Ralfs	*Cosmarium cucumis* Corda ex Ralfs	Benthic	-

Family	Species	Habitat	Saprobe
Spirogyraceae Bessey	*Spirogyra condensate* (Vaucher) Dumort*	Benthic	-
	Spirogyra crassa (Kützing) Kützing*	Benthic	β
	Spirogyra majuscula Kützing*	Planktonic	o-α
Zygnemataceae Kützing	*Zygnema cruciatum* (Vaucher) C.Agardh*	Benthic	x- β
	***Dinoflagellata* Fensome et al.**		
Protoperidiniaceae Bujak&E.H.Davies	*Protoperidinium granii* (Ostenf.) Balech*	Epiphytic, epipelic	-

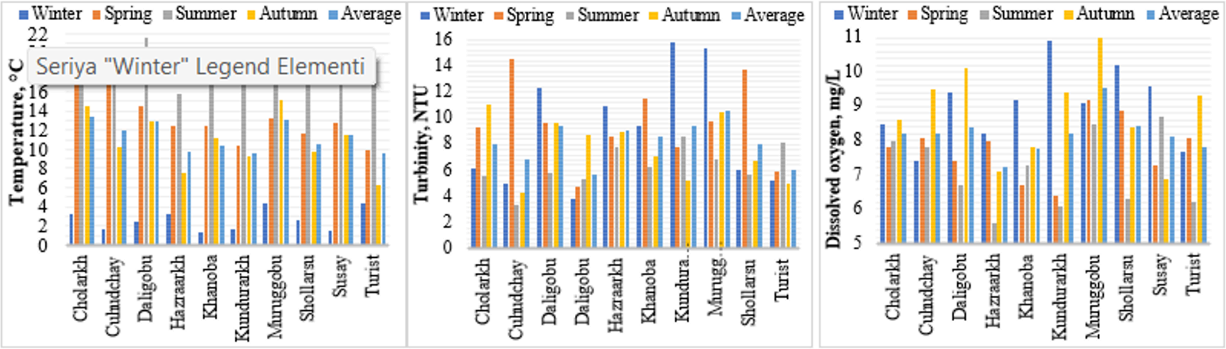
Table 2.

Seasonal values for main indices

Season	Winter	Spring	Summer	Autumn
D	0.129	0.131	0.129	0.120
SI	0.69	0.85	0.89	0.81

Figure 3.

Seasonal variation in physical parameters of stream. (a) Temperature; (b) Turbidity; (c) Dissolved Oxygen



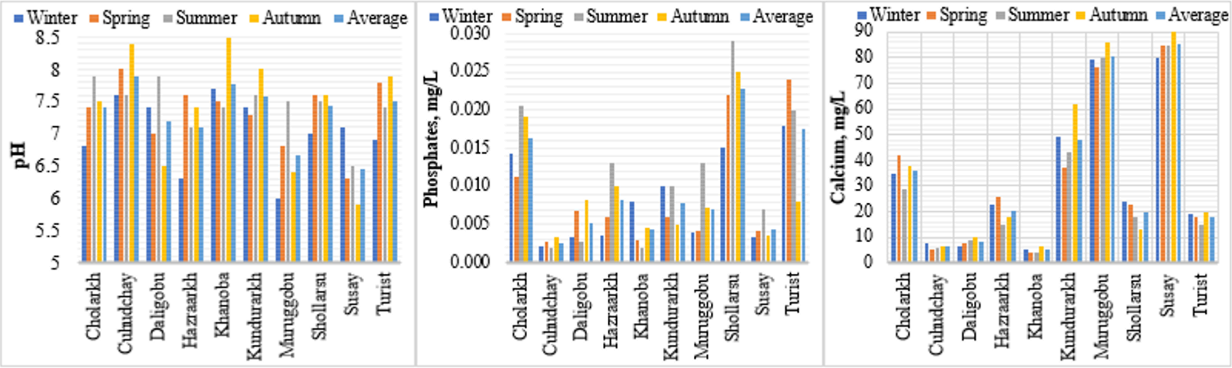
a

b

c

Figure 4.

Seasonal changes in general chemical properties. (a) pH; (b) Electrical Conductivity; (c) TDS



a

b

c

corresponds to global thermal optima for these algal groups (Reinl et al., 2023; Cottingham et al., 2021) (Figure 3).

Dissolved oxygen concentrations ranged from 5.6 to 11.3 mg L^{-1} (mean: 8.2 mg L^{-1}), with the majority of measurements falling within the 7.5–8.6 mg L^{-1} interval. These values reflect consistently well-aerated conditions across all sites and seasons. No significant decline in DO was observed, even during periods of elevated water temperature or increased algal biomass, indicating the absence of strong organic pollution and effective stream aeration (Wetzel, 2001). High dissolved oxygen levels supported diverse communities of diatoms and green algae, including *Staurosirella pinnata*, *Navicula cari*, and *Diatoma vulgaris*, all of which were most abundant at DO concentrations above 7.8 mg L^{-1}. Hypoxia-tolerant taxa were largely absent, further confirming favorable oxygen regimes throughout the study area. The pH of stream waters ranged from 5.9 to 8.5 (mean: 7.26), falling within the 6.9–7.6 interval. Acidic waters favored acidophilic taxa such as *Mougeotia* and *Pinnularia*, while neutral to alkaline conditions promoted greater diversity of diatoms and cyanobacteria. Peak diatom richness was observed at near-neutral pH, with taxa such as *Achnanthidium affine* and *Tryblionella victoriae* frequently occurring within the pH range of 6.0–7.5 (Tasnim et al., 2023; Zepernick et al., 2021) (Figure 3).

Electrical conductivity exhibited substantial spatial variation, ranging from 110 to 1970 µS cm⁻¹ (mean: 575 µS cm⁻¹, median: 315 µS cm⁻¹), and was closely mirrored by TDS (54–520 mg L⁻¹, mean: 219 mg L⁻¹, median: 157 mg L⁻¹) (Figure 4). These two parameters were strongly correlated ($r = 0.87$), reflecting parallel variation in dissolved mineral content across sites. Streams with moderate mineralization supported oligotrophic and sensitive diatom taxa such as *Gomphonema truncatum, Stauroneis smithii*, and *Navicula cryptotenella*. In contrast, sites with higher conductivity and TDS showed increased abundance of euryhaline, halotolerant species, particularly *Navicula oblonga, Fragilaria capucina*, and *Luticola mutica*, alongside filamentous green algae such as *Spirogyra majuscula*.

Lower mineralization correlated with the abundance of sensitive diatoms, while higher values supported β-mesosaprobic indicators (*Nitzschia palea, Gomphonema parvulum*). Turbidity ranged from 3.4 to 22.3 NTU (mean: 8.2 NTU) and total suspended solids (TSS) from 1 to 98 mg L⁻¹ (mean: 29 mg L⁻¹). Moderate to high turbidity favored sediment-tolerant filamentous cyanobacteria and green algae, whereas low turbidity supported a diverse assemblage of benthic diatoms (Sánchez et al., 2013; U.S. EPA, 2019).

Nutrient concentrations were consistently low to moderate, reinforcing the streams' oligotrophic to mesotrophic status. Soluble phosphate ranged from 0.002 to 0.029 mg L⁻¹ (mean: 0.009 mg L⁻¹), with the majority of samples falling within the 0.003–0.013 mg L⁻¹ interval, well below the threshold for phosphorus-limited bloom formation (Vuorio et al., 2020; Ballah et al., 2019). Nitrate varied from 0.08 to 0.48 mg L⁻¹ (mean: 0.26 mg L⁻¹), with most samples between 0.15 and 0.35 mg L⁻¹, and did not approach levels associated with cyanobacterial blooms (Hecky et al., 1993). Ammonium and nitrite concentrations remained consistently low across all sites, with minimal variation detected, further corroborating the absence of recent or ongoing organic pollution events (Elser et al., 1995). Nitrogen limitation likely favored diatom over cyanobacterial dominance, especially in spring and autumn.

The prevailing low-nutrient regime was reflected in the predominance of oligotrophic and sensitive diatom taxa such as *Gomphonema truncatum, Navicula cryptotenella, Stauroneis smithii, Achnanthidium minutissimum*, and *Fragilaria capucina*, as well as green algae like *Spirogyra majuscula* and *Zygnema* spp. Filamentous cyanobacteria, including *Oscillatoria margaritifera*, were only sporadically encountered, mainly at sites with slightly elevated nutrient concentrations (Figure 5).

Major ions including calcium (5.1–91 mg L⁻¹, mean 33 mg L⁻¹), sulphate (14–302 mg L⁻¹, mean 124 mg L⁻¹), and silicate (4–9.8 mg L⁻¹, mean 6.5 mg L⁻¹) varied spatially across the study area (Figure 6). Higher calcium concentrations consistently supported the occurrence of charophytes, particularly *Chara vulgaris* and *Chara contraria*, both indicators of clear, calcareous, low-nutrient waters (Herbst and Schubert, 2018). Sufficient silicate concentrations (typically >5 mg L⁻¹) enabled a robust and diverse diatom flora, including *Achnanthidium minutissimum, Fragilaria capucina*, and *Navicula oblonga*, with no evidence of silicate limitation. Sulphate-rich streams did not show a reduction in overall algal diversity but sometimes favored halotolerant taxa such as *Nitzschia palea* and *Gomphonema parvulum*, as well as green algae like *Ulothrix zonata* and *Spirogyra majuscula* (Kirkwood et al., 2006).

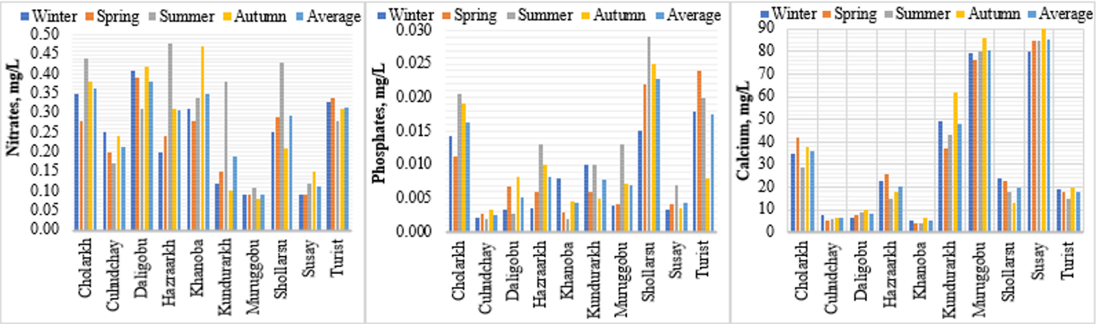
Only variables with normal or log-normal distribution were included to ensure robustness of the multivariate analysis. The first three principal components explained between 78% and 91% of total variance, depending on season (Figure 7).

- **Group 2:** Rivers Turist, Shollarsu, Khanoba, Cuhudchay, and Hazraarkh had low mineralization (TDS 54–194 mg L⁻¹), placing them among the least mineralized streams in the study area. pH conditions ranged from near-neutral to slightly alkaline, with no clear division into distinctly alkaline or acidic groups. Nutrient concentrations were generally low; however, Turist and Shollarsu exhibited relatively elevated phosphate (up to 0.018 mg L⁻¹) and nitrate (up to 0.33 mg L⁻¹) levels compared to other sites.
- **Group 3:** Kundurarkh River displayed intermediate ion concentrations (171–319 mg L⁻¹ TDS) and low nutrient content, while Daligobu River demonstrated pronounced intra-annual hydrochemical variability, shifting from low-mineralization in spring and summer to moderately mineralized, acidic waters in autumn (Wetzel, 2001).

The PCA gradients closely matched the main ecological drivers of algal community structure in the region.

Figure 5.

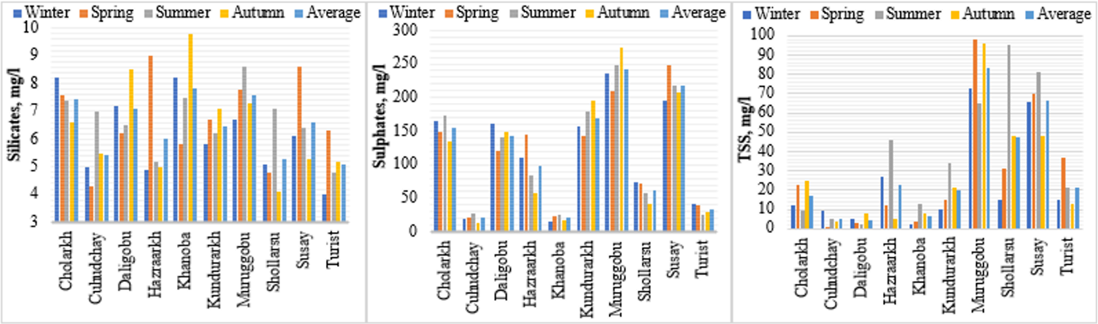
Seasonal variation of major ions and nutrients I. (a) Nitrate; (b) Phosphate; (c) Calcium



a b c

Figure 6.

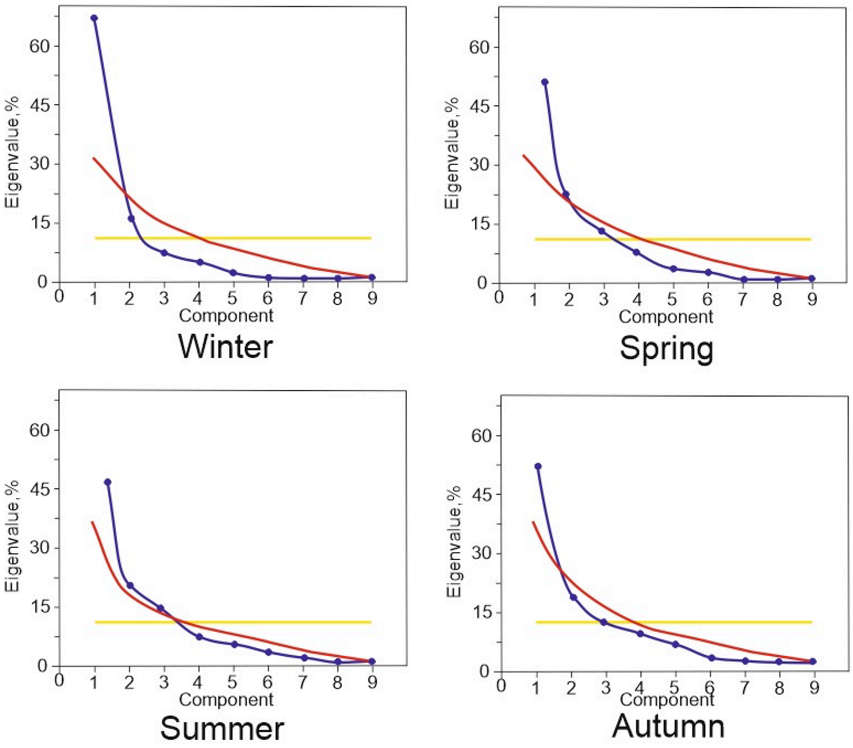
Seasonal variation of major ions and solids II. a) Slicate; b) Sulphate; c) Total Suspended Solids (TSS)



a b c

Figure 7.

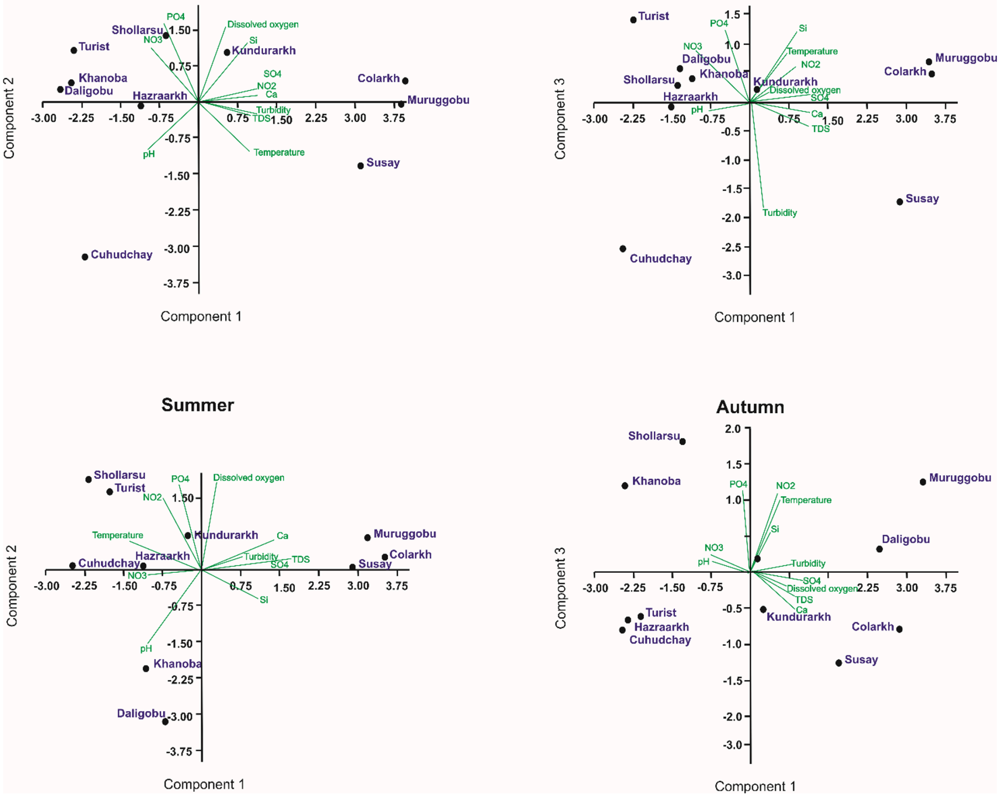
Scree plot of the first three principal components explaining 78–91% of total variance.



PC1's mineralization axis was strongly associated with the distribution of halotolerant and calciphilic taxa, such as *Nitzschia palea* and *Gomphonema parvulum*—both recognized β-mesosaprobic bioindicators tolerant of elevated

Figure 8.

Physical and hydrochemical characteristics of rivers on PCA ordination diagrams.



salinity and organic matter (Stenger-Kovács et al., 2023). Conversely, oligotrophic indicator taxa such as *Fragilaria* spp. and *Achnanthidium* spp. clustered in sites with low mineralization and conductivity (Stenger-Kovács et al., 2023).

PC2, representing nutrient enrichment, influenced taxonomic composition by modulating phosphate and nitrate availability—key factors in algal productivity. Elevated phosphate concentrations, although below bloom-triggering thresholds (<0.03 mg L^{-1}), coincided with increased abundances of pollution-tolerant cyanobacteria (*Oscillatoria* spp.) and green algae (*Chlorella vulgaris*), especially during summer low-flow periods (Barinova et al., 2017). In contrast, streams with lower nutrient loads favored sensitive Heterokontophyta and charophytes, signaling higher ecological quality (Oemke and Burton, 1986; Glibert, 2020)

Integration of these hydrochemical and biological gradients confirms that water chemistry, particularly mineral content and nutrient availability, is the primary determinant of algal assemblages in the Samur-Yalama rivers. While some spatial and seasonal variation occurs, most streams retain a diverse algal flora, with communities reflecting the generally good water quality and only minor evidence of anthropogenic enrichment or ecological stress (Kristiansen, 1996).

CONCLUSION

In conclusion, multivariate analyses revealed two primary hydrochemical gradients—mineralization (SO_4^{2-}, Ca^{2+}, TDS vs. DO, turbidity) and nutrient enrichment (PO_4^{3-}, NO_3^-, Si)—which together explained over 90 % the variance in water chemistry and delineated to three distinct stream groups. Seasonal surveys across ten rivers documented 46 algal taxa, including 37 newly recorded for the region. Sensitive diatoms (e.g., *Fragilaria capucina, Achnanthidium minutissimum*) dominated pristine headwaters, whereas tolerant taxa such as *Gomphonema parvulum* and *Nitzschia palea* prevailed in moderately enriched reaches. These results demonstrate a clear species–environment linkage and confirm the largely oligosaprobic status of Samur-Yalama streams. The study thus establishes a statistically robust baseline for algal-based biomonitoring and provides critical guidance for future conservation and water-management efforts in semi-arid South Caucasus catchments.

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