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Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

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Research Article

The Establishment of the Non-indigenous Cyclopoid Copepod *Oithona davisae* in Hamsilos Bay-Sinop, Southern Black Sea, Turkey

Funda Üstün[1], Tuba Terbıyık Kurt[2], Zeynep Hasançavuşoğlu[1]

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ABSTRACT

Hamsilos Bay, which is located within the first–degree natural protection area, is one of the most important regions on the southern Black Sea coast. Moreover, many fish species utilize this area for breeding and rearing activities. Small copepod *Oithona davisae* has been encountered in the Black Sea for two decades and spread rapidly the overall Black Sea, and become an important component in the pelagic ecosystem of the Black Sea. In this respect, the present study aims to characterize the distribution and abundance of *Oithona davisae* in Hamsilos Bay about environmental variables. Zooplankton samples were collected monthly from seven stations from July 2015 to June 2016 by using a plankton net with a mesh size of 112 µm and a diameter mouth opening of 50 cm. *O. davisae* was observed during the whole sampling period and was the dominant species from July 2015 to December 2015. The highest mean abundance of this species was recorded in September 2015 (3533.33 ind. m^{-3}). However, the lowest mean abundance was observed between January 2016 (170.68 ind. m^{-3}) and May 2016 (0.24 ind. m^{-3}). Copepodites 5–4 were predominant in the *O. davisae* population in all months and all stations. Adult copepods and their copepodites 5–4 were more abundant between July 2015 and December 2015, whereas the abundance of copepodites 3–1 was maximum between September 2015 and December 2015. The abundance of *O. davisae* was highly correlated with chlorophyll-α and physiochemical variables (temperature, dissolved oxygen, and salinity). In conclusion, this species, which is known to be distributed only in the autumn conditions in close areas, extended its temporal distribution and prevailed in the copepod communities during warmer periods. This could be attributed to the acclimatization success of this species in Hamsilos Bay ecosystems.

Keywords: *Oithona davisae*, non-native, invasive species, abundance, developmental stages, physiochemical variables

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INTRODUCTION

Marine ecosystems are influenced by human activities such as implantation, aquacultural facilities, transoceanic shipping, and fisheries activities. These activities lead to the entrance of non-indigenous species and the change in native communities throughout the world (Leidenberger et al., 2015). Non-indigenous species have an impact on native communities, habitats, and ecosystem processes. For this reason, regular monitoring of the introduction of non-native species into the ecosystem and its ecological effects is important to follow up on the changes that may occur in the environment and respond appropriately in time to the potential effects they can create (Brog, 2009).

The Black Sea, a semi-enclosed inland sea, is connected to the Aegean Sea and the Mediterranean Sea in the southwest through the Turkish Straits System and to the Sea of Azov in the north through the Kerch Strait (Ünlüata et al., 1990). The Black Sea is a very sensitive ecosystem that can be easily affected by alien (non-native) species due to its geographic location (Selifonova, 2011a). It is known that the introduction of non-indigenous species into the Black Sea started in the 19th century and led to great problems in the following decades (Shiganova, 1998). One of the alien species that entered the Black Sea probably with ballast waters (Gubanova et al., 2014) is the cyclopoid copepod *Oithona davisae*. Known to be native to the Seas of Japan and China (Razouls et al., 2023), *Oithona davisae* has successfully settled in many different marine and estuarine ecosystems such as the Barcelona Harbor in Spain (Saiz et al., 2003), Marmara Sea (Doğan & İşinibilir Okyar, 2016), Aegean Sea in Turkey (Terbıyık Kurt & Beşiktepe, 2019), İskenderun Bay in Levantine Sea in Turkey (Terbıyık-Kurt et al., 2022), Sacramento-San Joaquin estuary in California (Ferrari & Orsi, 1984), San Francisco Bay estuary in California (Lougee et al., 2002), Bilbao estuary in Spain (Uriarte et al., 2016). *O. davisae* was first found in 2001 in Sevastopol Bay in the Black Sea. Firstly, it was misidentified as *Oithona brevicornis* (Zagorodnyaya, 2002), but later it was identified as *O. davisae* upon re-examination of zooplankton specimens (Temnykh & Nishida, 2012). The first record of *O. davisae* was in Sinop in 2009 along the Black Sea coast of Turkey (Üstün & Terbiyik Kurt, 2016). Later, *O. davisae* expanded its distribution towards the eastern Black Sea (Trabzon coast) (Yıldız et al., 2017) in 2010 and the western Black Sea (İğneada coast) in 2012-2013 (Üstün et al., 2019). It is seen that *O. davisae* become to have a higher abundance and frequency, thus expanding its range on the Turkish coasts of the Black Sea after reporting by Üstün & Terbiyik Kurt, 2016 on the Sinop coast.

Hamsilos Bay had great significance on the southern Black Sea coast due to its location within the first–degree natural protection area (Anonymous, 2018). Furthermore, Hamsilos Bay is a breeding and rearing area for many fish species. In the present study, the temporal and spatial distribution of the abundance values of *O. davisae* in Hamsilos Bay, a protected area, and its relationship with environmental parameters have been first examined.

MATERIALS AND METHODS

Sampling area

Our study area, Hamsilos Bay, is 11 km from Sinop, a natural harbor located at the northernmost point of Turkey. While it looks like a fjord, it is a small ria formed by the submersion of a valley underwater (Akkan, 1975). The importance of Hamsilos Bay is demonstrated by its status as a natural area of protection, the absence of residential areas, and no dumping of any pollutants in this area, therefore is no stress factor harming the flora and fauna living here. Additionally, as it is an enclosed and protected area in terms of winds (mistral–northbound) and covered with *Zostera* facies in its deep structure (Ersoy Karaçuha, 2006), it provides shelter for various benthic and demersal living beings and makes the Bay a significant reproduction and shelter area for fish (Uygun, 2015).

Sampling and laboratory studies

The study was conducted monthly at seven stations in Hamsilos Bay from July 2015 to June 2016 (Table 1 and Figure 1). Environmental variables were simultaneously obtained after zooplankton samplings. The physical parameter (temperature, dissolved oxygen, and salinity) values of the surface seawater were recorded with the YSI 6600 MDS model multiparameter (YSI Incorporat-

Table 1. Information about sampling stations.

Station name	Information of Coordinate	Sampling Depth (m)
A	42°03'38''N – 35°02'31''E	2
B	42°03'36''N – 35°02'36''E	4
C	42°03'40''N – 35°02'36''E	9
D	42°03'45''N – 35°02'40''E	13
E	42°03'52''N – 35°03'14''E	30
F	42°04'5''N – 35°02'59''E	30
G	42°04'12''N – 35°02'45''E	30

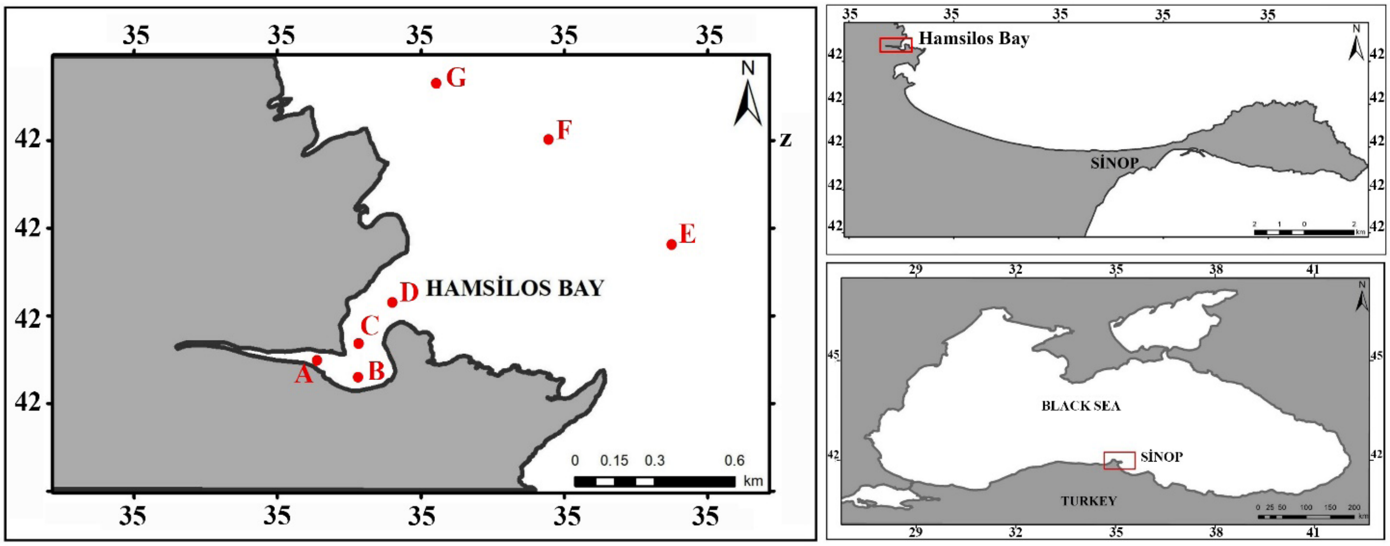

Figure 1. Location of sampling stations.

ed, Ohio, USA). The concentration of chlorophyll-*a* (µg. l^{-1}) was estimated according to Parsons et al. (1984).

A plankton net with a mouth opening of a diameter of 50 cm and a mesh size of 112 µm was vertically towed from the bottom to the surface of the water during the daytime. After sampling, the collected material was transferred into a bottle and preserved in a solution of borax-buffered 4% formaldehyde in seawater. *O. davisae* individuals were quantitatively examined using subsamples (two replicates) with a Stempel pipette (1 ml). Subsamples varied from 1/1 to 1/100 (usually 1/25 and 1/50) depending on the abundance of individuals in the samples. The abundance results of *O. davisae* were given in individual (ind.) m^{-3}. The consequences were presented as mean ± standard deviation.

In a zooplankton counting chamber, the development stages of *Oithona davisae* were identified and counted under a stereomicroscope. The present study did not include naupliar stages of this species because the mesh size of our zooplankton was inappropriate (Uye & Sano, 1995). According to Ferrari & Orsi (1984) and Temnykh & Nishida (2012), *O. davisae* was identified.

Analyses of Spearman correlation (SPSS 21, IBM Corp., Armonk, NY, USA) were applied to determine the correlation between the abundance of *O. davisae* and abiotic and biotic variables. The difference in abundance values of developmental stages of *O. davisae* between stations and months was tested by One-way ANOVAs with post hoc Tukey tests (SPSS 21, IBM Corp., Armonk, NY, USA).

RESULTS

Environmental parameters

A detailed description of the hydrological status of the sampling site was given by Üstün (2019) and Üstün & Birinci Özdemir (2019). During the sampling period, a clear seasonal cycle was observed in the mean temperature of surface seawater and fluctuated from 8.44 ± 0.04 °C (February 2016) to 25.71 ± 0.16 °C (August 2015) in Hamsilos Bay. The surface seawater means salinity ranged between 17.74 ± 0.07‰ (September 2015) and 18.96 ± 0.03‰ (May 2016). The dissolved oxygen values in surface seawater varied from 7.56 ± 0.36 mg. l^{-1} (July 2015) to 9.88 ± 0.13 mg. l^{-1} (February 2016). The mean concentration of chlorophyll-*a* fluctuated between 0.15±0.10 µg. l^{-1} (March 2016) and 0.71 ± 0.16 µg. l^{-1} (October 2015) in the surface seawater (Figure 2).

Seasonal dynamics of population abundance of *Oithona davisae*

The clear seasonal distribution pattern of *Oithona davisae* abundance was observed in the study area and ANOVA showed that differences between the abundance of *O. davisae* were statistically important in sampling months (F=14.961; p<0.05), but not at sampling stations (F=0.195; p>0.05). *O. davisae* had greater mean abundance values during the summer-autumn periods. The highest value for mean abundance was observed in September 2015 (3533.33 ± 1194.89 ind. m^{-3}). A small–scale decrease in mean abundance was observed in the period from September 2015 to December 2015 (1715.05 ± 938.67 ind. m^{-3}). In January 2016, mean abundance values started to decrease sharply and reached 170.68 ± 52.74 ind. m^{-3}. After January 2016, the decreasing trend continued gradually until May 2016 (0.24 ± 0.58 ind. m^{-3}) and reached its lowest level in May 2016 (Figure 3).

O. davisae concentrated in the inshore of the bay and the abundance of this species decreased at stations located outer of the bay. The maximum abundance value of *O. davisae* was noted in September 2015 at station B (5550 ind. m^{-3}) and the minimum abundance value was determined in May 2016 at station D (1.67 ind. m^{-3}). While there were high abundance values at stations inside the bay during July–August–September 2015, the abundance of this species at stations outside of the bay reached high values during October–November–December 2015. *Oithona davisae* was found only at station D in May 2016 (Figure 3, Table 2).

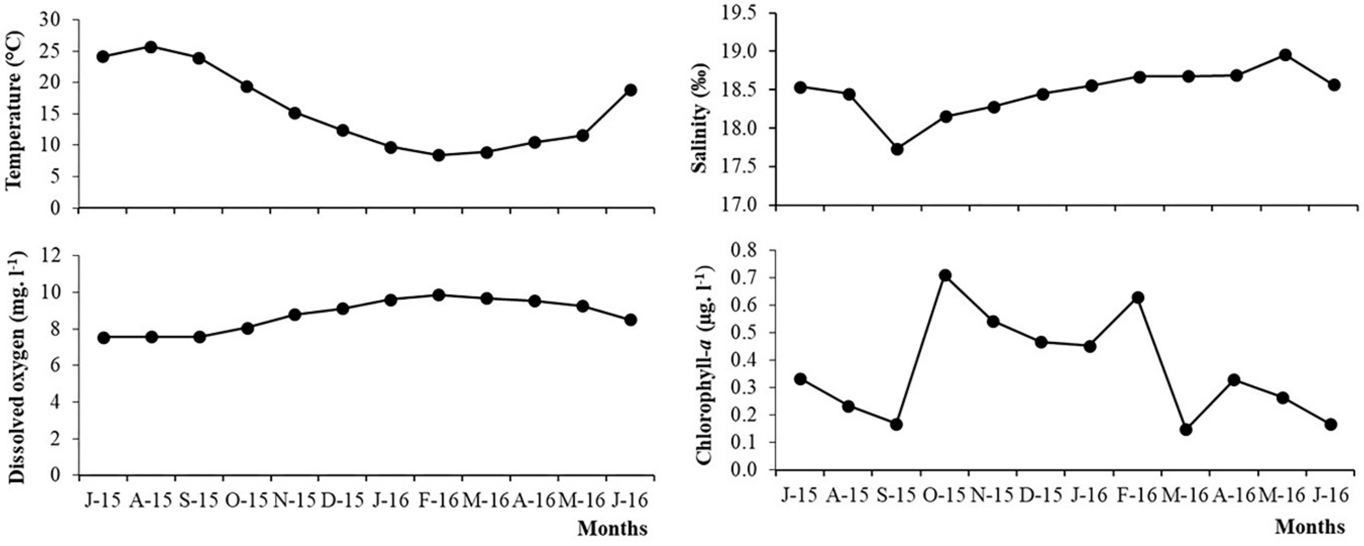

Figure 2. The monthly mean variation in environmental factors on surface seawater in Hamsilos Bay, Sinop.

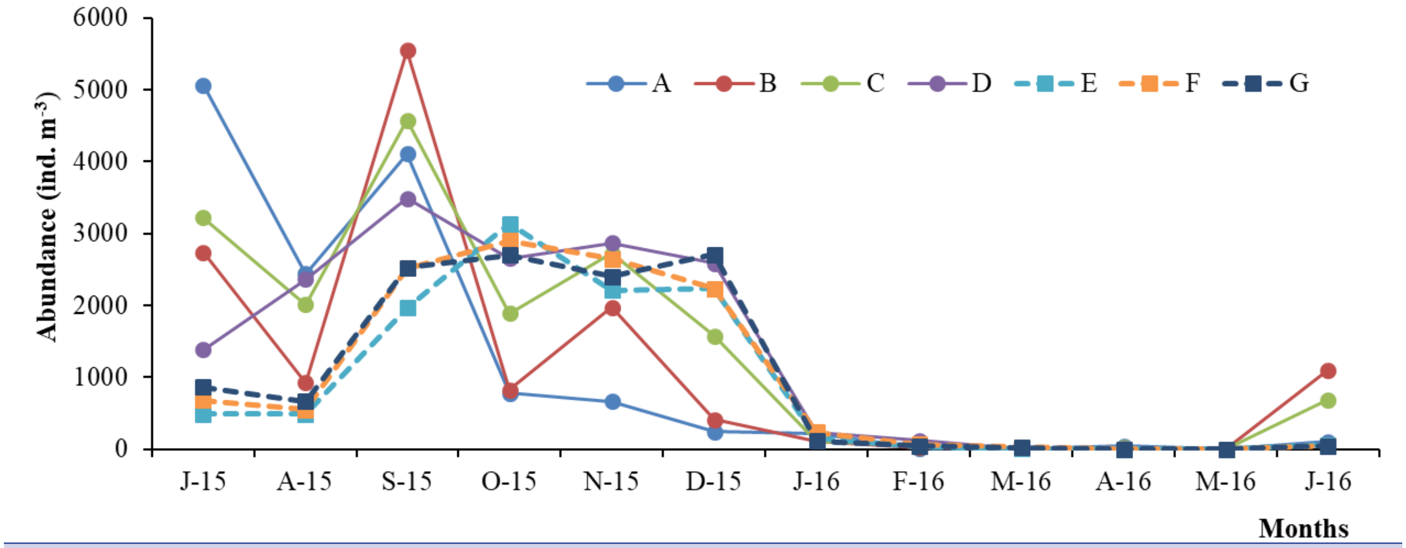

Figure 3. Spatial and temporal distributions in abundance (ind. m^{-3}) of *O. davisae* in Hamsilos Bay.

Table 2. Minimum, maximum, and mean of abundance values (ind. m^{-3}) of *O. davisae* according to stations in Hamsilos Bay.

Stations	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Minimum	10 (March 2016)	4 (April 2016)	12.5 (March 2016)	1.67 (May 2016)	4.17 (April 2016)	4.17 (April 2016)	2.5 (April 2016)
Maximum	5062.5 (July 2015)	5550 (September 2015)	4575 (September 2015)	3487.5 (September 2015)	3137.5 (October 2015)	2900 (October 2015)	2712.5 (December 2015)
Mean±STD	1142±1683.7	1569±1568.6	1408±1459.5	1315±1321.7	901±1094.7	994±1145.6	1008±1147.1

Population age structure

The majority of the population of *O. davisae* consisted of copepodite 5–4 (Figure 4). The abundance values of females varied from 0.83 ind. m^{-3} (June 2016; St F and G) to 625 ind. m^{-3} (July 2015; St A). The presence of females was more pronounced in September 2015 and October 2015 (Figure 5). The female abundance values among months differed significantly (F=11.850; p<0.05). Between stations, there were no statistically significant variations in female abundance (F=0.217; p>0.05). Abundance values of the male were lowest in January 2016 (2.5 ind. m^{-3}; St C) and highest in July 2015 (781.25 ind. m^{-3}; St A). Three peak periods (July 2015, September 2015, and December 2015) were observed in males (Figure 5). Male abundance significantly differed between months (F=5.899; p<0.05). In terms of male abundance, there were no statistical differences between stations (F=0.600; p>0.05). The mean sex ratio (female/male) values of *O. davisae* varied between 0.56 and 3.79. Female individuals dominated the community from August 2015 to February 2016 (Figure 6).

Copepodit 5–4 was present all over the research period (in May 2016 only in station D), with its maximum abundance of 4825 ind. m^{-3} at station B in September 2015 and its minimum abundance of 1.67 ind. m^{-3} at station D in May 2016. Copepodit 5–4 was dominated during the July 2015–December 2015 periods (Figure 5). Abundance values of copepodit 5–4 showed statistically significant differences among months (F=15.077; p<0.05). There were no statistically significant differences in copepodit 5–4 abundance among the stations (F= 0.255; p>0.05) (Figure 5).

Copepodit 3–1 abundance values ranged between 1 ind. m^{-3} (February 2016; St B) and 650 ind. m^{-3} (November 2015; St F). It contributed significantly to the *O. davisae* population between September 2015 and December 2015 (Figure 5). Abundance values of copepodit 3–1 showed statistically significant differences among months (F=13.723; p<0.05). No significant differences in copepodit 3–1 abundance were observed between stations (F=0.151; p>0.05).

Female, male, and copepodite 3–1 individuals were not encountered in March 2016 and May 2016. In April 2016, female and copepodit 3–1 were determined only at station A, and male at station C (Figure 5). Minimum and maximum mean abundance values were in the range of 0.71 ± 1.75 (April 2016) to 317.26 ± 48.40 (September 2015) ind. m^{-3}in female, 0.71 ± 1.75 (April 2016) to 262.5 ± 246.52 (July 2015) ind. m^{-3}in male, 0.24 ± 0.58 (May 2016) to 2818.75 ± 1163.20 (September 2015) ind. m^{-3}in copepodit 5–4, 1.43 ± 3.5 (April 2016) to 441.55 ± 176.88 (November 2015) ind. m^{-3} in copepodit 3–1 (Figure 5).

The abundance of developmental stages of *O. davisae* was well correlated with trophic elements and physiochemical variables. It was found that the abundance and distribution of developmental stages of *O. davisae* correlated negatively with salinity and

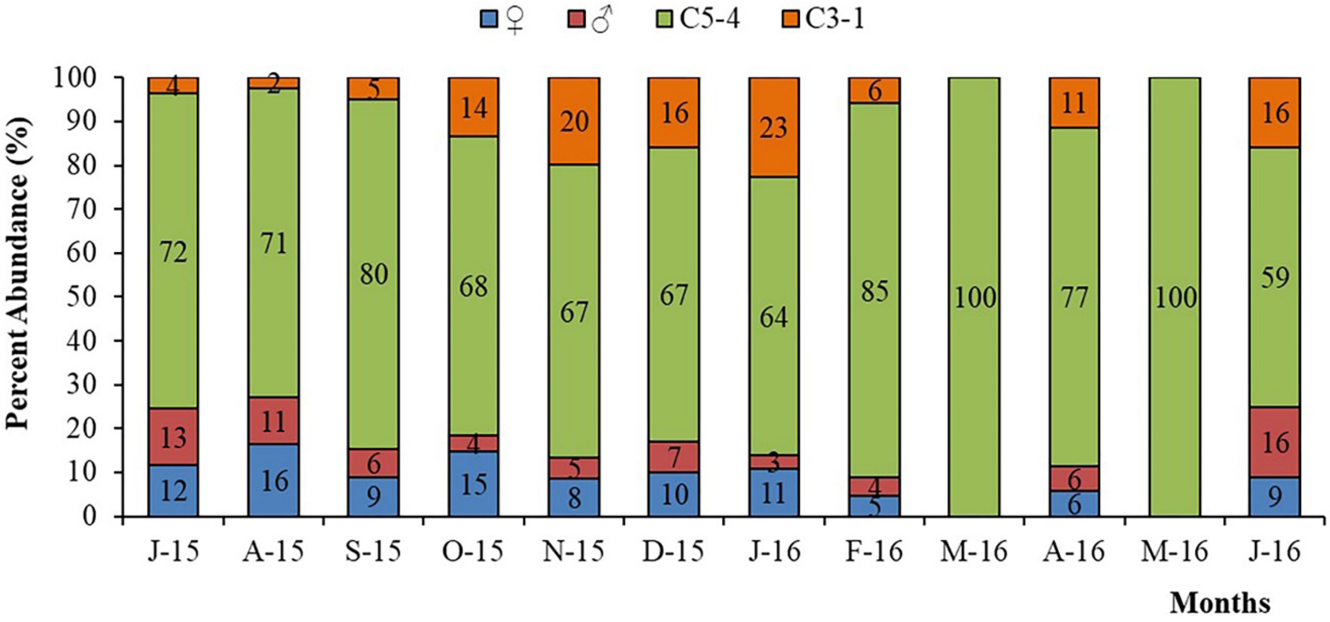

Figure 4. The percentage mean abundance contributions of different developmental stages of *O. davisae* in Hamsilos Bay.

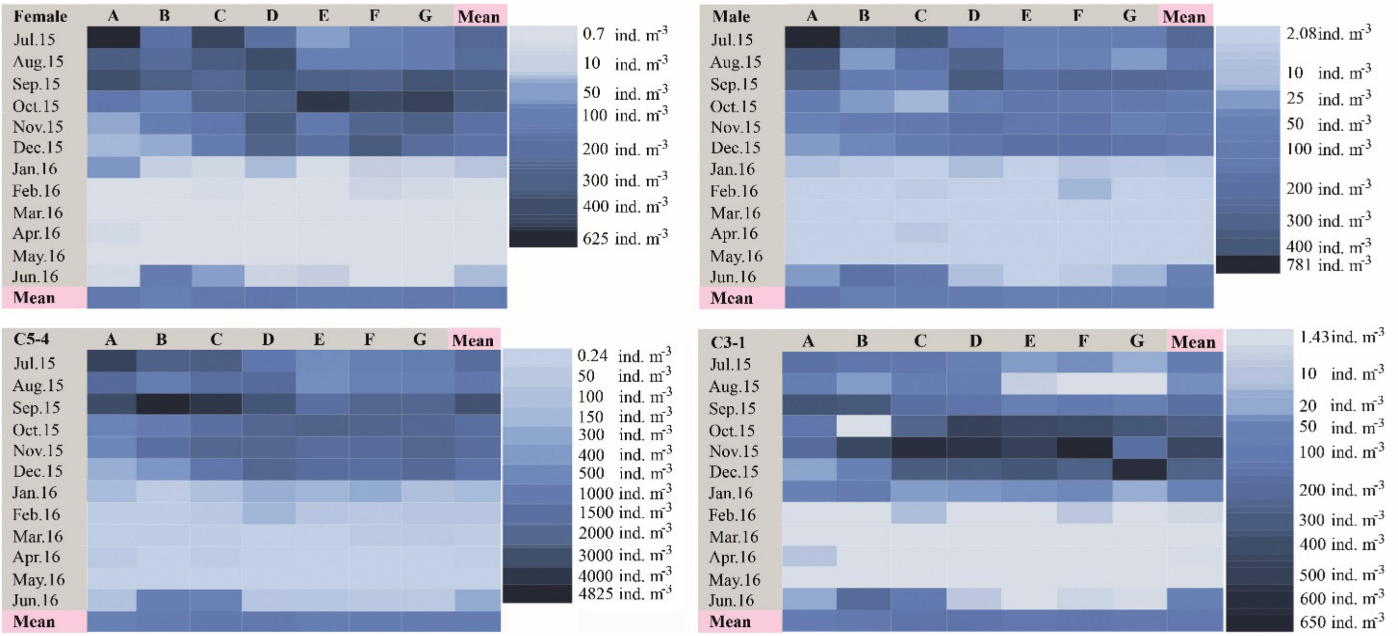

Figure 5. Temporal changes in mean abundance distributions of different developmental stages of *O. davisae* in Hamsilos Bay.

dissolved oxygen, but showed a positive correlation with chlorophyll-*a* and temperature. No correlation was found between the abundance of males and chlorophyll-*a* (Table 3).

DISCUSSION

Seven oithonid species were found in the Black Sea until now (Razouls et al., 2023). Most of these recorded oithonid species were known as questionable and had insufficient resources (Gubanova et al., 2014). Although *Oithona similis* are commonly distributed overall Black Sea (Gubanova et al., 2014), *Oithona davisae* expanded its distribution and became one of the most important components in the coastal community in the Black Sea (Gubanova et al., 2014), after the first observation of this species in Sevastopol Bay in the early 2000s (Zagorodnyaya, 2002). The abundance of this species in Sevastopol Bay already exceeded 40,000 ind. m^{-3} in October 2005 (Gubanova & Altukhov, 2007) and reached almost 100,000 ind. m^{-3} in October 2016 (Svetlichny

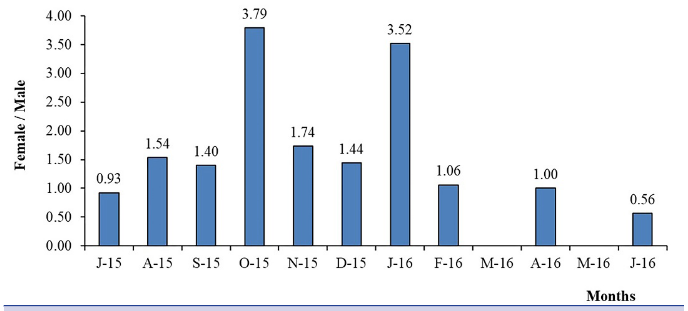

Figure 6. Mean sex ratio (male/female) values of *O. davisae* in Hamsilos Bay.

et al., 2018). Only eight years after the first appearance in the Black Sea, the evidence of the distribution of *Oithona davisae* was first observed on Turkey's coast in the Black Sea (Üstün & Terbiyik Kurt, 2016). It is seen that the presence of *O. davisae* is observed in the plankton every month and its abundance value increased when compared with the present study conducted in 2015–2016 in Sinop in respect of the species that were found to be present only in September–December period in Sinop in 2009. It was observed in other studies (Yıldız et al., 2017; Üstün et al., 2019; Yildiz & Feyzioğlu, 2020) carried out on the Turkish coasts of the Black Sea that the species reached values of maximum in the July–December (summer–autumn) period and demonstrated the lowest values in the January–May (winter-spring) period as is the case with the present study. Similar results were also revealed in the studies carried out on the coasts of Sevastopol Bay, Novorossiysk Bay, Romania, Bulgaria, and Georgia (Gubanova & Altukhov, 2007; Selifonova, 2011b; Timofte & Tabarcea, 2012; Mihneva & Stefanova, 2013; Seregin & Popova, 2016; Shvelidze, 2016; Svetlichny et al., 2018). Although the different plankton net sizes and sampling periods and depths do not allow us to compare the abundance values obtained from different studies, the studies demonstrated that the species has expanded its abundance and both temporal and spatial distribution since it was first found in the Black Sea.

In 2009, *O. davisae* was only distributed autumn period (Üstün & Terbiyik Kurt, 2016), however, it seems that *O. davisae* expanded its temporal distribution and was observed the following year on the Sinop coast. *O. davisae* to successfully inhabit and spread to new marine areas most probably due to having bioecological features, such as thermophilic (Ferrari & Orsi, 1984; Uye & Sano, 1995; Mihneva & Stefanova, 2013; this study), euryhaline (Svetlichny & Hubareva, 2014; Hubareva & Svetlichny, 2016), reproduction style (Svetlichny et al., 2016), food source (principally feeds on ciliates and heterotrophic dinoflagellates) (Uchima, 1988; Saiz et al., 2003; Khanaychenko et al., 2018), locomotor activity and energy metabolism (body mass density, sinking, and swimming speeds) (Svetlichny et al., 2016).

Oithona davisae is a long–lived species and demonstrates considerable variation in population size depending on temperature changes affecting food resources and the development of planktonic predators (Svetlichny et al., 2016). It is either absent (Takahashi & Uchiyama, 2007) or very scarce (Uye & Sano, 1995) during the winter–spring seasons among the planktons of the coastal waters of East Asia, its original habitat. The abundance of this cyclopoid copepod is low in the Black Sea during winter and spring and increases from summer to autumn with the increasing temperature (Gubanova &Altukhov, 2007; Selifonova, 2011b; Altukhov et al., 2014; Seregin & Popova, 2016). Capable of surviving the cold seasons in the Black Sea, fertilized females reproduce the next generation under favorable spring conditions (Svetlichny et al., 2016).

Table 3. Correlation between the abundance of *O. davisae* and developmental stages and environmental variables.

	O. davisae	**Female**	**Male**	**Copepodit 5–4**	**Copepodit 3–1**
Chlorophyll-a	.253 (*)	.232 (*)	.151	.246 (*)	.331 (**)
Temperature (°C)	.669 (**)	.752 (**)	.754 (**)	.685 (**)	.488 (**)
Salinity (‰)	-.771 (**)	-.795(**)	-.730(**)	-.810 (**)	-.734 (**)
Dissolved oxygen (DO mg. l^{-1})	-.710 (**)	-.763 (**)	-.770(**)	-.738 (**)	-.509 (**)

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

In the coastal waters of southeastern Japan, the original habitat of *O. davisae*, the species has been found to inhabit in seasonal temperature variations between 8.9 and 28.2 °C (Uye & Sano, 1995). The present study observed the highest abundance values between July 2015 and September–December 2015. The water temperature varied between 12–24 °C during this period. The lowest abundance values were recorded between February (8.4 °C–54 ind. m^{-3}) and May (11.6 °C–0.24 ind. m^{-3}). The results of this study agree with findings acquired in the Black Sea previously (Mihneva & Stefanova, 2013; Üstün & Terbiyik Kurt, 2016; Yıldız et al., 2017; Yildiz & Feyzioğlu, 2020). It was observed in the Black Sea that an intense increase in the population size of this species started when the surface water temperature reached 18–20 °C (Seregin & Popova, 2016). The analysis of seasonal variations of *O. davisae* recorded in samples collected from the coastal areas of Sinop suggested that temperature has a remarkable influence on the development time and abundance of the species. This study detected a strong positive correlation between temperature and *O. davisae* abundance. A negative correlation was calculated between *O. davisae* abundance values and surface seawater temperature in the seasonal study managed in İğneada (Üstün et al., 2019).

Exotic species that can successfully colonize new habitats are considered to have a wide salinity tolerance (Lee et al., 2003). *O.*

davisae has been detected in the salinity range of 28.6–32.3‰ in the Sea of Japan (Uye & Sano, 1995), in the salinity range of 12–19‰ in the San Francisco estuary (Ferrari & Orsi, 1984) and in the salinity range of 15.8–17.2‰ in the western Black Sea (Mihneva & Stefanova, 2013). In their experimental studies, Svetlichny & Hubareva (2014) and Hubareva & Svetlichny (2016) have shown that *O. davisae* can adapt well to high salinity and the species can live in the salinity range of 3–55‰ by keeping its body mass density constant and that *O. davisae* is (partly, 12–40‰) osmoregulatory species. The salinity ranged between 17.74–18.54‰ (mean: 18.27 ± 0.27‰) during the period when the values of the maximum abundance of the species were observed in our study. This study determined a negative correlation between salinity and abundance values of *O. davisae*. A positive correlation was calculated between the abundance values of *O. davisae* and surface seawater salinity in the seasonal study conducted in İğneada (Üstün et al., 2019). This contradictory report could be related to the joint and complex effect of the environmental variables.

In its original habitat, Asian waters, there is a congruence between the months with the highest abundance of the species and the months with the highest concentration of chlorophyll-*a* (Uye & Sano, 1995). It was revealed in our study that chlorophyll-*a* values ranged between 0.17–0.71 µg. l^{-1} (mean: 0.41 ± 0.19 µg. l^{-1}) during the period when the highest abundance values of the species were observed. A positive correlation was detected between abundance values of *Oithona* and chlorophyll-*a* in this study, however, such a case was not detected in the study conducted in Trabzon (Yıldız et al., 2017).

CONCLUSION

O. davisae has a high tolerance to different environmental conditions and physiological structures (osmoregulatory systems, high egg production rate, high metabolic activity, etc.) which facilitate to survival and reproduction of *Oithona davisae* in the Black Sea. In our study, it seems that *O. davisae* has expanded its spatial and temporal distribution along the Sinop coast in the Black Sea and has been inhabited by increasing its population, and biotic and abiotic factors affected the temporal distribution of this species. This study suggests that *O. davisae* has been successful in inhabiting and establishing a constant population in the southern Black Sea.

Note: This study was presented orally at the "13th International Conference on Copepoda" held in Los Angeles, California, from the 16th to the 21st of July 2017.

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REFERENCES

Akkan, E. (1975). *Sinop Yarımadasının jeomorfolojisi*. Ankara University Publisher, Ankara, Turkey, (in Turkish).

Altukhov, D.A., Gubanova, A.D. & Mukhanov, V.S. (2014). New invasive copepod *Oithona davisae* Ferrari and Orsi, 1984: seasonal dynamics in Sevastopol Bay and expansion along the Black Sea coasts. *Marine Ecology an Evolutionary Perspective*, 35 (Supplement s1), 28−34. https://doi.org/10.1111/maec.12168.

Anonymous, (2018). Ministry of forestry and water affairs (Orman ve Su Işleri Bakanligi), general directorate of nature conservation and National Parks (Doga Koruma ve Milli Parklar Genel Müdürlügü). http://hamsilos.tabiat.gov.tr/ (accessed 15 February 2018). (in Turkish).

Brog, M.A. (2009). Non-indigenous zooplankton − the role of predatory cladocerans and of copepods in trophic dynamics. [PhD thesis] University of Stockholms, Stockhol, Sweden.

Doğan, G. & İşinibilir Okyar, M. (2016). First report of a new invasive species *Oithona davisae* Ferrari and Orsi, 1984 (Copepoda: Cyclopoida) in the Sea of Marmara. *Turkish Journal of Fisheries and Aquatic Sciences*, 16, 469−473. https://doi.org/10.4194/1303-2712-v16_2_27.

Ersoy Karaçuha, M. (2006). Sinop Yarımadası kıyıları Zostera fasieslerinin Crustacea (Arthropoda) faunası ve ekolojik özellikleri (Crustacea (Arthropoda) fauna of Zostera facies in Sinop Peninsula coasts and their bioecological features). [Master's Thesis] University of Ondokuz Mayıs, Samsun, Turkey (Turkish with English Abstract).

Ferrari, F.D. & Orsi, J. (1984). *Oithona davisae*, new species, and *Limnoithona sinensis* (Burckhardt, 1912) (Copepoda: Oithonidae) from the Sacramento-san Joaquin Estuary, California. *Journal of Crustacean Biology*, 4(1), 106–126.

Gubanova, A. & Altukhov, D. (2007). Establishment of *Oithona brevicornis* Giesbrecht, 1892 (Copepoda: Cyclopoida) in the Black Sea. *Aquatic Invasions*, 2, 407–410. doi: 10.3391/ai.2007.2.4.10.

Gubanova, A., Altukhov, D., Stefanova, K., Arashkevich, E., Kamburska, L., Prusova, I., Svetlichny, L., Timofte, F. & Uysal, Z. (2014). Species composition of Black Sea marine planktonic copepods. *Journal of Marine System*, 135, 44–52.

Hubareva, E.S. & Svetlichny, L.S. (2016). Copepods *Oithona similis* and *Oithona davisae*: Two strategies of ecological–physiological adaptation in the Black Sea. *Oceanology*, 56(2), 241–247.

Khanaychenko, A., Mukhanov, V., Aganesova, L., Besiktepe, S. & Gavrilova, N. (2018). Grazing and feeding selectivity of *Oithona davisae* in the Black Sea: Importance of cryptophytes. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 937–949. http://doi.org/10.4194/1303-2712-v18_8_02.

Lee, C.E., Remfert, J.L. & Gelembiuk, G.W. (2003). Evolution of physiological tolerance and performance during freshwater invasions. *Integrative and Comparative Biology*, 43(3), 439–449. https://doi.org/10.1093/icb/43.3.439.

Leidenberger, S., Obst, M., Kulawik, R., Stelzer, K., Heyer, K., Hardisty, A. & Bourlat, S.J. (2015). Evaluating the potential of ecological niche modelling as a component in marine non-indigenous species risk assessments. *Marine Pollution Bulletin*, 97, 470–487.

Lougee, L.A., Bollens, S.M. & Avent, S.R. (2002). The effects of haloclines on the vertical distribution and migration of zooplankton. *Journal of*

Experimental Marine Biology and Ecology, 278, 111–134. https://doi.org/10.1016/S0022-0981(02)00326-X

Mihneva, V. & Stefanova, K. (2013). The non-native copepod *Oithona davisae* (Ferrari FD and Orsi, 1984) in the Western Black Sea: seasonal and annual abundance variability. *Bioinvasions Records*, 2(2), 119–124. http://dx.doi.org/10.3391/bir.2013.2.2.04.

Parsons, T.R., Maita, Y. & Lalli, C.M. (1984). A manual of chemical and biological methods for seawater analysis. Oxford, United Kingdom.

Razouls, C., Desreumaux, N., Kouwenberg, J. & de Bovée, F. (2023). Biodiversity of Marine Planktonic Copepods (morphology, geographical distribution and biological data). Sorbonne University, CNRS. http://copepodes.obs-banyuls.fr/en (accessed 09 March 2023).

Saiz, E., Calbet, A., Broglio, E. & Marı, P. (2003). Effects of small-scale turbulence on copepods: The case of *Oithona davisae*. *Limnology and Oceanography*, 48(3), 1304–1311. https://doi.org/10.4319/lo.2003.48.3.1304.

Selifonova, Z.P. (2011a). Ships' ballast as a primary factor for 'Mediterraneanization' of pelagic copepod fauna (Copepoda) in the Northeastern Black Sea. *Acta Zoologica Bulgarica*, 63(1), 77–83.

Selifonova, Z.P. (2011b). *Oithona brevicornis* Giesbrecht (Copepoda: Cyclopoida), invader into the Black Sea and in the Sea of Azov. *Russian Journal of Biological Invasions*, 2(2–3), 227–232. http://dx.doi.org/10.1134/s2075111711030131.

Seregin, S.A. & Popova, E.V. (2016). Long-term dynamics of the copepod invader *Oithona davisae* in coastal waters of the Black Sea. *Russian Journal of Biological Invasions*, 7(4), 374–382. https://doi.org/10.1134/s207511171604007x.

Shiganova, T.A. (1998). Invasion of the Black Sea by the ctenophore *Mnemiopsis leidyi* and recent changes in pelagic community structure. *Fisheries Oceanography*, 7, 305–310. https://doi.org/10.1046/j.1365-2419.1998.00080.x.

Shvelidze, T. (2016). First record of *Oithona davisae* Ferrari and Orsi, 1984 (Copepoda, Cyclopoida) and seasonal variations in the Georgian Black Sea coast. *Academia Journal of Scientific Research*, 4(11), 424–428. doi: 10.15413/ajsr.2016.0141.

Svetlichny, L. & Hubareva, E. (2014). Salinity tolerance of alien copepods *Acartia tonsa* and *Oithona davisae* in the Black Sea. *Journal of Experimental Marine Biology and Ecology*, 461, 201–208. http://dx.doi.org/10.1016/j.jembe.2014.08.012.

Svetlichny, L., Hubareva E., Khanaychenko A., Gubanova A. & Besiktepe, S. (2016). Adaptive strategy of thermophilic *Oithona davisae* in the cold Black Sea environment. *Turkish Journal of Fisheries and Aquatic Sciences*, 16(1), 77–90. https://doi.org/10.4194/1303-2712-v16_1_09.

Svetlichny, L., Hubareva, E. & İşinibilir, M. (2018). Population dynamics of the copepod invader *Oithona davisae* in the Black Sea. *Turkish Journal of Zoology*, 42(6), 684–693. https://doi.org/10.3906/zoo-1804-48.

Takahashi, T. & Uchiyama, I. (2007). Morphology of the naupliar stages of some *Oithona* species (Copepoda: Cyclopoida) occurring in Toyama Bay, southern Japan Sea. *Plankton Benthos Research*, 2(1), 12–27. https://doi.org/10.3800/pbr.2.12.

Temnykh, A. & Nishida, S. (2012). New record of the planktonic copepod *Oithona davisae* Ferrari and Orsi in the Black Sea with notes on the identity of "*Oithona brevicornis*". *Aquatic Invasions*, 7(3), 425–431. doi: http://dx.doi.org/10.3391/ai.2012.7.3.013.

Terbıyık Kurt, T. & Beşiktepe, Ş. (2019). First distribution record of the invasive copepod *Oithona davisae* Ferrari and Orsi, 1984, in the coastal waters of the Aegean Sea. *Marine Ecology*, 40(3), e12548. https://doi.org/10.1111/maec.12548.

Terbıyık-Kurt, T., Polat, S. & Guy-Haim, T. (2022). New invasive copepod species from the Levantine Sea, north-eastern Mediterranean: *Oithona davisae* (Ferrari and Orsi, 1984) (Cyclopoida: Oithonidae). *Bioinvasions Records*, 11(1), 215–226. https://doi.org/10.3391/bir.2022.11.1.22.

Timofte, F. & Tabarcea, C. (2012). *Oithona brevicornis* Giesbrecht, 1892 (Copepoda: Cyclopoida)-First record in the Romanian Black Sea waters. *Journal of Environmental Protection and Ecology*, 13(3A), 1683–1687.

Uchima, M. (1988). Gut content analysis of neritic copepods *Acartia omorii* and *Oithona davisae* by a new method. *Marine Ecology Progress Series*, 48, 93–97. https://doi.org/10.3354/meps048093.

Uriarte, I., Villate, F. &, Iriarte, A. (2016). Zooplankton recolonization of the inner estuary of Bilbao: influence of pollution abatement, climate and non-indigenous species. *Journal of Plankton Research*, 38(3), 718–731. https://doi.org/10.1093/plankt/fbv060.

Uye, S. & Sano, K. (1995). Seasonal reproductive biology of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. *Marine Ecology Progress Series*, 118, 121–128. https://doi.org/10.3354/meps118121.

Uygun, O. (2015). Balık larvalarının Sinop-Akliman kıyılarındaki kompozisyonu (Composition of fish larvae in Sinop-Akliman coast of the Black Sea). [Master'sThesis] University of Sinop, Sinop, Turkey (Turkish with English Abstract).

Ünlülata, Ü., Oğuz, T., Latif, M.A. & Özsoy, E. (1990). On the Physical Oceanography of the Turkish Straits. In Pratt, L.J. (Eds.), *The Physical Oceanography of Sea Straits* (pp. 25–60). NATO ASI Series, vol 318. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0677-8_2. ISBN : 978-94-010-6789-8.

Üstün, F. &, Terbiyik Kurt, T. (2016). First report of the occurrence of *Oithona davisae* Ferrari F. D. & Orsi, 1984 (Copepoda: Oithonidae) in the southern Black Sea, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 16(2), 413–420. https://dx.doi.org/ 10.4194/1303-2712-v16_2_21.

Üstün, F. (2019). Seasonal cycle of zooplankton abundance and biomass in Hamsilos Bay, Sinop, Southern Black Sea, Turkey. *Journal of Natural History*, 53(7-8), 365–389. https://doi.org/10.1080/00222933.2019.1592257.

Üstün, F., Bat L. & Şahin F. (2019). Composition, abundance and biomass of mesozooplankton in the southwestern Black Sea along the coast of İğneada, Turkey. *Biologia* 74, 851–862. https://doi.org/10.2478/s11756-019-00219-w.

Üstün, F. & Birinci Özdemir, Z. (2019). Seasonal distribution of gelatinous macrozooplankton in the Hamsilos Bay, Southern Black Sea, Turkey. KSU *Journal of Agriculture and Nature*, 22(4), 583–592. https://doi.org/10.18016/ksutarimdoga.vi.511807

Yıldız, İ., Feyzioglu, A.M. & Besiktepe, S. (2017). First observation and seasonal dynamics of the new invasive planktonic copepod *Oithona davisae* Ferrari and Orsi, 1984 along the southern Black Sea (Anatolian Coast). *Journal of Natural History*, 51(3–4), 127–139. http://dx.doi.org/10.1080/00222933.2016.1229060.

Yildiz, İ. & Feyzioğlu, A.M. (2020). Spatial and seasonal variation of the new invader *Oithona davisae* (Ferrari F. D. & Orsi, 1984) (Cyclopoida: Oithonidae) in the Southeast Black Sea. *Acta Zoologica Bulgarica*, 72(4), 517–524.

Zagorodnyaya, Y.A. (2002). Oithona brevicornis in the Sevastopol Bay: is it a single event or a new invader in the Black Sea Fauna? Marine Ecology Journal (Morskyj Ecologichny Journal), 61, 43. (in Russian).

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Research Article

Effect of Pozzolanic Cement Exposure in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Cement used in structures such as bridges, dams, and retaining walls built on the aquatic ecosystem harms different organisms. The current study reveals the effect of pozzolanic cement (PC) on Nile Tilapia (*Oreochromis niloticus*). The LC_{50} value for *O. niloticus* exposed to 6 different PC concentrations for 24 hours was calculated as 306.66 mg L^{-1}. Increasing PC concentration significantly increased the pH of water (<10) at the end of the study ($p<0.05$). The increase in pH level showed a positive correlation with the increase in fish mortality ($p<0.05$). In addition, PC negatively affected the hematological parameters of fish. Thus, the current study reveals the negative effects of PC on *O. niloticus* in the acute period. Future studies should focus on developing cement with environmentally friendly materials that will not affect the pH level of water.

Keywords: Aquatic toxicology, hematology, LC_{50}, pozzolanic cement

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INTRODUCTION

The construction industry is indispensable in that it meets the many visible and essential needs of humanity, such as new constructions, restorations, and post-earthquake reconstruction. Cement, as the basic material of the construction industry, is the second most produced material after steel, with a production of around 4.1 billion tons worldwide (Farfan et al., 2019). Since cement is the main material in the construction of many structures such as bridges, tunnels, dams, and retaining walls, the negative effects of possible effluents on natural water resources are inevitable (Kurtoglu et al., 2016). Even the cement wastes from construction sites that are far from the aquatic system eventually contaminate the aquatic ecosystems through rainwater and sewage systems. However, there are limited studies on the effect of cement in natural waters and habitats (Er & Kayış, 2022).

Natural water resources, which are generally the final destination of all pollutants, are the habitats of aquatic organisms. In order to ensure the continuity of biodiversity, it is necessary to protect organisms in water resources against anthropogenic pollutants and to investigate their resistance (Bashir et al., 2020; Gadzała-Kopciuch et al., 2004). Possible pollutants in the aquatic environment affecting water quality parameters such as pH, turbidity and dissolved oxygen are the primary factors that affect the living standards of organisms (Austin, 1998). Cement, which is often used in the construction industry, poses a danger to aquatic organisms because it has the potential to increase the pH of water. In a study where cement increased the pH level above 10.5 it was reported that the mortality rate of rainbow trout (*Oncorhynchus mykiss*) individuals was around 60% (Er et al., 2019). In general, a pH value between 9 and 10 can be harmful to fish, while pH values above 10 can be lethal to the rest (European Inland Fisheries Advisory, 1969).

Physical and chemical changes in the aquatic environment cause measurable physiological changes in fish (Fazio et al., 2013). This is because fish are in contact with the aquatic environment, which significantly affects blood homeostasis. Biomarkers are defined as a change in biological response, ranging from molecular to cellular and from physiological responses to behavioral changes (Depledge et al., 1995). As an indicator of water quality, the hematological profile in fish has been recently evaluated as a rapid analysis (Akram et al., 2021; Minaz et al., 2022a). In particular, automatic hematological assay is a significant diagnostic alternative to conventional methods today (Fazio, 2019). Blood parameters provide useful information about various body processes and are of great importance in assessing the harmful effects of anthropogenic pollution on aquatic environments (Guerrera et al., 2021).

The effect of cement as an anthropogenic pollutant on aquatic organisms has been discussed in several studies (Adamu et al., 2008; Er et al., 2019; Er & Kayış, 2022). Unpublished case studies of cement-related mortalities in aquaculture facilities are known. In this context, the impact of cement on Nile Tilapia (*Oreochromis niloticus*), one of the most cultured species in the world, is very significant. The current study focuses on the toxic effects of pozzolanic cement (PC), which is frequently used due to its qualities of permanent durability, easy workability and high impermeability. In our study the hematological response of *O. niloticus* against the potential sub-lethal concentration at the end of the acute period (24 hours) was also investigated.

MATERIALS AND METHODS

The toxic material (Pozzolanic Cement)

In the present study, the toxic effect of a commercial pozzolanic cement (PC) was evaluated (CEM IV/B (P) 32,5 R, Oyak Çimento, Türkiye). Pozzolan materials do not have individual binding properties. However, they have high binding abilities in aqueous ambient with calcium hydroxide. The PC in the current study includes 65-89% natural clinker (30% clay and 70% limestone) and 11-35% pozzolan materials. Therefore, the potential toxic material in the available study has a content of Silicon dioxide (SiO_2), calcium oxide (CaO), aluminum oxide (Al_2O_3), and iron oxide (Fe_2O_3).

Experimental design, fish, and water quality

This study took place in the toxicology laboratory, Recep Tayyip Erdoğan University, Rize, Türkiye. Nile Tilapia fish (50.4±4.4 g) were purchased from the Aquaculture Application and Research Center. The system design was triplicate configured with a 50 L aquarium and ten fish in each aquarium. For adaptation, the fish were placed in the aquariums ten days before the study. The study was carried out using rested tap water and in accordance with a 12-hour dark/light photoperiod. Aeration was distributed to the aquariums with air stones from a central pump system. Since PC is not a water-soluble material, it tends to aggregate at the bottom of the aquarium. In order to prevent this accumulation and keep the water homogeneous, a mixing system that provides water circulation was placed at the bottom of the aquarium. The fish were not fed in the acute period in order to provide stable water quality. All experimental studies were checked and approved by the Ethical Local Committee of the Recep Tayyip Erdogan University (Decision No: 2021/14).

Water quality parameters were measured at the beginning of the study to ensure their suitability for the living standards of the fish. Temperature (°C), pH and dissolved oxygen (mg L^{-1}) were measured with a portable multi-parameter (Hach, HQ40D 58258-00). Accordingly, water quality parameters were recorded as 17.1±0.6 °C, 6.51±0.2 and 7.65±1.4 mg L^{-1} for temperature, pH and dissolved oxygen, respectively.

Acute toxicity

Within the scope of the current study, the lethal concentration that killed 50% of the target population was determined. In this context, the fish were exposed to six different PC concentrations (100 mg L^{-1}, 200 mg L^{-1}, 300 mg L^{-1}, 400 mg L^{-1}, 500 mg L^{-1} and 600 mg L^{-1}) for 24 hours (Tablo 1). No PC was added to the control group but all other manipulative conditions were the same as for the PC groups. LC_{10}, LC_{50} and LC_{90} for 24 h values were determined as 255 mg L^{-1}, 306 mg L^{-1}, and 339 mg L^{-1} respectively.

Hematological analysis

Fish blood indicators were analyzed according to the automatic hematological assay method (Minaz et al., 2022a). Before the hematological analysis, five fish were randomly chosen from each group and transferred to an anesthesia tank that contained 60 mg L^{-1} clove oil. Afterward, 2.5 ml syringes were used to take blood samples from the caudal vein of the fish. Blood samples (0.3-0.5 mL) were fastish transferred to EDTA K3 tubes for the prevention of congelation. Finally, the following indicators were investigated using automatic hematological assay (Prokan-6800VET): leukocyte (WBC), erythrocyte (RBC), lymphocyte (LYM), monocyte (MID), granulocyte (GRAN), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The blood counter device was calibrated with blood from healthy fish prior to the study.

Statistical analyses

In the current study, all data were shown in mean ± standard deviation. The Kolmogorov-Smirnov test was used to determine normality distribution in the data set. According to the normality analysis, it was observed that all data sets showed normal distribution. Therefore, parametric tests were applied to determine statistical differences between the groups. Significant differences among groups were evaluated by a one-way ANOVA test. Also, the Tukey test was used to detect the differences in the groups. The Pearson Correlation test was performed to examine the statistical relationship between PC concentration, mortality rate and pH values. At the end of acute period, the Probit test was used to detect the LC_{50} value. P-values of less than 0.05 were considered statistically significant. All datasets were analyzed by SPSS 25 software package for Windows.

RESULTS AND DISCUSSION

Cement is the basic building block of many reinforced concrete structures in aquatic environments. When construction plans using cement are made to increase human welfare in an aquatic environment, most researchers typically focus on the fact that construction areas restrict the vital needs of organisms in the ecosystem, such as nutrition, reproduction and migration (Kocabaş et

Table 1. Lethal concentration of PC in *O. niloticus*.

Concentration ($mg\ L^{-1}$)	Total fish (n)	Number of dead fish (mortality%)
100	30	0 (0%)
200	30	0 (0%)
300	30	12 (40%)
400	30	27 (90%)
500	30	30 (100%)
600	30	30 (100%)
LC_{10} ($mg\ L^{-1}$)		255.08
LC_{50} ($mg\ L^{-1}$)		306.66
LC_{90} ($mg\ L^{-1}$)		339.40

Table 2. Correlation table between PC concentration, mortality rate, and pH.

		Concentration	Mortality Rate	pH
Concentration	Pearson Correlation	1		
	Sig. (2-tailed)			
Mortality Rate	Pearson Correlation	0.938^{**}	1	
	Sig. (2-tailed)	0.002		
pH	Pearson Correlation	0.825^{*}	0.759^{*}	1
	Sig. (2-tailed)	0.022	0.045	

* Correlation is significant at the 0.05 level (2-tailed);
** Correlation is significant at the 0.01 level (2-tailed)

al., 2013). Although many studies have been conducted on the effects of reinforced concrete structures on aquatic environments, the effect of cement on organisms has been ignored. Therefore, the current study focused on the effect of possible cement leakage from reinforced concrete structures on *O. niloticus* at the end of acute period.

Relationship between mortality rate and pH

The relationship between the mortality rate and pH are shown in Figure 1. Accordingly, while no death was observed up to 200 mg L^{-1} PC concentration at the end of 24 hours, death was observed in 40% at 300 mg L^{-1} PC, 90% at 400 mg L^{-1} PC, and in all individuals at 500 and 600 mg L^{-1}. Additionally, higher pH levels (pH level of up to almost 11) were observed with increasing PC concentration, while the pH was around 6.5 in the control group. At the LC_{50} level (306 mg L^{-1}), the pH level was estimated to be around 10.18. There was a significantly positive relationship between PC concentration, mortality rate and pH (Table 2, $p<0.05$).

Cement content tends to increase the pH level of water (Lee & Hur, 2005). However, studies have shown that this increase in pH level disappears within 24 hours in static water and returns to normal levels (Law et al., 2013). Therefore, a 24-hour LC_{50} value was determined in the current study. In a study conducted to determine the toxic effects of portland cement on tilapia (7.2 g), the LC_{50} value was found as 41.21 mg L^{-1} (Adamu et al., 2008). In the current study, this value was calculated as 307 mg L^{-1}. The difference between these two studies involving the same species may be due to the type of cement or the difference in fish size (34.8 g). In another study, the 96-hour LC_{50} value in rainbow trout exposed to different concentrations of PC was 440 mg L^{-1} (Kurtoglu et al., 2016). The reason for the high 96-hour LC_{50} value in the previous study may be related to the fact that the temperature was lower than in our study and more suitable for rainbow trout. It would be expected that the 24-hour LC_{50} value for rainbow trout, which is a more sensitive fish than tilapia, would be much higher.

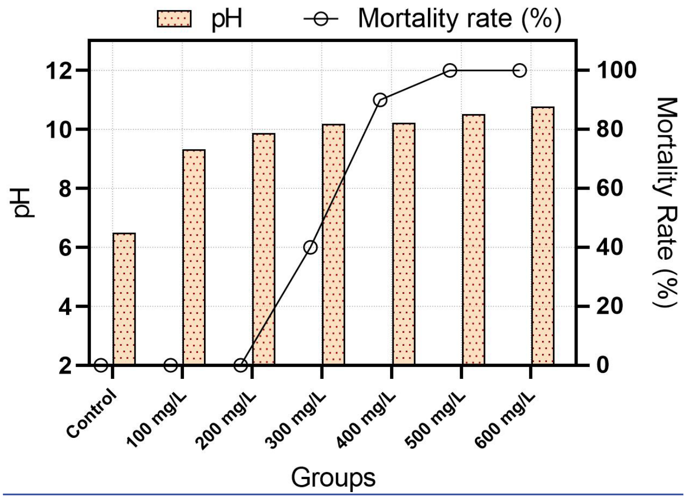

Figure 1. Mortality rate and pH depending on PC concentration.

In an aquatic ecosystem, pH is vital to organisms and can affect the solubility and toxicity of chemicals in the water. Although most aquatic organisms live in the pH range of 6.5-9, there may also be organisms living outside this range. However, high or low pH levels have negative effects on growth and reproduction. Cement tends to increase pH levels, resulting in toxicity to aquatic organisms. For instance, the increase in pH level caused a decrease in the EC_{50} (median-effective concentration) value for *Daphnia magna* (Rodrigues et al., 2020). In Atlantic salmon eggs, a low pH level (pH 2.78) caused the death of 98% of all individuals after 18 hours, while a high pH level (12.23) caused all individuals to die within 15 minutes (Foldvik et al., 2022). The toxicity ability of high pH level is much more effective. In the current study, the pH level showed a positive relationship with increasing concentration in the first 24 hours. However, pH levels in all groups returned to levels similar to the control group after 24 hours. In our previous study, a different group was designed whose pH level was reduced with HCL to reveal the toxic driving force of cement (Er & Kayış, 2022). At the end of the study, it was observed that there was no death in the group in which the pH level was manipulated. Therefore, it has been proven that the main driving force in cement toxicity studies is pH. The lethal effect of high pH level on organisms will contribute to the management of the contamination process both in nature and in aquaculture ambients. However, today's common belief is that in cases of cement-related contamination, losses should be determined by sampling fish rather than the characteristics of the water. Additionally, there is a deficiency regarding the time period for providing water samples.

Hematological evaluation

For hematological studies, 100 mg L^{-1} PC (T100) and 200 mg L^{-1} PC (T200) concentration groups, which were 1/3 and 2/3 times the LC_{50} value and in which all fish survived, were evaluated (Figure 2). Accordingly, significant differences were observed between groups for all hematological parameters except MCV. Significant decreases were observed between all groups in WBC values depending on increasing concentration ($p<0.01$). PC groups showed significantly higher RBC, HGB, and HCT values compared to the control ($p<0.01$). For LYM value, the T200 group was significantly lower than the other groups ($p<0.05$). Finally, significantly lower values were observed in the PC groups for MID, GRAN, MCH, and MCHC values ($p<0.01$).

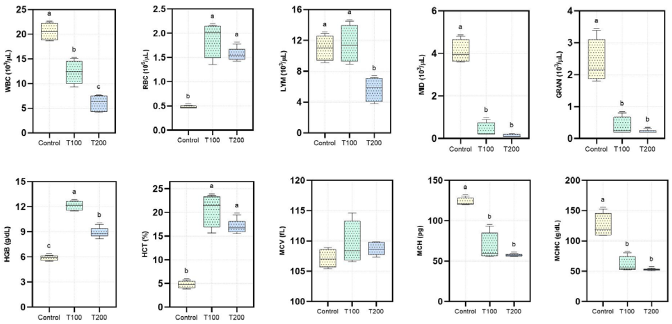

Figure 2. Hematological parameters of *O. niloticus* exposed to different PC concentrations.

One of the most important and rapid indicators for aquatic organisms encountering any toxic pollutant is hematological outputs (Ak et al., 2023; Fazio et al., 2017). This is because blood represents fish health depending on exposure to environmental stressors (Gabriel et al., 2011; Minaz et al., 2022a). Blood parameters may differ from normal levels in aquatic organisms during exposure to pollutants, transportation and anesthesia (Minaz et al., 2022b). In the current study, significantly decreased WBC values were noted in the increasing PC groups. WBCs are circulatory cells that assist in physiological responses and constitute different cell types such as lymphocytes and monocytes. Therefore, like WBC, lower MID and LYM in PC groups is an expected phenomenon. Although previous studies have rarely shown a tendency for WBC to increase in response to stressors, WBC tends to decrease in fish against many stressors (Esmaeili, 2021). Under stress conditions, the immune system is affected positively or negatively by many neuroendocrine elements (Khansari et al., 1990). The decrease in WBC, LYM and MID may be directly related to the decrease in cell production of the hematopoietic organs or in total leukocytes of the immune defense (Alam et al., 2021). It has previously been reported that exposure to herbicides as toxic substances reduces WBC values for the same fish species (Abdel-Warith et al., 2021). In general, exposure to toxic substances results in decreased leukocyte functions, subsequent inhibition of lymphocyte responses, apoptosis and cell death (Guzzi et al., 2012). RBC absorbs oxygen in the gills and carries it to the tissues. RBC in fish varies between 0.4×10^6 mm^{-3} and 5.2×10^6 mm^{-3} (Esmaeili, 2021). In the current study, this value remained within the general range. The immune system of fish may increase RBC under stress or due to infection. This is a defense mechanism to fight infection or toxic substances and it speeds up recovery. Like WBC, HGB is at higher levels in PC-exposed groups. The increase of HGB and HCT in fish may occur in order to increase the body's oxygen carrying capacity and respond to oxygen needs. However, this increase may vary under a particular fish species or in certain environmental conditions.

CONCLUSION

The release of cement, the basic building block of reinforced concrete structures, into the aquatic ecosystem has become an inevitable issue. Cement effluent entering the aquatic environment as a result of many developments such as agricultural activities and energy systems poses a danger to both the environmental environment and all organisms. In this context, the current study revealed the effects of pozzolanic cement exposure on Nile tilapia (*Oreochromis niloticus*). PC, which has a vital importance in the aquatic ecosystem due to its tendency to increase the pH level, had a toxic effect on *O. niloticus*. It also negatively affected the hematological system of the fish and created differences in blood parameters as a stress response. This study revealed the lethal and physiological effects of PC exposure during the acute period. Case studies are important to raise awareness by showing how the release of toxic PC material into natural waters affects the aquatic environment around the world. Future studies should focus on improving PC with supporting materials that do not increase the pH of water. In addition, using the current study as a reference, awareness should be raised for the construction industry and the use of environmentally friendly materials should be encouraged.

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Data Availability: All datasets used during the current study are available from the corresponding author on reasonable request.

REFERENCES

Abdel-Warith, A. W. A., Younis, E. M., Al-Asgah, N. A., Gewaily, M. S., El-Tonoby, S. M., & Dawood, M. A. O. (2021). Role of fucoidan on the growth behavior and blood metabolites and toxic effects of atrazine in nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Animals*, *11*(5), 1448. https://doi.org/10.3390/ANI11051448/S1

Adamu, K., Audu, B., & Audu, E. (2008). Toxicity and Histopathological Effects of Portland Cement Powder in Solution on the Structure of the Gill and Liver Tissues of the Nile Tilapia, *Oreochromis niloticus*. *Tropical Freshwater Biology*, *17*(1), 24–35. https://doi.org/10.4314/TFB.V17I1.20915

Ak, K., Er, A., & Minaz, M. (2023). Kültür Kökenli Karaca Mersin Balığı (*Acipenser gueldenstaedtii)*'nın Hematolojik Parametrelerinin Referans Değerlerinin Belirlenmesi. *Journal of Anatolian Environmental and Animal Sciences*, *8*(1), 88–94. https://doi.org/10.35229/JAES.1231567

Akram, R., Iqbal, R., Hussain, R., & Ali, M. (2021). Effects of bisphenol a on hematological, serum biochemical, and histopathological biomarkers in bighead carp (*Aristichthys nobilis*) under long-term exposure. *Environmental Science and Pollution Research 2021*, *1*, 1–16. https://doi.org/10.1007/S11356-021-17329-1

Alam, R. T. M., Abu Zeid, E. H., Khalifa, B. A., Arisha, A. H., & Reda, R. M. (2021). Dietary exposure to methyl mercury chloride induces alterations in hematology, biochemical parameters, and mRNA expression of antioxidant enzymes and metallothionein in Nile tilapia. *Environmental Science and Pollution Research*, *28*(24), 31391–31402. https://doi.org/10.1007/S11356-021-13014-5/FIGURES/2

Austin, B. (1998). The effects of pollution on fish health. *Journal of Applied Microbiology*, *85*(S1), 234S-242S. https://doi.org/10.1111/J.1365-2672.1998.TB05303.X

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*, 1–26. https://doi.org/10.1007/978-3-030-35691-0_1/TABLES/4

Depledge, M. H., Aagaard, A., & Györkös, P. (1995). Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. *Marine Pollution Bulletin*, *31*(1–3), 19–27. https://doi.org/10.1016/0025-326X(95)00006-9

Er, A., & Kayış, Ş. (2022). Acute Toxicity of Pozzolanic Cement on Two Crustacean Species, Water Flea (*Daphnia magna*) and *Gammarus komareki*. *Bulletin of Environmental Contamination and Toxicology*, *108*(2), 309–314. https://doi.org/10.1007/S00128-021-03345-X/FIGURES/5

Er, A., Oztek, O., & Kayİs, S. (2019). Effects of concrete additives on vital tissues of rainbow trout (*Oncorhynchus mykiss*): a histological examination. *Fresenius Environmental Bulletin*, *28*(5), 3829–3834.

Esmaeili, M. (2021). Blood Performance: A New Formula for Fish Growth and Health. *Biology 2021, Vol. 10, Page 1236*, *10*(12), 1236. https://doi.org/10.3390/BIOLOGY10121236

European Inland Fisheries Advisory, C. (1969). Water quality criteria for european freshwater fish—extreme pH values and inland fisheries. *Water Research*, *3*(8), 593–611. https://doi.org/10.1016/0043-1354(69)90048-7

Farfan, J., Fasihi, M., & Breyer, C. (2019). Trends in the global cement industry and opportunities for long-term sustainable CCU potential for Power-to-X. *Journal of Cleaner Production*, *217*, 821–835. https://doi.org/10.1016/J.JCLEPRO.2019.01.226

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, *500*, 237–242. https://doi.org/10.1016/J.AQUACULTURE.2018.10.030

Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M., & Faggio, C. (2013). Haematological and serum protein profiles of Mugil cephalus: Effect of two different habitats. *Ichthyological Research*, *60*(1), 36–42. https://doi.org/10.1007/S10228-012-0303-1/TABLES/4

Fazio, F., Saoca, C., Vazzana, I., & Piccione, G. (2017). Influence of Body Size on Blood Hemogram in Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792). *Veterinary Medicine*, *2*(3), 91–94. https://doi.org/10.17140/VMOJ-2-121

Foldvik, A., Holthe, E., Bremset, G., & Solem, Ø. (2022). Effects of Episodic Exposure to High-pH Water on Survival of Atlantic Salmon Eggs and Juveniles: Results from Laboratory and Field Studies. *Environmental Toxicology and Chemistry*, *41*(3), 771–780. https://doi.org/10.1002/ETC.5282

Gabriel, U. U., Akinrotimi, O. A., & Eseimokumo, F. (2011). Haematological Responses of Wild Nile Tilapia *Oreochromis niloticus* after Acclimation to Captivity. *Jordan Journal of Biological Sciences*, *4*(4), 225–230.

Gadzała-Kopciuch, R., Berecka, B., Bartoszewicz, J., & Buszewski, B. (2004). Some Considerations About Bioindicators inEnvironmental Monitoring. *Polish Journal of Environmental Studies*, *13*(5), 453–462. http://www.pjoes.com/Some-Considerations-About-Bioindicators-in-r-nEnvironmental-Monitoring,87684,0,2.html

Guerrera, M. C., Aragona, M., Porcino, C., Fazio, F., Laurà, R., Levanti, M., Montalbano, G., Germanà, G., Abbate, F., & Germanà, A. (2021). Micro and Nano Plastics Distribution in Fish as Model Organisms: Histopathology, Blood Response and Bioaccumulation in Different Organs. *Applied Sciences 2021, Vol. 11, Page 5768*, *11*(13), 5768. https://doi.org/10.3390/APP11135768

Guzzi, G., Pigatto, P. D., Spadari, F., & La Porta, C. A. M. (2012). Effect of thimerosal, methylmercury, and mercuric chloride in Jurkat T cell line. *Interdisciplinary Toxicology*, *5*(3), 159–161. https://doi.org/10.2478/V10102-012-0026-1

Khansari, D. N., Murgo, A. J., & Faith, R. E. (1990). Effects of stress on the immune system. *Immunology Today*, *11*(C), 170–175. https://doi.org/10.1016/0167-5699(90)90069-L

Kocabaş, M., Başçınar, N., Kutluyer, F., & Aksu, Ö. (2013). HES'ler ve Balıklar. *Türk Bilimsel Derlemeler Dergisi*, *1*, 128–131. https://dergipark.org.tr/tr/pub/derleme/issue/35088/389200

Kurtoglu, İ. Z., Kayis, S., Ak, K., Gencoglu, S., Duzgun, A., & Er, A. (2016). Histopathology of rainbow trout (*Oncorhynchus mykiss*) and sturgeon (*Acipenser baerii*) exposed to sublethal concentrations of cement. *Fresenius Environmental Bulletin*, *25*(9), 3523–3527.

Law, D. W., Setunge, S., Adamson, R., & Dutton, L. (2013). Effect of leaching from freshly cast concrete on pH. *Magazine of Concrete Research*, *65*(15), 889–897. https://doi.org/10.1680/MACR.12.00169

Lee, J. Y., & Hur, J. W. (2005). Acute Toxicity of Cement on Mortality of Pond Smelt (*Hypomesus olidus*). *Korean Journal of Environmental Biology*, *23*(1), 89–92.

Minaz, M., Er, A., Ak, K., Nane, İ. D., İpek, Z. Z., Kurtoğlu, İ. Z., & Kayış, Ş. (2022a). Short-term Exposure to Bisphenol A (BPA) as a Plastic Precursor: Hematological and Behavioral Effects on *Oncorhynchus mykiss* and *Vimba vimba*. *Water, Air, & Soil Pollution*, *233*(122), 1–12. https://doi.org/10.1007/S11270-022-05585-X

Minaz, M., Er, A., Ak, K., Nane, I. D., Ipek, Z. Z., Yalcın, A., Kurtoglu, I. Z., & Kayis, S. (2022b). Investigation of long-term bisphenol A exposure on rainbow trout (*Oncorhynchus mykiss*): Hematological parameters, biochemical indicator, antioxidant activity, and histopathological examination. *Chemosphere*, *303*, 135136. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135136

Rodrigues, P., Silvestre, J. D., Flores-colen, I., Viegas, C. A., Ahmed, H. H., Kurda, R., & de Brito, J. (2020). Evaluation of the Ecotoxicological Potential of Fly Ash and Recycled Concrete Aggregates Use in Concrete. *Applied Sciences 2020, Vol. 10, Page 351*, *10*(1), 351. https://doi.org/10.3390/APP10010351

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Research Article

Investigation of Benthic Macroinvertebrate Fauna and Water Quality of Animal Drinking Water Troughs by Multivariate Statistical Methods: The Case of Şarkışla

Menekşe Taş Divrik[1]

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ABSTRACT

This study was conducted in 2022 to investigate the water quality and benthic macroinvertebrate fauna of water troughs in Şarkışla, a district in the city of Sivas. Fifteen stations were selected from the most used water troughs in the district and sampling was carried out in the spring, summer, and autumn seasons during peak agricultural activities. As a result, a total of 17 taxa were identified: 4 taxa belonging to Oligochaeta, 6 species belonging to Chironomidae, 2 taxa belonging to Amphipoda, and one each belonging to Gastropoda, Hemiptera, Coleoptera, Ostracoda and Trichoptera larvae. All taxa identified are new records for the study area. In addition to benthic sampling, some physicochemical parameters of water (water temperature, pH, electrical conductivity, dissolved oxygen, total hardness, calcium, magnesium, chloride, salinity, total dissolved solids, phosphate, sulphate, nitrate nitrogen, nitrite nitrogen) were also analyzed. The similarity of the stations in terms of physicochemical parameters was analyzed by utilizing the Bray-Curtis similarity analysis. Accordingly, stations 2 and 4 were found to be the most similar, by 96.91%, and stations 3 and 13 were found to be the most different, by 66.46%. In addition, the relationship between the investigated physicochemical parameters was analyzed using the Pearson and Spearman Correlation Analysis. The relationship between the species and physicochemical parameters was revealed using the CCA Analysis.

Keywords: Water troughs, benthic macroinvertebrates, physicochemical parameters, statistical methods

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INTRODUCTION

Water troughs are artificially transformed examples of natural water sources. Water troughs are found all over Turkey. Sometimes they are located in villages and sometimes they are found on the roadsides. They were first built to meet the water needs of animals. They can also be used to control water flow, agricultural irrigation, drinking water, and groundwater (Külköylüoğlu et al., 2013; TODAİE, 2011). Water is vital for all living creatures on this planet. In animals, body temperature regulation, nutrient uptake, and waste removal occur with water. Water troughs are artificial water structures created to meet the water needs of animals. They are open-water storage structures of various sizes to accumulate and hold water for animal consumption (Denktaş, 2000; Tay, 2011). These structures are an important source of water for domestic cattle and sheep in cultivated areas and also for wild animals such as wolves, deer, rabbits, birds, etc. in rural and wild areas (Özer, 2010). These water structures, especially in rural areas, are very important to meet the water needs of animals while grazing. Depending on the number of animals relying on the water trough, they can be of different sizes. Water troughs are sometimes located in forested areas, sometimes on higher parts of the landscape, and sometimes on roadsides. The water in these structures is open to contamination. Especially in the summer months algae layers can form on the inside of the water troughs, due to increased weather temperature and stagnation of the water body.

Benthic macroinvertebrates are often used as indicators of the biological status of water bodies. They are reliable indicators as they spend all or most of their life in water. They are easy to collect due to their high tolerance of pollution (Kazancı et al., 1997). Benthic macroinvertebrates are usually found attached to rocks and vegetation, or they are buried in the sand and sediments at the bottom of the water trough.

To date, studies conducted on water troughs belong to Özkan (2006a; 2006b), Külkoylüoğlu ve ark. (2012), Külköylüoğlu et al. (2013), Başak et al., (2013); Külköylüoğlu et al., (2017); Özer & Dikmen (2021); Ataman et al., 2023; Sanbur (2023).

This study aims to determine the water quality and benthic macroinvertebrate fauna of water troughs in the district of Şarkışla using multivariate statistical analyses.

MATERIALS AND METHODS

Study area

Şarkışla is located in the southwest of Sivas province, within the borders of the Upper Kızılırmak region of central Anatolia. Its surface area is 2250 km wide and is at an altitude of 1180 m above sea level.

Şarkışla is the 7th largest district in terms of surface area among the 17 districts of Sivas (https://sarkisla.gov.tr). The economy of Şarkışla is based on agriculture and animal husbandry. The district offers a rich variety of agricultural products due to the presence of large agricultural land and agricultural basins. Field crops include cereals, fodder crops, industrial crops, and edible legumes. Sugar beet and potatoes are among the most cultivated crops. The district creates a suitable environment for animal husbandry with its climate and the presence of pasture areas. Cattle and ovine breeding is common in the district (Oran Kalkınma Ajansı, 2017). Animal husbandry is intensively practiced in the region where this research was conducted. Natural water troughs are made of concrete, metal or wood and are generally rectangular in shape. Water troughs exist for the benefit of both humans and animals.

The coordinates of the selected sampling stations and information about the water troughs are detailed in Table 1.

Sampling

The study was carried out at 15 stations in the spring, summer and autumn of 2022, during peak agricultural activity. The stations could not be reached in winter due to bad weather conditions. For this reason, sampling could not be performed during the winter season. pH levels, total dissolved solids, and electrical conductivity of the water samples were measured and recorded during the field study. To analyze other physicochemical parameters, water samples were taken in dark-coloured glass bottles of 1 liter. At the same time as taking water samples, benthos samples were also collected from the bottom part of the water troughs using hand mud scoops of different sizes. The samples were stored and preserved with 70% ethyl alcohol.

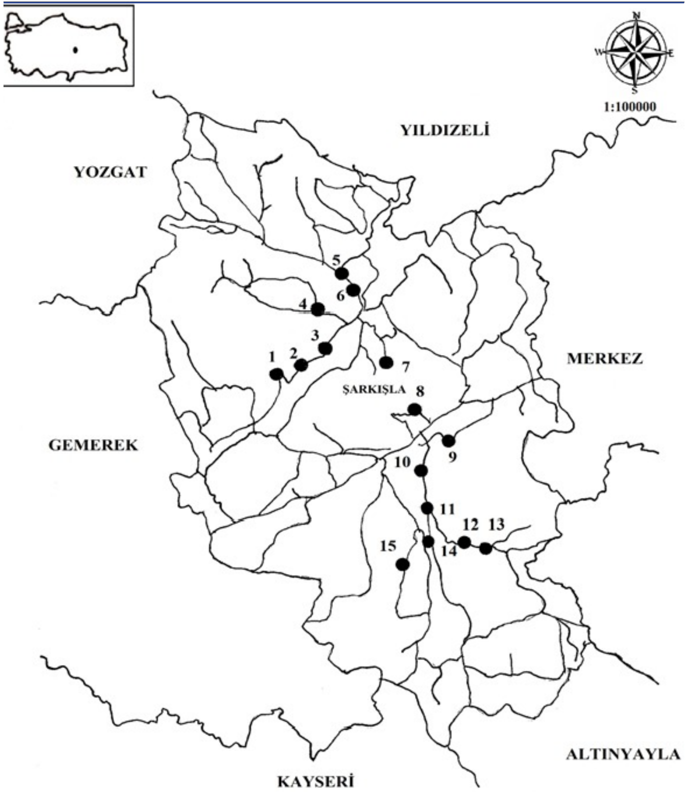

Figure 1. Study area and sampling locations.

Figure 2. Two-partition concrete water trough.

The physicochemical parameters of the water were measured using various titrimetric and spectrophotometric methods (Egemen & Sunlu, 1999).

The benthos samples brought to the laboratory were separated from the debris and placed in 70% ethyl alcohol for species iden-

Table 1. Geographical coordinates and information about the water troughs sampled.

Though Number	Stations	Coordinates	Information about the water troughs
1	Çiçekliyurt	39°26'00"N 36°17'58"E	2- partitions concrete water trough
2	Alaçayır	39°27'08"N 36°19'48"E	3- partitions metal water trough
3	İlyashacı Village	39°27'46"N 36°19'58"E	2- partitions concrete water trough
4	Emlakkaracaören	39°29'24"N 36°2128"E	Karacaören Meryemin Ahmet Fatma Cemal Koçtürk Hayratı, 4- partitions concrete water trough
5	Akdağmadeni Şarkışla roadside water trough	39°25'50"N 36°24'19"E	2- partitions concrete water trough, surrounded by agricultural land
6	Belen Tepesi roadside water trough	39°25'04"N 36°24'01"E	Muhlis Doğan Hayratı, 3- partitions concrete water trough
7	Cemel Altınyayla road	39°19'12"N 36°30'11"E	5- partitions concrete water trough, surrounded by agricultural land
8	Elmalı Village entrance	39°26'00"N 36°17'58"E	3- partitions concrete water trough, surrounded by agricultural land
9	Maksutlu Village	39°21'38"N 36°27'40" E	Kalembey Oğullarından Kaya Kızı Nalan Polat Hayratı, 2- partitions concrete water trough
10	Üyük (Hüyük) Village	39°22'21"N 36°17'58"E	5- partitions concrete water trough
11	Döllük Village	39°20′80″N 36°27′89″E	2- partitions concrete water trough
12	Cemel roadside water trough	39°18'16"N 36°27'36"E	Asım oğlu Ahmet Demirci ve Cemel Belediyesi Hayratı, 4- partitions concrete water trough, surrounded by agricultural land
13	Cemel Village road	39°20'09"N 36°26'20"E	4- partitions concrete water trough. In addition, daily agricultural labourers live in the surrounding area.
14	Hamlar	39°17'55"N 36°27'24"E	4- partitions concrete water trough, surrounded by agricultural land. Wheat, barley, chickpea, and sunflower are among the most cultivated crops.
15	Aşılık (Osmanpınarı)	39°16'08"N 36°27'48"E	4- partitions concrete water trough, surrounded by agricultural land. Wheat and barley are among the most cultivated crops.

tification. The collected benthos samples were identified to the smallest taxonomic group possible. Kathman & Brinkhurst (1998), Milligan & Michael (1997) and Timm (1999) were consulted the for identification of Oligochaeta. Oliver et al., (1978), Saether (1980), Cranston (1982), Pinder & Reiss (1983), and Fittakau & Roback (1983) were consulted for the identification of Chironomidae. For the identification of Amphipoda, the help of subject experts was obtained.

Statistical analyses

All obtained results were transformed by LogBase10 in Microsoft Office Excel 2010 and SPSS 9.0 for Windows to use statistical techniques (Krebs, 1999). The relationship between the physicochemical parameters was evaluated by applying the Spearman and Pearson Correlation Analysis (Krebs, 1999). The similarity of physicochemical parameters in terms of stations was analyzed by Bray-Curtis Cluster analysis using BioDi-versity Pro 2.0 (McAleeceet al., 1997). The statistical relationship between the benthic macroinvertebrates and environmental parameters Canonical Correspondence Analysis (CCA) was determined using the Past Version 3.14. (Hammer et al., 2001).

RESULTS AND DISCUSSION

A total 17 taxa were identified in this study. These included four taxa belonging to Oligochaeta, six to Chironomidae, two to Amphipoda, and one each to Gastropoda, Hemiptera, Coleoptera, Ostracoda and Trichoptera larvae. All taxa identified are new records for the study area.

In the study, the habitat and station information on taxa as well as information about seasons is as follows. The highest number of taxa was found in the spring season (14 taxa), followed by the summer (10 taxa) and finally the autumn (7 taxa). *Nais elinguis* are abundant in organically polluted waters. They can be found in fast-flowing rivers as well as in muddy, foul-smelling, poorly oxygenated, and cold waters under wide environmental conditions (Timm, 2003). In the study, *Nais elinguis* were found in the spring season at stations 4 and 6. *Nais simplex* is an almost cosmopolitan species, found in fresh waters (Timm et al., 2002). *N. simplex* was found in water samples collected from soft muddy ground. *N. simplex* was found only at station 11 in the spring and summer seasons. *Chaetogaster diaphanus* is found among plants in brackish and fresh waters and in rivers with generally clear water. It is a predatory species that eats small invertebrates such as Oligochaetae, Chironomidae larvae, Cladocera and Copepoda (Timm, 2003). *C. diaphanus* was found in the spring season at station 11. Timm (2013) reported that species belonging to the genus *Potamothrix* sp. are present in freshwater, brackish water and euryhaline forms. *Potamothrix* sp. was found in the spring season at station 13.

Chironomus riparius is a common species and usually lives in eutrophic conditions (Thienemann, 1954). *C. riparius* was found at stations 2, 6, 9, 11, 12 in the spring season (Table 2). It was found at stations 1, 8, 13, 14 in the summer season, and at station 2 in the autumn season (Table 2). The most common species encountered during sampling was *Chironomus anthracinus* (Table 2). This species is the most tolerant species of the Chironomidae family and is very common (Taşdemir et al., 2009). In this study, *C. anthracinus* was found in all three seasons and in all stations. *C. anthracinus* was found at stations 3, 5, 6, 8, 9, 10, 11, 12, 14 and 15 in the spring season (Table 2); at stations 1, 2, 4, 7, 8, 9, 12, 13, 14 and 15 in the summer season (Table 2); and at stations 2, 3, 4, 5, 10, 12 and 13 in the autumn season (Table 2). *Chironomus viridicollis* lives in the muddy parts of stagnant waters (Özkan 2006a). In the study, it was detected at stations 8 and 9 in the spring season and at station 8 in the summer season (Table 2). Taşdemir et al., (2010) reported that *Cricotopus intersectus* was found in stagnant waters. In this study, *C. intersectus* were found at stations 1, 3, 5, 9, 10, 11, 12 in the spring season; at stations 1, 2, 3, 5, 9, 10, 11, 14 in the summer season, and at station 8 in the autumn season (Table 2). Özkan (2007) reported that *Paracladius conversus* is seen on mud-aquatic stream plants. *P. conversus* was found at station 3 in the spring and at station 7 in the summer seasons. Özkan (2007), reported that *Tanytarsus gregarilus* is found in mud in stagnant waters, and found in sandy, muddy, and stony ground in streams. *T. gregarilus* was found at stations 3, 6, 9, 13, 14, 15 in the spring season (Table 2). In the summer season, it was found at stations 1, 7, 8, 14, 15 (Table 2).

Gastropods are more abundant in spring and summer. Their tolerance to very hard and salty waters is quite high (Robert & Diilon, 1999). Gastropoda individuals were found at station 8 in the spring; at station 15 in the summer; and at stations 12 and 13 in the autumn (Table 2). *Niphargus* sp. is reported to be the largest of all freshwater amphipod genera (Väinölä et al., 2008). Usually, these individuals inhabit caves or groundwater (Karaman & Ruffo, 1986; Sket, 1999). *Niphargus* sp. was found at station 1 in the autumn season. *Gammarus pseudocsyritia* has a wide tolerance range to temperature (usually 5–21 °C), so it was reported that this adaptation to different water temperatures is the main reason for its wide presence (Zamanpoore et al., (2011). *G. psedosyriacus* was found at station 5 in the the spring season (Table 2). Hemipterans are found in lentic habitats or in backwater or pool areas of streams (http://lakes.chebucto.org/). Hemiptera was found at station 12 in the autumn season. Coleopterans larvae are organisms sensitive to pollution. They can be found in moderately polluted waters (Haldar et al., 2016). Coleoptera was found at station 5 in the spring season (Table 2).

Ostracods live in all kinds of aquatic environments including the base of oceans, in rivers, in lakes and even in swamps. Some ostracods live in temporary water bodies and can survive when the pond dries up in summer (www.bgs.ac.uk). Ostracoda was found

Table 2. Benthic macroinvertebrate taxa identified from the water troughs and their seasonal distribution according to stations.

Taxonomic Groups /Seasons	Spring Season	Summer Season	Autumn Season
Oligochaeta			
Nais elinguis Müller, 1773	4,6	-	-
Nais simplex Piguet, 1906	11	11	-
Chaetogaster diaphanus (Gruithuisen, 1828)	11	-	-
Potamothrix sp.	13	-	-
Chironomidae			
Chironomus riparius Meigen, 1804	2,6,9,11,12	1,8,13,14	2
Chironomus anthracinus Zetterstedt, 1860	3,5,6,8,9,10,11,12,14,15	1,2,4,7,8,9,12,13,14,15	2,3,4,5,10,12,13
Chironomus viridicollis Wulp, 1877	2,9	8	-
Cricotopus intersectus (Staeger,1839)	1,3,5,9,10,11,12	1,2,3,5,9,10,11,14	8
Paracladius conversus (Walker, 1856)	3	7	-
Tanytarsus gregarilus Kieffer, 1909	3,6,9,13,14,15	1,7,8,14,15	-
Gastropoda	8	15	12,13
Amphipoda			
Niphargus sp.	-	-	1
Gammarus psedosyriacus Karaman&Pinkster, 1977	5	-	-
Hemiptera	-	-	12
Coleoptera	5	-	-
Ostracoda	-	7,10	3,7,12,13
Trichoptera larvae	3,8	7	-
Total number of taxa	14	10	7

at stations 7 and 10 in the summer season (Table 2). It was found at stations 3, 7, 12 and 13 in the autumn season (Table 2). Trichopterans are indicator species of clean waters. They live in all kinds of aquatic environments, including rivers, lakes, ponds, and swamps (Haldar et al., 2016). Trichoptera larvae were found at stations 3 and 8 in the spring and at station 7 in the summer (Table 2). The benthos samples from the water troughs and the detected species are given in Table 2.

During the study period, water temperature values were measured between 6 °C (spring season, stations 3, 7, 10, 12, 13) and 16.5 °C (summer season, station 13) (Table 3). pH values were found to vary between 7.23 (spring season, station 13) and 8.67 (autumn season, station 11) (Table 3). Electrical conductivity values were found to vary between 359 µS/cm (autumn season, station 7) and 994 µS/cm (spring season, stations 8 and 13) (Table 3). During the study period, dissolved oxygen value was measured between 1.52 mg/L (autumn season, station 12) and 6.66 mg/L (spring season, stations 5 and 15) (Table 3). TH values were recorded between 18 FS° (spring season, station 15) and 37 FS° (spring season, station 2). Ca values were found to vary between 5.20 mg/L (autumn season, station 11) and 123.9 mg/L (summer season, station 5) (Table 3). Mg values were found to vary between 1.22 mg/L (spring season, station 12) and 34 mg/L (summer season, station 13) (Table 3). Cl values were found to vary between 0.99 mg/L (summer season, stations 2 and 3) and 53.9 mg/L (summer season, station 10) during the study period (Table 3). Salinity values were measured between 0.008 ‰ (autumn season, 2nd, 3rd, 6th stations) and 0.09 ‰ (spring, 10th station) (Table 3). TDS values were found to vary between 140 ppm (spring, 7th and 10th stations) and 486 ppm (spring season, 8th station) (Table 3). PO_4 values varied between 0.0005 mg/L (autumn season, station 1) and 0.87 mg/L (autumn season, station 11) (Table 3). It was found that the measured SO_4 values varied between 0.12 mg/L (autumn, station 1) and 5.34 mg/L (autumn season, station 8) (Table 3). NO_3-N values varied between 5.83 (summer season, 11th station) and 54.5 mg/L (summer season, 13th station) (Table 3). The NO_2-N values measured in the study varied between 0.002 mg/L (spring season, 11th station) and 0.65 mg/L (summer season, 4th station). (Table 3). Some physicochemical data measured from the water troughs according to seasonal average values of the stations are presented in Table 3.

According to TSWQR (2016), 1st class refers to high-quality or "very good" water. This quality of water can be used for animal production and farm needs. When the results obtained are evaluated, it is determined that all stations are of 1st class quality in terms of pH, sulfate and water temperature (SKKY, 2004). In terms of electrical conductivity, the stations are between 1st and 2nd class water quality (TSWQR, 2016). In terms of NO_3-N, it was determined that stations 2, 5, 8, 9, 10, 11 and 12, were of 3rd class water quality and the other stations were of 4th class water quality. The high NO_3-N values of the waters may be attributed to the presence of agricultural land around some stations. Leaving animal faeces in grazing areas can also increase the amount of nitrate in groundwater. In terms of dissolved oxygen, stations 3, 4 and 8 are in the 4th class water quality grade and the other stations are of between 2nd and 3rd class water quality. When the chloride values in the water samples were examined, it was found that stations 9 and 10 were between 1st and 2nd class water quality grades and the other stations were of 1st class water quality (TSWQR, 2016).

D.O., Ca, Salinity and NO_3-N values were found to be high in the spring season. W.T., pH, E.C., Mg, Cl, PO_4, SO_4 were found in high amounts in the summer season (Table 4). As the air temperature increases in the summer, the water temperature also increases. The heated water dissolves the rock layers through which it passes and causes both pH and E.C. values to be higher. It was determined that T.H., TDS, NO_2-N values were high in the autumn season. Average values according to seasons and water quality parameters are given in Table 4.

When the average physicochemical values of the sampling stations are compared using the Bray-Curtis similarity index it is found that stations 2 and 4 are the most similar stations (96.91%), followed by station 9 (96.72%). Stations 3 and 13 are the least similar stations (66.46%) (Figure 3). Stations 2 and 4 are the most similar stations. It is very likely that the water coming to these stations comes from the same source. The elevation of the 3rd station is higher than that of the 13th station. The difference in elevation may impact water properties depending on the rock layers the water is passing through.

The average physicochemical values across the three seasons are also analyzed using the Bray-Curtis similarity index. Accordingly, spring and summer seasons were 95.90% similar, followed by the autumn season with 95.89% similarity to both spring and summer. Summer and autumn seasons were found to be the least similar seasons with a rate of 95.82% (Figure 4). It was determined that the similarity is very high across all three seasons.

The taxa and physicochemical values were also analyzed utilizing the CCA Analysis. The eigenvalues of the first two axes were calculated as 0.486 and 0.388, respectively. In the analysis, the two axes explain 40.59% of the variance of the species, 22.56% (Axis 1) and 18.03% (Axis 2). The distributions of *N. elinguis*, *Potamothrix* sp., and Gastropoda were affected by NO_2-N, NO_3-N, TDS, E.C., and salinity. Environmental variables do not affect the distributions of *C. intersectus*, *P. conversus*, *G. psedosyriacus*, Coleoptera, and Trichoptera. While W.T., Cl, PO_4, D.O., SO_4, and pH are effective in the distribution of *C. riparius*, *C. viridicollis*, *N. simplex*, *C. diaphanus*, *Niphargus* sp., Ostracoda, and Hemiptera; T.H., Mg, and Ca are not effective. The species *C. anthracinus* is affected by T.H., Mg, and Ca (Figure 5).

In addition, Pearson correlation analysis was applied to the normally distributed physicochemical values. Accordingly, a significant positive correlation was found between Ca and TH levels. Similarly, a significant negative correlation was found between pH and Ca levels. The relationship and correlation coefficients between the physicochemical parameters analyzed are given in Table 5.

Finally, the Spearman correlation analysis was applied to the values that did not show a normal distribution. Accordingly, a significant negative correlation was found between D.O. and NO_2-N; NO_2-N and $PO_{4;}$; W.T. and D.O. There is a significant positive correlation be-

Table 3. Physicochemical data measured at sampling stations in terms of seasons.

	Parameters	Unit	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th
Spring Season	W.T.	°C	7	7.5	6	6.5	7	7	6	7	6.5	6	7	6	6	6.5	6.5
	pH		7.85	7.70	7.89	7.73	7.64	7.69	7.72	7.68	7.63	7.78	7.83	7.55	7.23	7.40	7.79
	E.C.	µs/cm	398	652	366	645	521	394	362	994	455	701	381	398	994	655	408
	D.O	mg/L	4.76	4.18	3.80	3.42	6.66	4.32	5.71	2.85	4.76	5.90	5.52	5.71	5.33	6.09	6.66
	T.H	°F	20	37	19	32.8	27.6	21.6	20	35.6	27.8	27	18.6	31.2	35.2	28.4	18
	Ca	mg/L	48	59.3	38.4	83.36	96.19	73.74	68.13	102.6	84.16	83.36	50.55	76.15	103	74.54	48.08
	Mg	mg/L	6.83	5.42	4.73	12.29	16.68	12.68	11.7	16.29	13.7	11.36	7.75	1.22	16.48	11.22	6.3
	Cl	mg/L	6.99	6.99	2.99	25.99	2.99	1.99	3.99	8.99	27.99	3.99	4.99	23.99	19.99	21.99	7.99
	Salinity	‰	0.02	0.01	0.03	0.03	0.02	0.01	0.01	0.04	0.03	0.09	0.03	0.05	0.07	0.06	0.03
	TDS	ppm	183	321	181	316	248	173	140	486	250	245	140	249	247	249	152
	PO_4	mg/L	0.62	0.20	0.62	0.32	0.39	0.67	0.71	0.74	0.43	0.38	0.61	0.49	0.67	0.48	0.78
	SO_4	mg/L	0.08	0.59	0.20	1.38	1.10	0.26	0.48	4.80	1.16	0.99	0.12	1.72	3.20	1.63	0.34
	NO_3-N	mg/L	52.7	24.9	37.6	34.28	10.94	29.06	33.04	40.04	26.66	23.65	30.16	18.56	17.04	28.17	29.9
	NO_2-N	mg/L	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.003	0.01	0.002	0.02	0.01	0.02	0.01
Summer Season	Parameters	Unit	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th
	W.T.	°C	16	15.5	16	15	15	14.5	16	15	15.5	15	14.5	16	16.5	15	15.5
	pH		7.91	7.71	7.98	7.86	7.81	7.72	7.91	7.86	7.80	7.91	7.70	7.88	7.86	7.94	7.90
	E.C.	µs/cm	395	644	370	641	552	458	788	641	728	705	378	640	737	643	412
	D.O	mg/L	3.04	2.66	2.47	1.90	2.09	3.72	4.37	1.52	5.71	4.76	3.61	3.80	4.56	4.76	5.14
	T.H	°F	20	35.4	18.8	32	29.8	21.7	40	29	26.8	29	17.9	30.4	34	30	19
	Ca	mg/L	48.09	68.13	40.08	80.16	123.9	80.15	60.92	91.3	20.04	113.8	48.7	74.54	97.7	61.7	48.8
	Mg	mg/L	6.83	7.95	5.17	11.71	16.4	13.60	5.08	15.1	1.64	29	9.53	30.4	34	30	19
	Cl	mg/L	2.99	0.99	0.99	1.99	8.99	4.99	4.99	20.99	36.9	53.9	2.99	26.9	31.9	26.9	9.99
	Salinity	‰	0.008	0.04	0.02	0.05	0.03	0.009	0.01	0.05	0.02	0.04	0.03	0.02	0.02	0.04	0.02
	TDS	ppm	158	250	161	283	222	334	293	429	330	319	158	239	317	288	179
	PO_4	mg/L	0.65	0.18	0.64	0.37	0.41	0.69	0.70	0.73	0.55	0.50	0.87	0.50	0.68	0.53	0.79
	SO_4	mg/L	5.13	1.46	2.40	1.73	1.01	0.13	0.35	3.71	0.97	0.73	0.36	1.51	2.78	1.59	0.74
	NO_3-N	mg/L	32.79	21.79	33.04	35.9	13.24	26.52	30.8	13.7	14.1	6.08	5.82	12.4	54.5	26.4	39.8
	NO_2-N	mg/L	0.04	0.05	0.009	0.65	0.04	0.03	0.04	0.32	0.04	0.03	0.02	0.04	0.04	0.05	0.04

W.T: Water Temperature. E.C: Electrical Conductivity. D.O: Dissolved Oxygen. T.H: Total Hardness. TDS: Total Dissolved Solids

Table 3 (Continue). Physicochemical data measured at sampling stations according to seasons.

	Parameters	Unit	1st	2 nd	3 rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13 th	14 th	15 th
Autumn Season	W.T.	°C	8	8.5	8	9	9.5	9.5	8.5	10	10.5	10	10	11	11	10.5	11
	pH		7.85	7.70	7.82	7.75	7.74	7.52	7.61	7.79	7.76	7.80	8.67	7.62	7.66	7.80	7.70
	E.C.	µs/cm	404	612	365	567	509	416	359	886	737	581	395	639	656	630	442
	D.O	mg/L	2.66	2.47	2.28	1.71	2.09	3.23	3.04	3.80	2.47	2.85	3.42	1.52	1.90	2.28	2.66
	T.H	°F	26.2	38	22.4	37	30	26.8	24.4	35	31	24.4	20.6	30.6	32.6	32	24.4
	Ca	mg/L	57.7	41.68	44.08	72.14	89.77	86.57	72.14	97.79	53.70	62.52	5.20	80.16	84.96	65.73	57.71
	Mg	mg/L	7.66	0.89	5.27	8.54	14.53	14.53	12.68	15.27	5.52	9.90	8.70	12.05	12.84	8.54	6.25
	Cl	mg/L	7.99	8.99	7.99	2.99	8.99	7.99	10.99	9.99	28.99	44.98	3.99	12.99	13.99	14.99	2.99
	Salinity	‰	0.01	0.008	0.008	0.01	0.01	0.008	0.02	0.01	0.05	0.02	0.01	0.01	0.02	0.05	0.03
	TDS	ppm	202	306	182	283	254	208	179	443	368	290	305	319	348	178	300
	PO_4	mg/L	0.0005	0.01	0.006	0.004	0.01	0.001	0.003	0.012	0.005	0.003	0.02	0.012	0.008	0.009	0.01
	SO_4	mg/L	0.12	0.06	0.09	0.93	1.07	0.01	0.17	5.34	1.44	0.65	0.19	1.66	3.33	1.83	1.27
	NO_3-N	mg/L	17.04	10.96	17.93	13.94	15.82	27.79	0.90	7.75	12.39	11.95	15.72	11.51	30.20	10.84	24.34
	NO_2-N	mg/L	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.08	0.05	0.05	0.05	0.05
Average values of the stations	**Parameters**	**Unit**	**1st**	**2 nd**	**3 rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**	**11th**	**12th**	**13 th**	**14 th**	**15 th**
	W.T.	°C	10.3	10.5	10	10.1	10.5	10.3	10.1	10.6	10.8	10.3	10.5	11	11.1	10.6	11
	pH		7.87	7.89	7.89	7.78	7.73	7.64	7.74	7.77	7.73	7.83	8.06	7.68	7.58	7.71	7.79
	E.C.	µs/cm	399	636	367	618	527	423	503	840	640	662	385	559	796	643	421
	D.O	mg/L	3.48	3.10	2.85	2.34	3.61	3.75	4.37	2.72	4.31	5.50	4.18	3.67	3.93	4.37	4.82
	T.H	°F	22.06	36.8	20.06	33.93	29.13	23.36	28.13	33.2	28.53	26.8	19.03	30.73	33.93	30.13	20.46
	Ca	mg/L	51.26	56.37	40.85	78.55	103.2	80.15	67.06	97.23	52.63	86.56	34.81	76.95	95.22	67.32	51.53
	Mg	mg/L	7.10	4.75	5.05	10.84	15.87	13.60	9.82	15.53	6.95	16.7	8.66	14.55	21.10	16.58	10.51
	Cl	mg/L	5.99	5.65	3.99	10.32	6.99	4.99	6.65	13.32	31.29	34.29	3.99	21.29	21.96	21.96	6.99
	Salinity	‰	0.01	0.01	0.01	0.03	0.02	0.009	0.01	0.03	0.03	0.05	0.02	0.02	0.03	0.05	0.02
	TDS	ppm	181	292	174	294	241	238	204	452	316	284	201	269	304	238	210
	PO_4	mg/L	0.42	0.13	0.42	0.23	0.27	0.45	0.35	0.49	0.32	0.29	0.50	0.33	0.45	0.34	0.52
	SO_4	mg/L	1.77	0.70	0.89	1.34	1.06	0.13	0.33	4.61	1.19	0.79	0.22	1.63	3.10	1.68	0.78
	NO_3-N	mg/L	34.17	19.21	29.52	28.04	13.33	27.79	21.58	20.49	17.71	13.89	17.23	14.15	33.91	21.80	31.34
	NO_2-N	mg/L	0.03	0.03	0.01	0.23	0.03	0.03	0.03	0.12	0.02	0.02	0.03	0.03	0.03	0.04	0.03

W.T: Water Temperature. E.C: Electrical Conductivity. D.O: Dissolved Oxygen. T.H: Total Hardness. TDS: Total Dissolved Solids

Table 4. Water quality parameters of water troughs according to seasonal average values

	Spring	Summer	Autumn	Average
W.T. (°C)	6.57	15	9.7	10.42
SKKY, 2004	**I**	**I**	**I**	**I**
pH	7.67	8	7.8	7.82
TSWQR,2016	**I**	**I**	**I**	**I**
E.C.(µs/cm)	554.93	582	546.5	561.14
TSWQR,2016	**I-II**	**I-II**	**I-II**	**I-II**
D.O (mg/L	5.04	4	2.6	3.88
TSWQR 2016	**II-III**	**II-III**	**IV**	**II-III**
T.H (°F)	26.65	28	29	27.88
Ca (mg/L)	72.64	71	64.8	69.48
Mg (mg/L)	10.31	16	9.5	11.94
Cl (mg/L)	11.46	16	12.6	13.35
SKKY, 2004	**I**	**I**	**I**	**I**
Salinity (‰)	0.04	0.01	0.01	0.02
TDS (ppm)	238.67	264	277.7	260.12
PO_4 (mg/L)	0.54	1	0.01	0.52
SO_4 (mg/L)	1.2	2	1.2	1.47
SKKY, 2004	**I**	**I**	**I**	**I**
NO_3-N (mg/L)	29.11	24	15.3	22.80
TSWQR,2016	**IV**	**IV**	**II-III**	**IV**
NO_2-N (mg/L)	0.01	0.01	0.1	0.04
SKKY, 2004	**II**	**II**	**IV**	**IV**

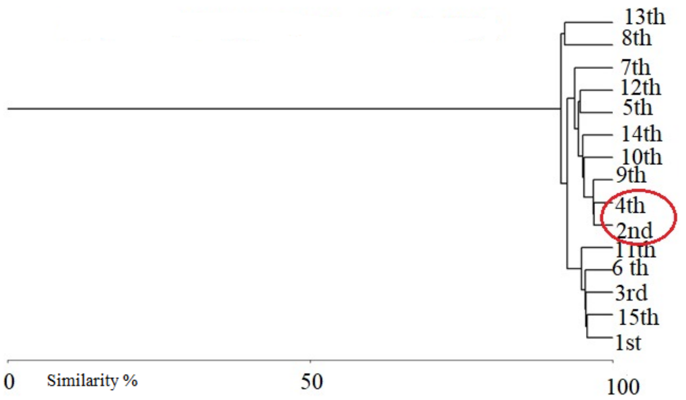

Figure 3. Bray Curtis Similarity dendogram in terms of physicochemical values.

tween W.T. and NO_2-N; D.O. and PO_4; E.C. and Cl; E.C. and SO_4; E.C. and TDS; Cl and salinity; Cl and TDS. As temperature increases in aquatic systems, oxygen concentration decreases. This causes the release of phosphorus from the sediment (IPCC, 2008). The results support the literature. Significant positive correlation was found between E.C. and Cl, and between SO_4 and TDS. The conductivity of water increases in the presence of inorganic dissolved solids such as Cl, NO_3, SO_4 and PO_4 anions or Na, Mg, Ca, Fe and Al cations (Spellman, 2003). Salinity consists of four cation groups such as Ca, Mg, Na, K and four aion groups such as HCO_3, CO_3, SO_4 and Cl (Egemen & Sunlu 1999). Cl, SO_4 and TDS were all found to be significantly positively correlated with E.C. The results are consistent with the literature. Correlation coefficients are given in Table 6.

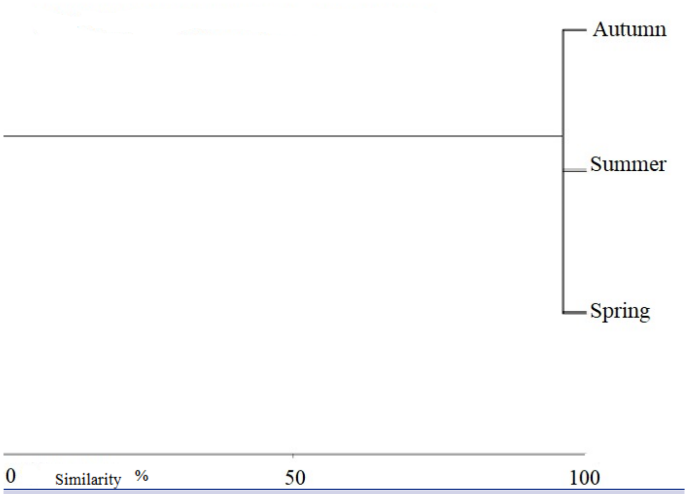

Figure 4. Bray-Curtis similarity dendogram in terms of seasons.

When the study is compared with previous studies, it can be said that some similar features are observed. Özkan (2006a) taxonomically examined samples from six different localities in Bozcaada between 1999-2002. The same study also identified a total of 14 species and made diagnostic keys of the species. All species are reported as new records for Bozcaada. *Chironomus salinarius* K was reported as a new species for the Chironomidae fauna of Turkey. *C. thummi*, *C. anthracinus*, *C. viridicollis*, *Einfeldia pagana* and *Polypedilum aberrans* were recorded as species emerging from the water troughs. Özkan (2006a) reported that *C. ripar-*

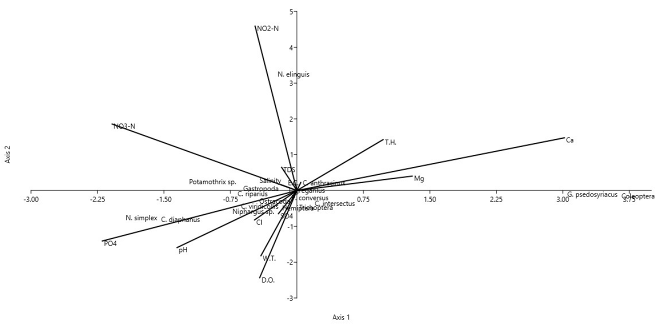

Figure 5. CCA Analysis of physicochemical values with taxa.

Table 5. Pearson Correlation Analysis.

	Ca	TH	pH	NO_3-N
Ca	1			
TH	.468**	1		
pH	-.500**	-.294	1	
NO_3-N	-.084	-.173	.092	1

****: correlation is significant at 0.01 level ($p < 0.01$);**
-: no statistically significant correlation was detected
**. Correlation is significant at the 0.01 level (2-tailed).

Külköylüoğlu et al. (2013) collected samples from water troughs in Bolu, Erzincan, Gaziantep, Kahramanmaraş, Ordu and Van provinces at different times between 2006 and 2010. They identified 32 Ostracoda species in total from 105 water troughs and investigated the composition and diversity of these species. The species belonging to Ostracoda were reported as *Heterocypris incongruens*, *Ilyocypris bradyi*, *Psychrodromus olivaceus*, *Candona neglecta*. The authors of the above-mentioned study identified water troughs as important structures to be studied due to their high-altitude locations and the unique species diversity to be found in them. They stated that zooplankton, phytoplankton and molluscs of different taxonomic groups found in water troughs should also be studied. In this study, ostracoda were found at stations 7 and 10 in the summer season and at stations 3, 7, 12 and 13 in the autumn season. However, the exact species could not be identified.

Başak et al. (2013) collected samples belonging to Copepoda and Cladocera groups from zooplankton in Ankara province. They reported 12 new zooplankton species for Ankara. The most common species found was *Eucyclops serrulatus*.

Külkoylüoğlu et al. (2017) investigated the diversity and distribution of Ostracods between Karabük and Düzce provinces. They reported that the most widespread species was *Heterocypris incongruens*.

Table 6. Spearman Correlation Analysis.

	NO_2-N	Mg	D.O.	E.C.	W.T.	Cl	Salinity	SO_4	TDS	PO_4
NO_2-N	1									
Mg	.137	1								
D.O.	-.600**	.069	1							
E.C.	.206	.299*	.010	1						
W.T.	.594**	.175	-.424**	.209	1					
Cl	.110	.330*	.111	.471**	.054	1				
Salinity	.272	.313*	-.055	.292	.356*	.443**	1			
SO_4	.209	.323*	-.170	.527**	.287	.253	.380*	1		
TDS	.371*	.170	-.259	.731**	.231	.421**	.202	.342*	1	
PO_4	-.425**	.155	.488**	-.027	.052	-.116	.031	.129	-.197	1

*: correlation significant at 0.05 level ($p < 0.05$);
****: correlation is significant at 0.01 level ($p < 0.01$);**
-: no statistically significant correlation was detected

ius and *C. anthracinus* lived mostly in the muddy bottom of stagnant waters, while *C. viridicollis* lived in the mud of stagnant waters. In this study, *C. riparius*, *C. anthracinus* and *C. viridicollis* species were found. Our study supports the literature in terms of both habitats and species.

Özkan (2006b) reported that *Paracladius conversus* and *Tanytarsus gregarilus* species were observed in stagnant water bodies in Gökçeada. *Paracladius conversus* and *T. gregarilus* were also identified in this study, in line with the larger literature.

In their study, Külkoylüoğlu et al. (2012) investigated whether water troughs constructed as artificial sources are beneficial for biodiversity and for the ecological characteristics of Ostracods in water troughs in some regions of Turkey.

Özer & Dikmen (2021) analyzed water troughs across the world, but especially in Erzurum, Turkey. They classified the water troughs according to type of material, number of sections, aesthetic features, and shapes, and made some suggestions on the protection and development of these structures.

Ataman et al. (2023) investigated the diversity and seasonal cycles of freshwater Ostracoda in man-made water troughs. They reported that *Heterocypris incongruens*, *Heterocypris salina* and *Ilyocypris bradyi* species were found in the water troughs.

Sanbur (2023) investigated the diatom species diversity of rural fountains and water troughs in various regions of Eskişehir. The conclusion of the study showed that 200 diatom taxa from the water troughs had been identified. The researcher reported that diatom diversity was high in the water troughs.

As a result of this multivariate statistical study carried out on water samples collected from 15 water trough stations across the Şarkışla region of Sivas, statistically significant relationships were found showing that altitude and agricultural activity impact water quality. These factors contribute to variations in water quality from the 1st to the 4th water quality grades. Within agricultural activities, the water quality of troughs may deteriorate due to pesticide applications. Pesticides can cause deterioration of groundwater quality and deterioration of the physical, chemical, and biological structure of water through irrigation and precipitation due to rain and snow. In areas where livestock breeding is intensive, other factors polluting groundwater resources should be identified and water troughs should be evaluated based on integrated basin management In order to identify this, it is very important to carry out studies of this kind at regular intervals to monitor the water quality and benthic macroinvertebrate fauna of surface and groundwater resources and to catalogue and maintain the hydrobiological diversity of Turkey.

Conflict of interest: The author declares that there is no conflict of interest.

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REFERENCES

Ataman, A., Külköylüoğlu, O., Özdemir, C. (2023). İnsan yapımı yalaktaki tatlı su Ostracoda çeşitliliği ve mevsimsel döngüleri, 10.Ulusal Limnoloji Sempozyumu, Çankırı Karatekin Üniversitesi, 4-6 Eylül. Ankara.

Başak, E., Aygen, C., & Külköylüoğlu, O. (2013). Taxonomy, distribution, and ecology of crustacean zooplankton in trough waters of Ankara (Turkey). Turkish Journal of Zoology, 38: 1-10. hptss//doi:10.3906/zoo-1301-7.

Cranston, P. S. (1982). A Key to the Larvae of the British., Orthocladiinae. Freshwater Biological Association Scientific Publication 4, 152 pp.

Denktaş, M. (2000). Karaman Çeşmeleri. *Kıvılcım Yayınları*, Kayseri.

Egemen, O. & Sunlu, U. (1999). *Water quality*. Ege University Fisheries Faculty, Issue number: 14, İzmir. ISBN: 9754831416.

Fittakau, E. J. & Roback, S. S. (1983). The Larvae of Tanypodinae (Diptera: Chironomidae)of Holoarctic Region:Keys and Diagnnoses. *Entomologica Scandinavica Suppl*. 19, 33-110.

Haldar, R., Kosankar, S. & Sangolkar, L. N. (2016). Ecological signifcance of macro invertebrates as an indicator of environmental pollution. *International Journal of Engineering Science and Computing*, 6(11), 3302-3307.

Hammer, Ø, Harper, D. A.T. & Paul D. R., (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, vol. 4, issue 1, art. 4: 9pp.

IPCC (2008). Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, [BATES, B.C., KUNDZEWICZ, Z.W., WU S. and PALUTIKOF, J.P. (eds.)]. Geneva, 210 pp.

Karaman, G. S. & Ruffo, S. (1986). Amphipoda: Niphargus group (Niphargidae sensu Bousfield, 1982). In: L. BOTOSANEAU (ed.), Stygofauna mundi, a faunistic, distributional, and ecological synthesis of the world fauna inhabiting subterranean waters (including marine interstitial): 514-534. (E. J. Brill/Dr. W. Backhuys, Leiden)

Kathman, R. D. & Brinkhurst, R. O. (1998). *Guide to The Freshwater Oligochaetes of North America*, Aquatic Resources Center, Tennessee, USA.

Kazancı, N., Girgin, S., Dügel, M. & Oğuzkurt, D. (1997). Akarsuların Çevre Kalitesi Yönünden Değerlendirilmesinde ve İzlenmesinde Biyotik İndeks Yöntemi. *Türkiye İç Suları Araştırmaları Dizisi: II*, Ankara.

Külköylüoğlu, O., Akdemir, D., Sarı, N., Yavuzatmaca, M., Oral, C., & Başak, E. (2013) "Distribution and ecology of Ostracoda (Crustacea) from troughs in Turkey," *Turkish Journal of Zoology*: Vol. 37: No. 3, https://doi.org/10.3906/zoo-1205-17. Available at: https://journals.tubitak.gov.tr/zoology/vol37/iss3/3.

Külköylüoğlu, O., Yılmaz, S., & Atmaca, Y. (2017). Comparison of Ostracoda (Crustacea) species diversity, distribution and ecological characteristics among habitat types. Fundamental and Applied Limnology. 190 (1), 63-86. https//:doi:10.1127/fal/2017/0872.

Külköylüoğlu, O., Akdemir, D., Sarı, N., Yavuzatmaca, M., Oral C., & Başak, E.(2012). Distribution and ecology of Ostracoda (Crustacea) from troughs in Turkey. *Turkish Journal of Zoology*, *37*: 277-287. https://doi:10.3906/zoo-1205-17

Krebs, C. J. (1999). Ecological Methodology. Addison Wesley Longman, Menlo Park, California.

McAleece, N. Gage, J. D. G., Lambshead, P. J. D. & Paterson, G. L. J. (1997). BioDiversity Professional statistic analysis software. Jointly developed by the Scottish Association for Marine Science and the Natural History Museum London.

Milligian, D. & Michael, R. (1997). Identification Manual for The Aquatic Oligochaeta of Florida Volume I, Freshwater Oligochaetes. State of Florida Department of Environmental Protection Tallahassee, Florida.

Oliver, D., R., McClymont, D., & Roussel, M., E. (1978). A Key to Some Larvae of Chironomidae (Diptera) From the Mackenzie and Porcupine River Watersheds, Biosystematics Research Institute, Ottowa, Canada.

Oran Orta Anadolu Kalkınma Ajansı, Sivas Yatırım Rehberi, Temmuz, 2017.

Özer, S. & Dikmen, O. (2021). Investigation of Water Troughs, One of the Disappearing Values of Turkish Culture, According to Their Condition of Use. *Atatürk Univ. Ziraat Fak. Derg.,* 52 (3): 238-252, http://doi: 10.17097/ataunizfd.863288.

Özer, S. (2010). Geçmişten günümüze kent-çevre ilişkisi içinde çeşmeler. *Sanat Dergisi*, 0 (13): 129-134.

Özkan, N. (2006a). "The Larval Chironomidae (Diptera) Fauna of Bozcaada (Tenedos)". *G. U. Journal of Science*. Vol. 19, No. 2. 57–67, Ankara.

Özkan, N. (2006b). "The Larval Chironomidae (Diptera) Fauna of Gökçeada (Imbroz)", *G. U. Journal of Science*. Vol. 19, No. 2. 69-75, Ankara.

Özkan, N. (2007) "Larvae of Çanakkale Region and Their Distribution". *Trakya Uni. J. Sci*. 8 (2) :123-132.

Pinder, K. & Reıss F. (1983). The Larvae of Chironominae (Diptera: Chironomidae) of the Holoarctic Region- Keys and Diagnoses. *Entomologica Scandinavica Suppl*. 19, 293-435, Lund Sweden.

Robert, T .& Dillion, J. R. (1999). The Ecology of Freshwater Molluscs, 561, 367-464.

Sanbur, A. (2023). Eskişehir ili köy çeşmesi ve su yalaklarında bulunan diyatomelerin tür çeşitliliği ve dağılımı. Yüksek Lisans tezi, Bursa Uludağ Üniversitesi, Fen Bilimleri Enstitüsü, 68 sayfa, Eskişehir.

Seather, O. A (1980). Glossary of Chironomid Morphology Terminology (Diptera: Chironomidae), *Entomologica Scandinavica Suppl.* 14, 51 pp. Lund Sweden.

Sket, B. (2008). Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. Hydrobiologia, 595: 241-255.

SKKY, 2004, Su Kirliliği Kontrol Yönetmeliği, Yayımlandığı Resmi Gazete, 31 Aralık Cuma 2004, Sayı:25687.

Spellman, F.R. (2003). Handbook of Water and Wastewater Treatment Plant Operations. CRC press, USA, ISBN:1-56670-627-0.

Taşdemir, A., Ustaoğlu, M.R. & Balık, S. (2009). Contrubution to the Knowledge of Chironomidae (Diptera) Fauna of the Gediz Delta (Menemen-İzmir-Turkey), *Journal of FisheriesSciences.com*, 3(1):32-39, https://doi.org/:10.3153/jfscom.2009006.

Taşdemir, A., Yıldız, S., Özbek, M., Ustaoğlu, M.R., Balık, S.2010. The Macrobenthic (Oligochaeta, Chironomidae, Amphipoda) Fauna of Tahtali reservoir (Izmir), Journal of FisheriesSciences.com, 4(4):376-383, https://doi:10.3153/jfscom.2010040.

Tay, L. (2011). Kırsehır Fountaıns. *Adiyaman University Journal of Social Sciences*, 4 (6): 204-221.

Thienemann, A. (1954). Chironomus, Verbreitung, und Wirtschaftliche Bedeutung der Chironomiden. Binnengewasser 20: 1954.

Timm, T., & Veldhuijzen van Zanten, H.H. (2002). Freshwater Oligochaeta of North-West Europe. World Biodiversity Database, CD-ROM Series. University of Amsterdam: Expert Center for Taxonomic Identification.

Timm, T. 2003. Veldhuijzen Van Zanten, H.H. Freshwater Oligochaeta of North-West Europe.

Timm, T. (1999). A Guide to the Estonian Annelida Naturnalist's *Handbooks 1*, Estonian Academy Publishers, Tartu-Talinn.

Timm, T. (2013)."The genus Potamothrix (Annelida, Oligochaeta, Tubificidae): a literature review. Estonian Journal of Ecology, vol. 62, no. 2, pp. 121+. Gale Academic OneFile, link.gale.com/apps/doc/A334486526/AONE?u=anon~b9b60d87&sid=googleScholar&xid=0cd8f639. Accessed 10 June 2023.

Todaie, (2011). Köyler. Institute of Public Administration for Turkey and Middle East, Ankara. Available from www.yerelnet.org.tr.

Väinölä, R., J. Witt, D. S., Grabowski, M, Bradbury,. J. H., Jazdzewski, K. & TSWQR (2016). Turkish Surface Water Quality Regulation Official Gazzette No, 29797.

Zamanpoore, M., Poeckl, M., Grabowski, M., Schiemer, F. (2011). Taxonomic review of freshwater Gammarus from Iran. *Zootaxa*, 3140: 1–14.

Ostracods, Retrieved August, 8, 8, 2023, from https://www.bgs.ac.uk/discovering-geology/fossils-and-geological time/ostracods/#environment, accessed: 10.10.15.

Order Hemiptera, Retrieved September, 20, 9, 2023, from http://lakes.chebucto.org/ZOOBENTH/BENTHOS/vi.html, accesses: 10.10.15.

Şarkışla Kaymakamlığı, Retrieved August, 15, 2023, from http://sarkisla.gov.tr, accessed:10.10.15.

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Research Article

Bio-indicator Bacteria Levels in Riva Stream, an Important Stream in İstanbul, Türkiye

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ABSTRACT

Bacterial pollution is the presence of harmful bacteria in aquatic ecosystems, which can cause health problems if ingested. These bacteria originate from a variety of sources, including human and animal waste, agricultural runoff, and untreated sewage. This study aimed to assess the levels of bioindicator bacteria and their associations with various environmental parameters in surface water samples. The samples were collected seasonally from five stations along Riva Stream between March 2018 and January 2019. Indicator bacterial levels were determined using membrane filtration. Temperature, salinity, pH, dissolved oxygen, and conductivity values of the stations were measured in situ during sampling using a multiparameter (YSI). As a result of the sampling, maximum total coliform, fecal coliform, and intestinal enterococci levels were recorded as 24 x 10^3 CFU/100ml, 18 x 10^3 CFU/100ml and 24 x 10^2 CFU/100 ml, respectively. During sampling, the indicator bacterial levels were above the limit at all stations. When the FC/IE ratios were analyzed, the maximum FC/IE ratio was recorded as 52.10 during the winter sampling period at the station. The detection of bioindicator bacteria levels above the standard limit throughout the sampling period (especially during the summer period) was associated with a high level of domestic waste in the region. The results showed that more frequent and detailed monitoring studies should be conducted in this region.

Keywords: Total coliform, Fecal coliform, Intestinal enterococci, Environmental parameters, Riva stream

INTRODUCTION

Microbial communities have a significant impact on the regulation of water quality and biogeochemical cycling in freshwater ecosystems. It is well-known that bacteria play a pivotal role in the decomposition and stabilization of organic matter in both natural environments and biological sewage treatment processes. Comprehending the ecology and evolution of microbial communities and the implications of these changes for public health, it is imperative to evaluate physical and chemical parameters, including salinity, temperature, and pH (Ibekwe et al., 2012).

Accelerating urbanization, increased population density, and ineffective sewerage infrastructure systems have important implications for public health (Mallin, 2000; 2001; Garbossa, 2017). Water sources contaminated with sewage and human fecal contamination are related to the occurrence of infectious waterborne diseases (McKee and Cruz, 2021; Manetu and Karanja, 2021; Kahn et al., 2018).

Coliform bacteria, especially enteric bacteria, serve as indicators of the presence of bacterial pathogens. Although fecal coliforms themselves are generally not directly harmful to human health, their detection indicates the presence of fecal waste that may harbor harmful pathogens (Farrell, 2013). The microbial water quality standard includes the evaluation of bacterial indicator organisms, which often includes the coliform group, fecal streptococci, enterococci, and *Escherichia coli* (E. coli) (Wen et al., 2020; Davene et al., 2020; Augustyn et al., 2016; APHA, 2013). When pollution originates from human waste, the fecal coliform/fecal streptococcal ratio (FC/IS ratio) is greater than 4, between 0.1 and 4.0 when it originates from domestic animals such as cattle, and below 0.1 in wild animals (Clinton et al., 2006, Benhassane et al., 2019, Gerba, 2009).

The Marmara Sea, an inland sea between Asia and Europe and the gateway to the Aegean and Black Seas through the Bosphorus (Bosphorus) and the Dardanelles (Dardanelles), is important for the fisheries sector, maritime transport, and ecosystem health (Altuğ et al., 2009). The Riva Stream flows through Riva village, Istanbul and it is approximately 33 km long. It is located between the Black Sea and the exit of the Marmara Sea and is one of the largest rivers in the region. Ömerli Dam, the largest drinking water reservoir on Riva Stream, was established by the General Directorate of State Hydraulic Works (DSİ) in 1972 (Tarkan, 2010; Özuluğ et al., 2005). The area around Riva Stream and Riva Beach offers opportunities for a variety of outdoor activities such as hiking, picnics, camping and water sports. Due to these features, it has become a popular touristic and recreational destination.

Studies indicating the presence of pollutants and including topics such as fishing, mollusks, indicator bacteria and eutrophication have emerged in previous studies on the Riva Stream (Dökümcü and Koşal Şahin, 2022; Altuğ et al., 2016; Garipağaoğlu et al., 2015; Tarkan, 2010; Özuluğ et al., 2005; Dinseven and Çurgunlu, 1988).

This study monitoried the level of indicator organisms associated with bacterial pollution at determined stations in Riva stream and determined the relationship of bio-indicators with variable environmental parameters.

MATERIALS AND METHODS

Surface water (0-30 cm) was collected seasonally from five stations between March 2018 and January 2019. The samples were delivered to a laboratory under cold chain conditions and were analyzed on the same day as the sampling. The stations are shown in Figure 1 and listed in Table 1.

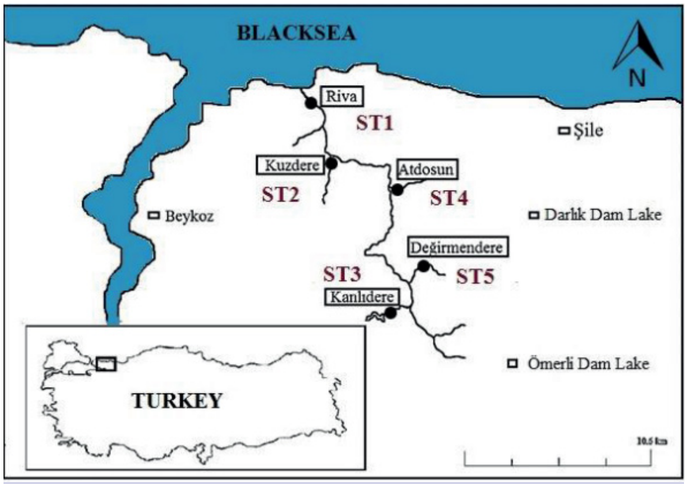

Figure 1. The positioning of Riva Stream and the designated sampling sites.

Table 1. The geographical coordinates of the sampling stations along Riva Stream.

Station No	Station Name	Coordinate
S 1	Riva Stream	N 41.12763° E 29.13521°
S 2	Kuzdere	N 41.12763° E 29.13521°
S 3	Kanlıdere	N 41.07127° E 29.16175°
S 4	Atdosun Stream	N 41.10403° E 29.16333°
S 5	Değirmendere	N 41.08222° D 29.17393°

The membrane filtration method was used to determine indicator bacteria levels. The100ml samples filtered through sterile filters were placed in Endo-NKS (sartorius) medium for total coliform bacteria, m-FC - NKS (Sartorius) for fecal coliform and azide - NKS (Sartorius) medium for intestinal enterococci. The media were incubated for 24 hours at 37±0.1°C for total coliforms and intestinal enterococci, and at 44±0.1°C for fecal coliforms. The colonies grown at the end of the incubation were counted and recorded as CFU/100ml. The pink-red colonies exhibiting a yellow-green metallic sheen in the m-Endo medium were considered as potential indicators of coliforms. The Cytochrome oxidase test (API Strep, BioMereux) was performed on the pink-red colonies with a yellow-green metallic sheen, which were evaluated as suspicious for coliform, and oxidase-negative colonies were taken into numerical evaluation. The blue colonies observed in the m-FC medium were considered as potential indicators of fecal coliforms. Cytochrome oxidase (API Strep, bioMérieux) and indole tests were performed on these colonies with suspected fecal coliform. Oxidase negative and indole positive colonies were evaluated numerically (APHA, 2013). To determine if the bacteriological contamination was of human or animal origin (such as cattle), the ratio of fecal coliform bacteria levels (FC) to intestinal enterococci (IE) bacteria levels was evaluated. Saturated oxygen (mg/L), water temperature (°C), salinity (‰), conductivity (µS/cm) and pH values were measured in situ during sampling using multiparameters (YSI). Spearman's rank correlation statistical analysis was performed to determine the relationship between environmental parameters and indicator bacterial levels. The significance level for differences between the parameters was set at 0.05. The differences between the measured environmental parameters and bacterial levels were examined by two-way ANOVA test. Statistical analyses were performed using SPSS 28.0.

RESULTS AND DISCUSSION

The variable environmental parameters of the sampling areas, including dissolved oxygen, temperature, salinity, pH, and conductivity, are shown in Table 2.

Table 2. Physicochemical parameters on Riva Stream.

Stations	Months	Temp (°C)	Salinity (‰)	pH	O_2 (mg/L)	Conductivity (µS/cm)
Station 1	Mar-18	11.47	1.25	8.67	4.82	2.44
	Aug-18	24.50	2.40	7.70	3.46	1.12
	Oct-18	18.10	1.40	7.14	3.47	3.07
	Jan-19	5.40	0.20	7.91	11.50	0.97
Station 2	Mar-18	10.47	0.34	8.82	9.70	0.41
	Aug-18	21.50	0.00	7.69	5.82	0.60
	Oct-18	17.70	0.00	7.07	4.70	0.44
	Jan-19	5.80	0.00	8.04	13.76	0.32
Station 3	Mar-18	11.46	0.17	8.88	10.08	0.31
	Aug-18	16.6	0.00	6.32	6.78	0.04
	Oct-18	15.7	0.00	7.16	6.80	0.02
	Jan-19	6.5	0.00	9.63	12.39	0.15
Station 4	Mar-18	11.0	0.16	8.59	10.34	0.58
	Aug-18	21.6	1.20	7.57	8.83	0.01
	Oct-18	15.9	0.00	7.20	6.02	0.04
	Jan-19	4.4	0.00	8.96	12.65	0.25
Station 5	Mar-18	11.97	0.27	7.92	7.58	0.55
	Aug-18	24.9	0.40	7.51	5.52	0.39
	Oct-18	16.2	0.30	7.40	6.76	1.10
	Jan-19	5.4	0.00	8.10	12.57	0.48

pH values 6.32 to 9.63, dissolved oxygen values 3.46 to 13.76mg/L, temperature values 4.4-24.9°C, salinity values and ppt values 0 to 2.4 recorded as the maximum and minimum, respectively.

The detected total coliform levels in the samples are shown in Figure 2.

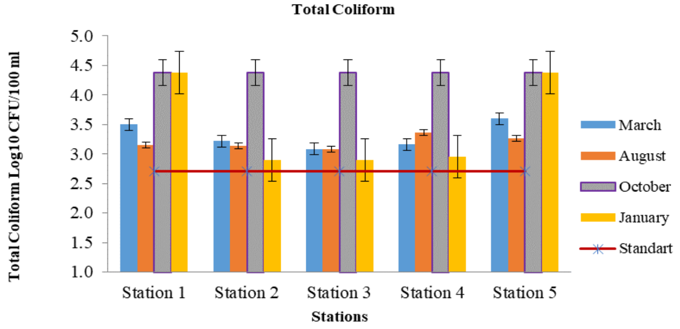

Figure 2. Total coliform bacteria levels are determined in water samples by seasons and stations.

The levels of coliform bacteria in samples from all stations were found to exceed national limit values. The highest total number of coliform bacteria was detected as 24x10^3 CFU/100ml in October and January 2018.

The detected fecal coliform levels in the samples are shown in Figure 3. The fecal coliform levels were higher than the recommended national limit values. Fecal coliform counts were recorded at a minimum of 10 CFU/100ml and a maximum of 18 × 10^3 CFU/100ml during the study period.

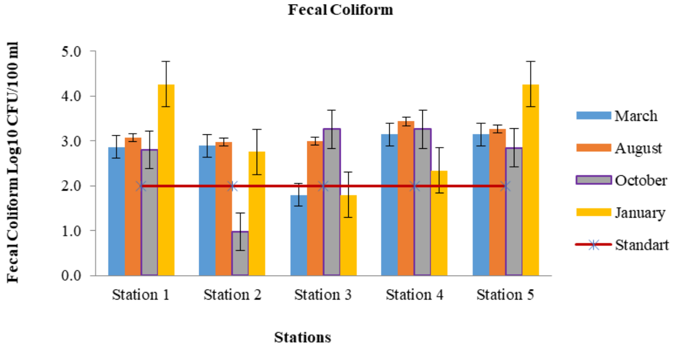

Figure 3. Fecal coliform levels are determined in water samples by seasons and stations.

The detected intestinal enterococci levels in the samples are shown in Figure 4. The levels of intestinal enterococci were higher than the recommended national limit. Intestinal enterococci counts were recorded at a minimum of 38 CFU/100ml and a maximum of 24 × 10^2 CFU/100ml during the study period.

The fecal coliform/ intestinal enterococci ratios (FC/IE ratio) analyzed in the surface water samples during the study period are shown in Table 3. Data presented in Table 3 shows that the FC/IE counts recorded a minimum 0.04 and maximum 52.10 during the study period.

Values are shown in bold. If the FC/IE ratio is greater than 4, the pollution is considered as human origin, and if the FC/IE ratio is between 0.1 and 4, the pollution is considered as animal origin.

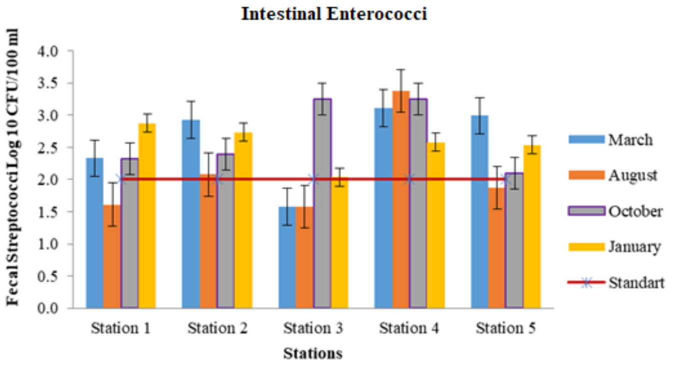

Figure 4. Intestinal enterococci bacteria levels are determined in water samples by seasons and stations.

Table 3. Fecal coliform/Intestinal Enterococci ratio at all stations.

Stations	FC/IE			
	Mar-18	Aug-18	Oct-18	Jan-19
S 1	3.38	**28.34**	2.95	**23.89**
S 2	0.91	**7.78**	0.04	1.05
S 3	1.64	**25.84**	1.00	0.57
S 4	1.08	1.12	1.00	0.58
S5	1.42	**24.21**	**5.60**	**52.10**

Many river and stream studies conducted worldwide pointed out that the pollution rate is above the WHO and EPA standards. Shittu et al. (2008) reported that the total coliform count of Abeokuta, Southwest Nigeria, exceeded the EPA's maximum contamination standards for coliform bacteria in drinking water. Viau et al. (2011) reported that Enterococci were associated with bacterial pathogens from the five indicators tested in Hawaiian Oahu coastal streams. Musyoki et.al. (2013) reported that the microbiological contamination of the Nairobi and Athi rivers was unacceptably high, according to Kenya standards and WHO guidelines for drinking water and agricultural use. Akkan et al. (2019) reported that the Total Coliform (TC), Fecal Coliform (FC) and Fecal Streptococcus (FS) rates of surface water samples in Yağlıdere were 45%, 71.66% and 56.66%, respectively. Altug et al. (2016) reported that the total coliform and fecal coliform counts were higher than advised national limit values in Riva stream.

The data obtained within the scope of the study was analyzed in accordance with national and international water quality standards. The standard values for the European Parliament are a total coliform count 500 CFU/100ml (2.69/100 ml based on log10), fecal coliform count 100 CFU/100ml (2.0/100ml based on log10), and intestinal enterococci counts 100 CFU/100ml (2.0/100ml based on log10 value), which are indicated in dark red in the graphs below (European Parliament, 2006). Standard values for sea water used for recreational purposes were determined by the National Regulation published on 31 December 2004. According to this, limit values were established as 1000 cfu/100ml for total coliform, 200 cfu/100ml for fecal coliforms and 100 cfu/100ml for fecal enterococcus (Official Gazette 2004).

When total coliform, fecal coliform and intestinal enterococci data were examined, it was determined that the bacteriological pollution level in the Riva River was above the standard limits in all seasons. During the sampling period, the highest levels of total coliforms were recorded in summer, which was associated with increased human activities. Fecal coliform counts peaked at a maximum of 18 x 10^3 CFU/100ml throughout the study, while intestinal enterococci counts ranged from a minimum of 38 CFU/100ml to a maximum of 24 x 10^2 CFU/100ml. The fact that the levels of total coliforms, fecal coliforms and fecal enterococci consistently exceeded the recommended national limits draws attention to the persistent presence of bacteriological pollution sources in the region.

Environmental variables significantly impact the levels of indicator bacteria in rivers (Herrig et al., 2019; Islam et al., 2017). Indicator bacteria such as *E. coli* and fecal coliforms which are used to measure the presence of fecal contamination in water can indicate the potential presence of other harmful bacteria and viruses (Saxena et al., 2015;Lin and Ganesh, 2013. Temperature is an important factor in bacterial growth in aquatic ecosystems. Warm temperatures can accelerate bacterial growth, leading to higher levels of indicator bacteria in the water. Conversely, cold temperatures can slow bacterial growth and reduce the levels of the indicator bacteria (Kunili and Ateş, 2021). During the summer months, when temperatures are warmer and recreational use of rivers is more common, there is typically an increase in the levels of indicator bacteria owing to higher levels of human and animal waste entering the water. In our study, no significant relationship was found between the total, fecal coliform levels, and water temperature. This situation is due to the fact that there is a continuous input of pollution to the study area. Heavy rainfall washes pollutants, including bacteria, from the surrounding land into the river, leading to increased levels of the indicator bacteria. This is particularly true for urban areas, where rainwater runoff can carry pollutants from streets and parking lots into nearby rivers (Shehane et al., 2005). Higher flow rates dilute the bacteria in water, thereby reducing the levels of indicator bacteria. However, during periods of high flow, bacteria is more easily resuspended from the riverbed and carried downstream, potentially increasing the levels of the indicator bacteria (Crump et al., 2007). Agricultural and urban land use contributes to higher levels of indicator bacteria in rivers. Agricultural runoff contains animal waste and fertilizers, whereas urban runoff contains a variety of pollutants, including pet waste, oil and grease from roads, and chemicals from industrial areas (Gotkowska-Płachta, et al., 2016; Paule-Mercado et al.,2016).

When examining the relationship between measurements of various environmental parameters, a significant correlation was found between the pH values and temperature values ($r = 0.005$, $p < 0.05$). Furthermore, pH values exhibited variations at the different stations (< 0.001), but no seasonal variation was observed. A significant correlation was identified between the dissolved oxygen values, temperature, pH, and conductivity

Table 4. Spearman's rank correlations between environmental parameters and indicator bacteria levels in Riva stream (N 20; marked correlations are significant at p< 0.05).

Correlations

		Depth	Width	FV	Temp	pH	Cond	DO	Salinity	TC	FC	IE
D	PC	1										
	Sig. (2-tailed)											
	N	20										
W	PC	.110	1									
	Sig. (2-tailed)	.645										
	N	20	20									
FV	PC	-.133	-.319	1								
	Sig. (2-tailed)	.576	.171									
	N	20	20	20								
T	PC	-.354	.066	-.283	1							
	Sig. (2-tailed)	.126	.782	.227								
	N	20	20	20	20							
pH	PC	.313	-.023	.500*	-.607**	1						
	Sig. (2-tailed)	.179	.924	.025	**.005**							
	N	20	20	20	20	20						
Cond	PC	.188	.802**	-.290	.101	-.034	1					
	Sig. (2-tailed)	.427	**<.001**	.215	.672	.888						
	N	20	20	20	20	20	20					
DO	PC	.149	-.358	.372	-.826**	.600**	-.461*	1				
	Sig. (2-tailed)	.530	.122	.106	**<.001**	**.005**	**.041**					
	N	20	20	20	20	20	20	20				
Salinity	PC	.040	.715**	-.249	.489*	-.080	.591**	-.527*	1			
	Sig. (2-tailed)	.866	**<.001**	.289	**.029**	.737	**.006**	**.017**				
	N	20	20	20	20	20	20	20	20			
TC	PC	-.061	.169	-.349	-.029	-.462*	.219	-.204	-.137	1		
	Sig. (2-tailed)	.799	.477	.131	.904	**.041**	.354	.388	.566			
	N	20	20	20	20	20	20	20	20	20		
FC	PC	.078	.253	-.226	-.381	-.015	-.003	.373	-.131	.453*	1	
	Sig. (2-tailed)	.743	.281	.337	.098	.950	.991	.105	.582	**.045**		
	N	20	20	20	20	20	20	20	20	20	20	
IE	PC	-.238	-.250	-.102	.038	-.137	-.350	.104	-.055	.165	.063	1
	Sig. (2-tailed)	.312	.289	.668	.873	.566	.130	.662	.818	.487	.791	
	N	20	20	20	20	20	20	20	20	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

F.V. Flow Velocity °C; Temperature Cond; Conductivity (μS/cm) D.O; Dissolved Oxygen ‰; Salinity/ TC: Total Coliform levels, FC: Fecal Coliform Levels, IE: Intestinal Enterococci levels

values. Additionally, a significant difference was noted in the dissolved oxygen values among different stations. In the analysis of water temperature values, a disparity was observed between stations ($p < 0.05$), while no significant difference was observed between seasons. The total coliform values displayed significant differences among stations. Overall, environmental variables have a significant impact on the levels of indicator bacteria in rivers. Monitoring these variables and understanding their effects can help water managers and policymakers develop strategies to reduce bacterial pollution in rivers and protect public health.

CONCLUSION

Riva Stream is important due to its ecosystem structure and it is also a popular place for tourism and recreation activities. This study, carried out between March 2018 and January 2019, investigated the seasonal levels of indicator bacteria in Riva stream. It was determined that indicator bacteria levels throughout the study area exceeded national and international standard values throughout the year in every season. Human activities were identified as the primary source of pollution. Seasonal sampling from the Riva stream provided important data for the evaluation of the pollution load carried by the stream and the possible introduc-

Table 5. Two-way ANOVA between bio-indicator bacteria and environmental parameters.

	Stations		Seasons	
	F.	**Sig.**	**F.**	**Sig.**
Depth	1.245	.326	1.645	.215
Width	.003	1.000	4628.520	<.001
Flow Velocity	2.192	.129	2.015	.144
Temperature	75.211	<.001	.071	.990
pH	10.356	<.001	.088	.985
Conductivity	.529	.669	8.256	<.001
Dissolved Oxygen	17.106	<.001	.712	.596
Salinity	1.207	.339	4.494	.014
Total Coliforms	13.309	<.001	.345	.844
Fecal Coliforms	2.056	.147	.804	.542
Intestinal Enterotococci	.288	.834	2.476	.089

tion of bacteria to the Black Sea coast. Bacterial pollution, which was found to flow from both sides of Riva Stream to the Black Sea poses a potential risk for recreational activities in the region. To improve water quality, it is imperative to implement a comprehensive management strategy and adopt measures to address water quality issues. Additionally, improving national awareness of the problems associated with fecal pollution in freshwater sources is crucial to maintaining overall ecosystem health.

Conflict of interests: The authors have no conflicts of interest to declare.

Ethics committee approval: Ethics committee approval is not required.

REFERENCES

Akkan, T., Mehel, S. & Mutlu, C. (2019). Determining the level of bacteriological pollution level in Yağlıdere Stream, Giresun. Journal of Limnology and Freshwater Fisheries Research, 5(2), 83-88. https://doi.org/10.17216/limnofish.450722

Altug, G., Cardak, M., Ciftci, P.S. & Gurun, S. (2009). An Important Water Route Between Mediterranean and Black Seas and Bacterial Pollution (Canakkale and Istanbul Straits, Turkey) - Proceeding of the 3rd WSEAS Int. Conference on Waste Management, Water Pollution, Air Pollution, Indoor Climate. 466–471.

Altug, G., Gurun, S., Ciftci Türetken, P. S. & Kalkan, S. (2016). Levels of bio-indicator bacteria transported to the Black Sea by the Riva Stream, Istanbul, Turkey. International Journal of Marine Science and Ocean Technology (IJMO), 3(4), 45-49. DOI: 10.19070/2577-4395-160008

APHA. (2013). Standard methods for the examination of water and wastewater, 22nd ed. American Public Health Association, Washington, D.C., Retrieved January 9, 2018, from https://www.standardmethods.org/PDF/22nd_Ed_Errata_12_16_13.pdf

Augustyn, Ł., Babula, A. M., Joniec, J., Stanek-Tarkowska, J., Hajduk, E. & Kaniuczak, J. (2016). Microbiological indicators of the quality of river water, used for drinking water supply. Polish Journal of Environmental Studies, 25(2), 511-519. DOI: https://doi.org/10.15244/pjoes/60899

Benhassane, L., Zerdani, I., Fadlaoui, S., Mounjid, J., Loudiki, M., Oubraim, S. (2019). Monitoring Impacts of Human Activities on Bouskoura Stream (Casablanca, Morocco): Bacteriology and Parasitology. Bulletin de l'Institut Scientifique, Rabat, Section Science de la vie, 41, 15-32.

Clinton, B.D., Vose, J.M. (2006). Variation in Stream Water Quality in an Urban Headwater Stream in the Southern Appalachians. Water Air Soil Pollut 169, 331–353 https://doi.org/10.1007/s11270-006-2812-x

Crump, B.C., Adams, H.E., Hobbie, J.E., and Kling, G.W. (2007) Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. Ecology 88: 1365–1378.

Devane, M.L., Moriarty, E., Weaver, L., Cookson, A., Gilpin, B. (2020). Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring, Water Research, 185, 116204, https://doi.org/10.1016/j.watres.2020.116204.

Dinseven, E. & Çurgunlu, E. (1988) Determination of the Trophic status of the Riva stream for monitoring Eutrophication. Istanbul University Journal of Fisheries & Aquatic Sciences, 2:35-52.

Dökümcü, N. & Koşal Şahin, S. (2022). The Seasonal Distribution of Molluscan (Bivalvia and Gastropoda) Biodiversity in Riva Stream (Istanbul) . Aquatic Sciences and Engineering, 37 (2) , 69-73. DOI: 10.26650/ASE2021962620

EPA. (2006). EPA microbiological alternate test procedure (ATP) protocol for drinking water, ambient water, wastewater and sewage sludge monitoring methods.

European Parlament. (2006). Directive 2006/7/EC of the European parlament and of the council of 15 February 2006.

Farrell JB. (2013). Fecal pathogens control during composting. In: Science and engineering of composting: Design, environmental, microbiological and utilization aspects edit. Hoitink HAJ, Keenerg HM. 1993;282-300.

Garbossa, L. H., Souza, R. V., Campos, C. J., Vanz, A., Vianna, L. F. & Rupp, G. S. (2017). Thermotolerant coliform loadings to coastal areas of Santa Catarina (Brazil) evidence the effect of growing urbanisation and insufficient provision of sewerage infrastructure. Environmental monitoring and assessment, 189(1), 27. DOI: 10.1007/s10661-016-5742-0

Garipağaoğlu, N. , Şahin, C. , Şahin, C. , Çeker, A. & Şenol, C. (2015). Çayağzı (Riva) Havzası'nın Doğal Ortam Koşulları (Jeolojik-Jeomorfolojik-Hidrografik Açıdan) Ve Sürdürülebilir Kullanım Üzerindeki Rolü. Marmara Coğrafya Dergisi, 0 (31) , 48-81. DOI: 10.14781/mcd.73355

Gerba, C.P. (2009) "Indicator microorganisms." In Environmental microbiology, pp. 485-499. Academic Press. https://doi.org/10.1016/B978-0-12-370519-8.00023-7

Gotkowska-Płachta, A., Gołaś, I., Korzeniewska, E. et al. (2016). Evaluation of the distribution of fecal indicator bacteria in a river system depending on different types of land use in the southern watershed of the Baltic Sea. Environ Sci Pollut Res 23, 4073–4085 https://doi.org/10.1007/s11356-015-4442-6

Herrig, I., Seis, W., Fischer, H. et al. (2019). Prediction of fecal indicator organism concentrations in rivers: the shifting role of environmental factors under varying flow conditions. Environ Sci Eur 31, 59 https://doi.org/10.1186/s12302-019-0250-9

Ibekwe, M.A., Leddy, M. B., Bold, R. M. & Graves, A. K. (2012). Bacterial community composition in low-flowing river water with different sources of pollutants. FEMS microbiology ecology, 79(1), 155-166. DOI: 10.1111/j.1574-6941.2011.01205.x

Islam, M.M.M., Hofstra, N. & Islam, M.A. (2017). The Impact of Environmental Variables on Faecal Indicator Bacteria in the Betna River Basin, Bangladesh. Environ. Process. 4, 319–332 https://doi.org/10.1007/s40710-017-0239-6

Khan, K., Lu, Y., Saeed, M.A., Bilal, H., Sher, H., Khan, H., Ali, J., Wang, P., Uwizeyimana, H., Baninla, Y., Li, Q., Liu, Z., Nawab, J., Zhou, Y., Su, C., Liang, R. (2018). Prevalent fecal contamination in drinking water resources and potential health risks in Swat, Pakistan, Journal of Environmental Sciences, 72, 1-12, https://doi.org/10.1016/j.jes.2017.12.008.

Kunili, İ.E., Ateş, A. S. (2021). Effects of seasonal changes and environmental factors on bioindicator bacteria levels in Çardak Lagoon, Çanakkale Strait, Turkey. Oceanological and Hydrobiological Studies. 50(3): 299-309. https://doi.org/10.2478/oandhs-2021-0025

Lin J., & Ganesh, A. (2013). Water quality indicators: bacteria, coliphages, enteric viruses, International Journal of Environmental Health Research, 23:6, 484-506, DOI: 10.1080/09603123.2013.769201

Mallin, M.A., Ensign, S.H., McIver, M.R., Shank, G.C. & Fowler, P.K. (2001). Demographic, landscape, and meteorological factors controlling the microbial pollution of coastal waters. Hydrobiologia, 460(1), 185–193. https://doi.org/10.1023/A:1013169401211

Mallin, M. A., Williams, K. E., Esham, E. C. & Lowe, R. P. (2000). Effect of human development on bacteriological water quality in coastal watersheds. Ecological applications, 10(4), 1047-1056. DOI: 10.2307/2641016

Manetu, W.M., Karanja, A.M. (2021). Waterborne Disease Risk Factors and Intervention Practices: A Review. Open Access Library Journal. 8, e7401. DOI: 10.4236/oalib.1107401

McKee, A.M., Cruz, M.A. (2021). Microbial and Viral Indicators of Pathogens and Human Health Risks from Recreational Exposure to Waters Impaired by Fecal Contamination. Journal of Sustainable Water in the Built Environment. 7(2): https://doi.org/10.1061/JSWBAY.0000936

Musyoki, A.M., Suleiman, M.A., Mbithi, J.N. & Maingi, J.M. (2013) Water-Borne Bacterial Pathogens in Surface Waters of Nairobi River and Health Implications to Communities Downstream Athi River. International Journal of Life Sciences Biotechnology and Pharma Research, 3, L4-L10.

Özuluğ, M., Acıpınar, H., Gaygusuz, O., Gursoy, C., Tarkan, A.S. (2005) Effects of human factor on the fish fauna in a drinking-water resource (Omerli Dam Lake-Istanbul, Turkey). Research journal of agriculture and biological sciences. 1(1): 50-55.

Paule-Mercado, M.A.,J.S. Ventura, S.A. Memon, D. Jahng, J.-H. Kang, C.-H. Lee. (2016). Monitoring and predicting the fecal indicator bacteria concentrations from agricultural, mixed land use and urban stormwater runoff, Science of The Total Environment,550: 1171-1181. https://doi.org/10.1016/j.scitotenv.2016.01.026.

Saxena, G., Bharagava, R.N., Kaithwas, G., Raj, A. (2015). Microbial indicators, pathogens and methods for their monitoring in water environment. J Water Health 13 (2): 319–339. https://doi.org/10.2166/wh.2014.275

Shehane, S.D., V.J. Harwood, J.E. Whitlock, J.B. Rose. (2005). The influence of rainfall on the incidence of microbial faecal indicators and the dominant sources of faecal pollution in a Florida river, Journal of Applied Microbiology, 98 (5): 1127–1136, https://doi.org/10.1111/j.1365-2672.2005.02554.x

Shittu, O. B., Olaitan, J. O. & Amusa, T. S. (2008). Physico-chemical and bacteriological analyses of water used for drinking and swimming purposes in abeokuta, nigeria. African Journal of Biomedical Research, 11(3). DOI: 10.4314/ajbr.v11i3.50739

Tarkan, A.S. (2010). Effects of Streams on Drinkable Water Reservoir: An Assessment of Water Quality, Physical Habitat and Some Biological Features of the Streams. Journal of FisheriesSciences.com. 4(1): 8-19.

Wen, X., Chen, F., Lin, Y., Zhu, H., Yuan, F., Kuang, D., Jia, Z., Yuan, Z. (2020). Microbial Indicators and Their Use for Monitoring Drinking Water Quality—A Review. Sustainability 12, 2249. https://doi.org/10.3390/su12062249

Viau, E. J., Goodwin, K. D., Yamahara, K. M., Layton, B. A., Sassoubre, L. M., Burns, S. L., ... & Boehm, A. B. (2011). Bacterial pathogens in Hawaiian coastal streams—associations with fecal indicators, land cover, and water quality. Water research, 45(11), 3279-3290. DOI: 10.1016/j.watres.2011.03.033

Yayintaş, Ö. T., Yilmaz, S., Türkoğlu, M., Şolakoğlu, F. A. & Şakir, F. (2007). Seasonal variation of some heavy metal pollution with environmental and microbiological parameters in sub-basin of Kocabas Stream (Biga, Canakkale, Turkey) by ICP-AES. Environmental monitoring and assessment, 134(1), 321-331. https://doi.org/10.1007/s10661-007-9623-4

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Research Article

Seasonal Feeding Biology of Catfish, *Pachypterus atherinoides* (Bloch, 1794) with Special Reference to Lentic and Lotic Ecosystem

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ABSTRACT

The study of food and feeding biology is extremely crucial in fishery. *Pachypterus atherinoides* (Bloch, 1794) is a good food and ornamental catfish in the world. The present study aims to examine the seasonal variation of feeding habits of *P. atherinoides* in both lentic and lotic ecosystems. The study was conducted over two years in selected lentic and lotic aquatic ecosystems of the Jhargram and Paschim Medinipur districts of West Bengal, India. The study reveals that *P. atherinoides* shows carni-omnivorous nature. Pearson's correlation represents gastro-somatic index, relative gut length, and hepato-somatic index have significant positive correlation among them. Based on posthoc tests, it has been observed that the gastro-somatic index, relative gut length, and hepato-somatic index vary significantly both seasonally and in both lentic and lotic ecosystems. This study would help aquaculturists in the culture, captive breeding, and conservation of *P. atherinoides* and also help researchers in the biometric study of another fish.

Keywords: Carni-omnivorous, Catfish, Feeding biology, Lentic, Lotic, *Pachypterus atherinoides*

INTRODUCTION

The study of food and feeding behaviors is extremely crucial in fishery biology. Fish mostly obtain their energy from food, which has a big impact on their population size, growth rate, and overall health. Because they enable fish to consume all of the potential food in water bodies without competing with one another and living in communities with other fish, diet and feeding habits are crucial in aquaculture (Victor *et al.*, 2014). To determine the fullness of the stomach and the feeding status of the species, the gastrosomatic index (GaSI) is used and the relative gut length (RGL) is applied to determine the eating habits and to measure a certain relationship with the length and life stage of the fish species. The RGL value is often used to predict a wide range of food groups and show which foods different fish species like to eat. The study of fish-eating habits and gut content analysis has several objectives, including learning about their food preferences, determining whether they obtain enough food seasonally, and determining when feeding intensity peaks and troughs. The study of a species' feeding ecology can indicate how the creature has evolved ecologically to manage the challenge. It is also vital for the species' propagation to get a better understanding of its feeding behavior, as this information can be used to exploit natural fish food. The HSI measures the liver weight to body weight ratio. An indicator of eating and metabolism status is the HSI biomarker. Changes in HSI and GSI values are good indicators of fish with compromised reproductive function. HSI and GSI can go up during Vitelogenesis. *Pachypterus atherinoides* (Bloch, 1794), often known as Indian potasi, is a common catfish belonging to the family Schibeidae found in rivers and ponds throughout India (except Kerala), Pakistan, Bangladesh, Nepal, Myanmar, Bunna (Menon, 1999). It has high nutritional and also ornamental value. The fish meal of the species contains energy 343

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Kcal, moisture 16 gm, protein 58gm, fat 09 gm, mineral 17 gm, calcium 1597 mg, phosphorus 595 mg, Iron 41 mg each 100 gm (Gopalan *et al*., 2004). Aquarists are attracted to it because of its bright color and small size. This species has been gradually decreasing due to pollution, habitat destruction, selective captive breeding, removal from aquatic bodies for their carnivorous nature, and also the high preferences of exotic catfish [African catfish *Clarius gariepinus* (Burchell, 1822) and Thai catfish *Pangasius sutchi* due Fowler, 1937]. Globally , there has been very little research on the feeding biology of *Pachypterus atherinoides* (Bloch, 1794). In India, except for Gogai *et al*., 2020, there is no study on the feeding biology of this species. However, they did not observe seasonal feeding behavior and habitat/ecosystem differentiation, and they did not study both lentic and lotic ecosystems. In West Bengal, some aspects of various indigenous fish species have been studied by different researchers (Dasgupta, 2004; Chattopadhyay *et al*., 2014; Gupta and Banerjee, 2014; Jana et al., 2021; Jana *et al*., 2022A; Jana *et al*. 2022B; Sit *et al*. 2020; Chanda and Jana., 2021; Sahil *et al*., 2022; Sit *et al*. 2022A; Sit *et al*. 2022B; Sit *et al*., 2023A; Sit *et al*., 2023B) but not to observe the feeding habit of the said species. The interspecific interaction and productivity of water bodies can be determined by studying fish feeding habits. Fish nutrition and feeding have an impact on their development, reproduction, and health (Srivastava *et al*., 2014). Most research on the food and feeding habits of fish from diverse habitats has shown that those species varied in time and place, as well as at different stages of growth, emphasizing the need for more research into a species' food and feeding habits.

MATERIALS AND METHODS

Collection of fish specimens: Specimens were collected every fifteen days from lentic (selected Ponds) and lotic (selected River sites) ecosystems/habitat of Paschim Medinipur and Jhargram districts, West Bengal, India, during the Pre-monsoon/Summer (March-June), monsoon (July-Oct), and Post-monsoon/Winter (Nov-Feb) seasons since March 2020 to February 2022 (Figure 1).

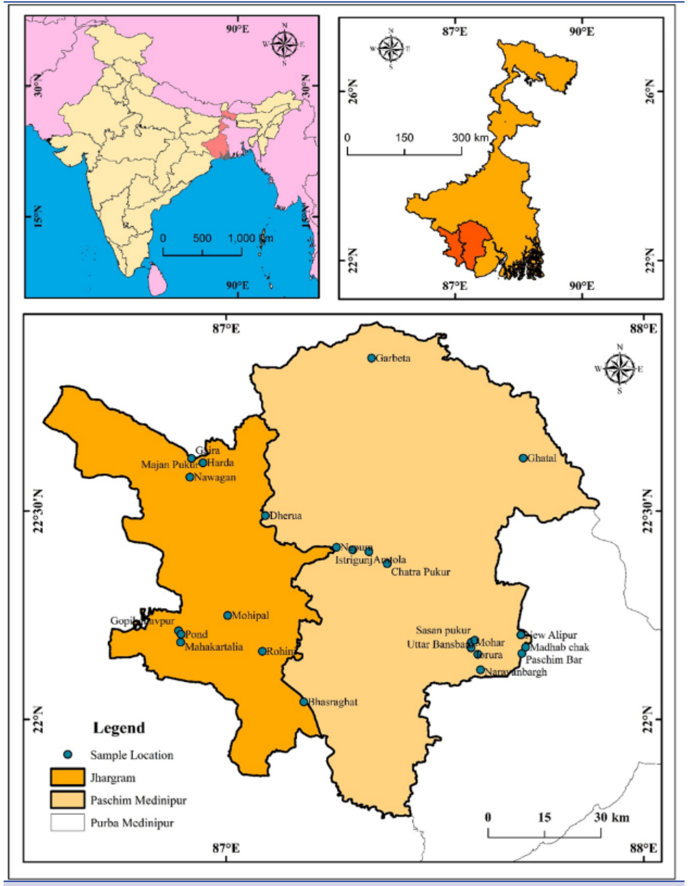

Figure 1. Specimens collection Sites.

Measured length and weight: Seasonally and ecosystem/habitat-wise, the total weight and total length of each specimen were measured by a digital weighing machine with 0.01 gm accuracy and a digital slide caliper instrument with 0.01 mm accuracy, respectively.

Dissection and Internal organ measured: All internal organs were collected by dissection with the help of scissors, forceps, needles, and a brush. The liver and digestive tract were dissected out and kept in a vial with a labeled 10% formalin solution. Digital slide calipers were used to measure the length of the digestive tract. After that, the stomach was separated from the digestive system. An electronic balance was used to weigh the liver and stomach.

Observed stomach fullness: Stomach distention per species, as determined by season, has been observed. According to Nagar and Sharma (2016), the stomach's distension was graded as 'full', '3/4 full', '1/2 full', '1/4 full', and 'empty' by eye assessment.

Gastrosomatic index (GaSI): The following formula (Desai, 1970) was used to figure out the Gastrosomatic Index (GaSI), or the amount of food eaten each month:

$$\text{GaSI} = \frac{\textit{Weight of the stomach}}{\textit{Weight of fish}} \times 100$$

Relative gut length (RGL): The following formula was used to compute the Relative Gut Length (RGL) (Al-Hussain, 1949).

$$\boldsymbol{RLG} = \frac{\textit{Length of gut}}{\textit{Total body length}}$$

Hepatosomatic index (HSI): HSI was determined by Rajaguru's 1992 formula.

$$\boldsymbol{HSI} = \frac{\textit{Weight of liver}}{\textit{Weight of fish}} \times 100$$

Food content analysis: To identify the various foods that the fish swallowed, the stomachs were dissected, and 1 mL of the food contents were taken in a glass vial and examined under a light microscope (XSP L101). Hynes's (1950) frequency of occurrence method was used to look at the foods, and the following equation was used:

$$\text{Percentage of occurrence of a food type} = \frac{\textit{Number of guts where the food occurred}}{\textit{Total number of guts analyzed}} \times 100$$

Data analysis

Finally, data were analyzed (Descriptive statistics, MANOVA, Pearson's Correlation, Post Hoc test,) with the help of Microsoft Excel (2019), SPSS (2021), and Origin Pro (2023) software systems.

RESULTS AND DISCUSSION

Seasonally, habitat-wise maximum, minimum, and average values of gastrointestinal length and weight of *P. atherinoides* in both districts were represented in Table 1. GaSI values were highest during the Summer season (6.65 ± 1.11, 7.06 ± 1.81, 7.95 ± 1.90, and 7.08 ± 1.56), followed by the Winter (6.09 ± 1.05, 4.85 ± 1.37, 6.40 ± 1.01, and 6.09 ± 1.41) and the monsoon (2.93 ± 1.00, 4.70 ± 1.53, 6.16 ± 0.96, and 5.41 ± 1.42) in both habitats (pond and river) of two districts (Table 2 and Figure 2-3). Gogoi *et al.* (2020) recorded the average GaSI value of *P. atherinoides* was 2.683±0.495 gm, with the highest GaSI value (2.832±0.754) during the winter-spring season (Feb-April) and minimum (2.525±0.363 gm) during the summer-rainy (May-July) in the Subansiri river, Assam. In the present study, the value of GaSI is greater than that of Gogoi *et al.* The GaSI values change seasonally, and the highest during pre-breeding and lowest during breeding have been similar to the studies of Gupta and Banerjee (2014), Kurbah and Bhuyan (2018), Sharma *et al.* (2018) and Gogoi *et al.* (2020) but not similar to the study of Chaturvedi and Saksena (2013). GaSI values indicated maximum food intake during the pre-spawning season and minimum during the breeding season. GaSI ranges from 2.91 to 6.08, representing the voracious nature of fish (Lanthaimeilu and Bhattacharjee, 2018), so the present result indicates the species has been the same (Table 2). In the present study, the RGL values ranged from 0.52 ± 0.057 to 0.572 ± 0.059, and seasonal fluctuation was observed in the ecosystem of both districts for *P. atherinoides* (Table 2 and Figure 3). Gogoi *et al.* (2020) observed that the average RGL value was 0.486±0.046 with a minimum of 0.440±0.058 mm and a maximum of

Table 1. Gastro- intestinal length and gastro-intestinal weight of *Pachypterus atherenoides* in both ecosystem of Jhargram and Paschim Medinipur districts.

District	Habitat	Season	GiL (cm)				GiWt (gm)			
			Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
JHARGRAM	POND	*Pre-monsoon*	2.20	5.80	4.1094	0.90531	0.16	0.47	0.2988	0.09967
		Monsoon	3.60	6.80	5.0188	0.95189	0.04	0.29	0.1763	0.09879
		Post-monsoon	2.20	5.10	3.8594	0.77078	0.04	0.39	0.2338	0.06757
	RIVER	*Pre-monsoon*	2.6	5.8	4.4281	0.90313	0.19	0.47	0.3175	0.08281
		Monsoon	3.80	6.90	5.2219	0.89973	0.08	0.30	0.1806	0.06345
		Post-monsoon	2.40	5.60	4.1938	0.81119	0.06	0.45	0.2603	0.09654
PASCHIM MEDINIPUR	POND	*Pre-monsoon*	2.20	5.90	4.1594	0.90371	0.09	0.45	0.2500	0.11843
		Monsoon	3.60	6.80	5.0344	0.95161	0.06	0.31	0.1522	0.07417
		Post-monsoon	2.30	5.10	3.9219	0.76189	0.04	0.33	0.1931	0.07464
	RIVER	*Pre-monsoon*	2.60	5.90	4.5000	0.92632	0.10	0.44	0.3097	0.09163
		Monsoon	3.90	6.90	5.2375	0.89542	0.04	0.38	0.1991	0.09730
		Post-monsoon	2.40	5.60	4.2063	0.81317	0.04	0.31	0.2013	0.06748

Table 2. Gastro Somatic index (GaSI) and Relative gut length (RGL) of *P. atherinoides* in both ecosystem of Jhargram and Paschim Medinipur districts.

District	Habitat	Season	GaSI				RGL			
			Min	Max.	Mean	SD	Min.	Max.	Mean	SD
JHARGRAM	POND	*Pre-monsoon*	4.167	8.861	6.65298	1.116203	0.400	0.659	0.52981	0.057801
		Monsoon	0.727	4.578	2.93009	1.001402	0.474	0.63	0.5432	0.049304
		Post-monsoon	3.417	7.947	6.09778	1.053266	0.395	0.622	0.53590	0.058751
	RIVER	*Pre-monsoon*	5.324	14.331	7.95290	1.906884	0.464	0.949	0.57953	0.091541
		Monsoon	3.095	7.500	6.16186	.967922	0.451	0.633	0.55654	0.048127
		Post-monsoon	4.310	9.845	6.40334	1.012078	0.421	0.691	0.57232	0.059477
PASCHIM MEDINIPUR	POND	*Pre-monsoon*	3.217	12.925	7.06904	1.812687	0.393	0.678	0.53297	0.057550
		Monsoon	2.727	8.989	4.70767	1.539878	0.468	0.630	0.54480	0.047737
		Post-monsoon	2.639	6.860	4.85507	1.370543	0.411	0.614	0.54326	0.053157
	RIVER	*Pre-monsoon*	3.689	10.553	7.08806	1.563371	0.441	0.702	0.57125	0.058865
		Monsoon	2.597	8.491	5.41102	1.425112	0.446	0.641	0.55706	0.049073
		Post-monsoon	3.003	8.616	6.09162	1.412353	0.421	0.691	0.56461	0.060830

N=128 Min=Minimum; Max=Maximum; SD=Standard Deviation

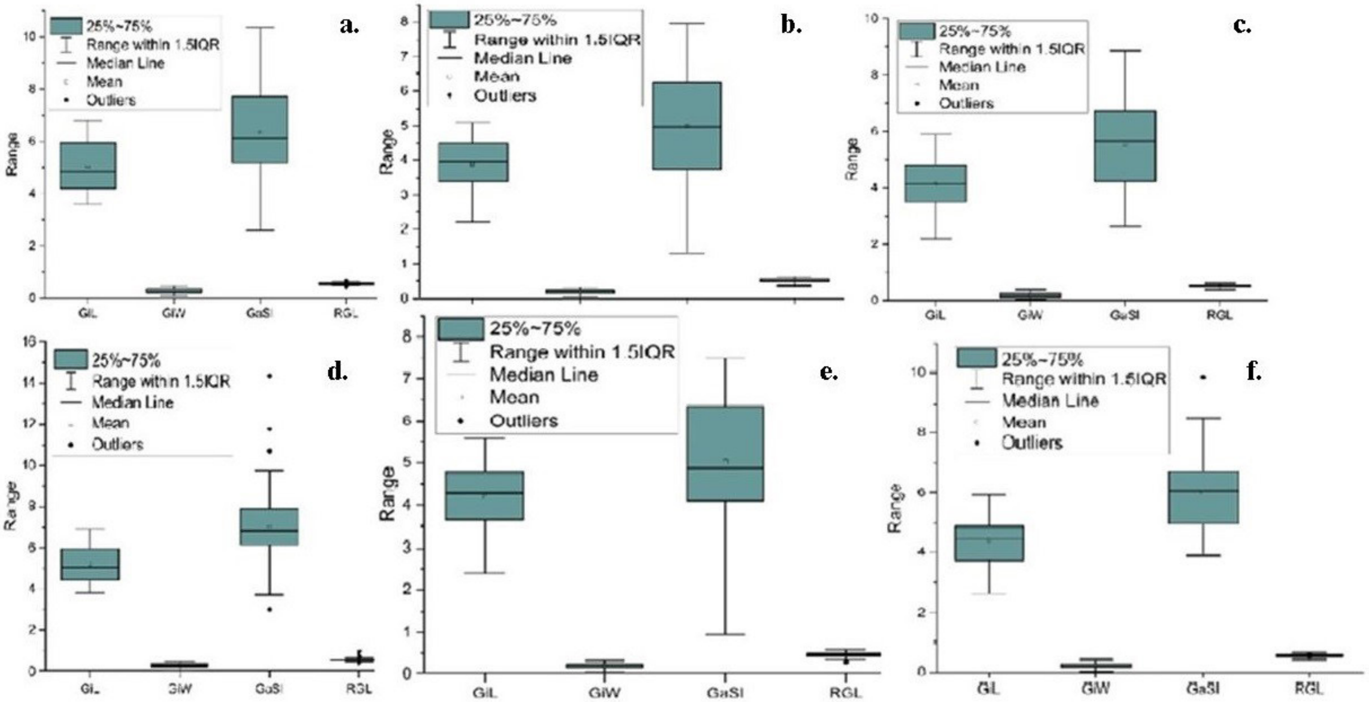

Figure 2. Seasonally GiL, GiWt, GaSI and RGL in both habitat in Jhargram (a,b &c) and Paschim Medinipur (d,e &f) district; a & d-Summer; b & e-Monsoon; c & f-Winter.

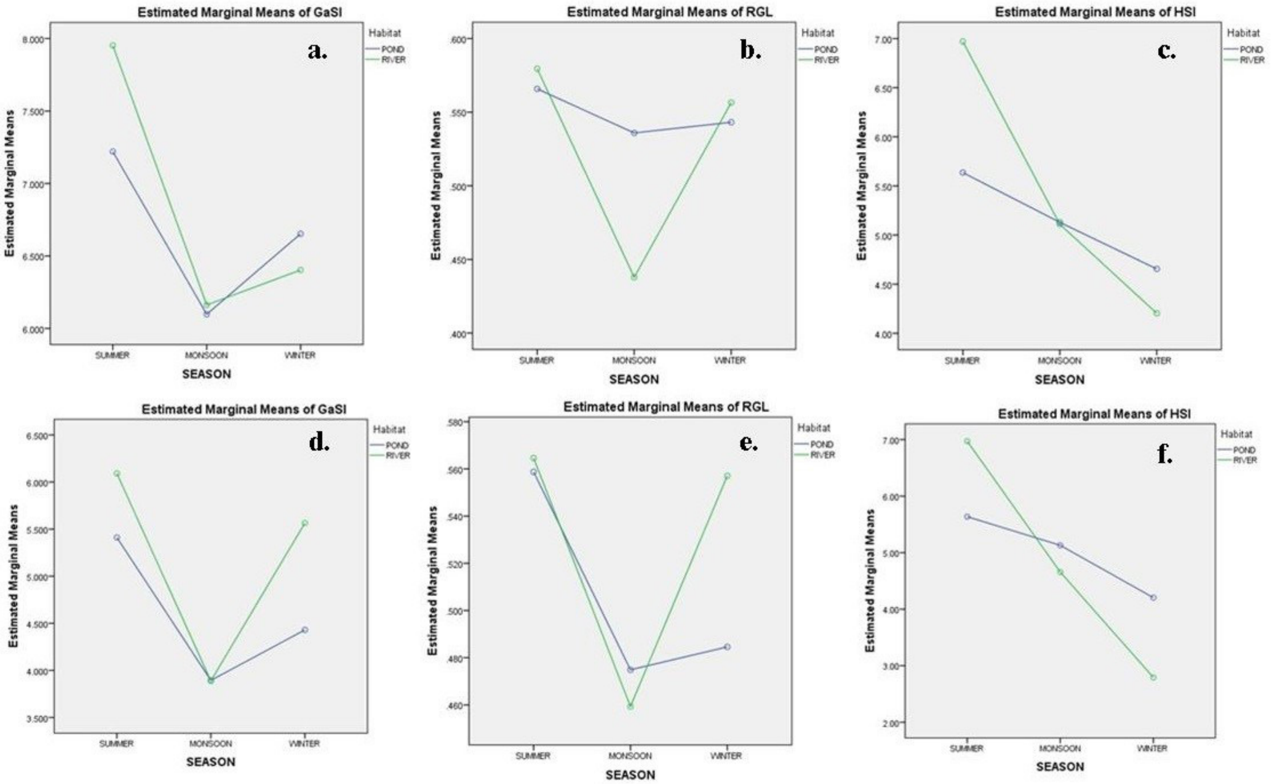

Figure 3. Seasonal variation of GaSI, RGL, HSI in both habitat in *Jhargram* (a,b &c) and Paschim Medinipur (d,e &f) district; a & d-Summer; b & e-Monsoon; c & f-Winter.

0.511±0.029 mm for *P. atherinoides*. RGL values above 0.8 indicated herbi-omnivorous, 0.7 to 0.8 represented carni-omnivorous, below 0.7 was carnivorous fish, and above 1.6 always herbivorous fish (Koundal *et al*., 2013), RGL ranged from 0.60 to 0.87 representing omnivorous nature (Dinh *et al*., 2018), the average RGL value 1.08±0.16 indicated carni-omnivorous nature (Mojumder *et al*., 2020), RGL ranged between 0.6 to 0.67 with mean SD 0.64 ± 0.003 indicated carnivorous nature (Alam *et al*., 2020) and very low (<0.32) indicated species as highly carnivorous nature (Renjit Kumar and Roshni, 2021). The present finding agrees with those of Koundal *et al*. (2013), indicating that fish are carnivorous. However, in the current study, the gut contents of *P. atherinoides* show that it eats both plant- and animal-based feeds, including phytoplanktons, zooplanktons, crustaceans, larvae, molluscs, eggs, scales, protozoa, plant parts, and so on. This research revealed the largest proportion of zooplankton accepted as animal food (Figures 4-5). The enormous variety of feeding behaviors that fish have developed is due to evolution, which produced structural adaptations for getting food from an equally large variety of environmental situations (Gupta, 2015). HSI values of female *P. atherinoides* ranged from 4.203 ± 0.979 to 6.97 ± 3.32 with the maximum during Summer (5.63 ± 1.62 and 6.97 ± 3.325) followed by monsoon (7.38 ± 5.23 and 5.11 ± 0.933), Winter (4.65 ± 1.625 and 4.203 ± 0.979) and in male HSI values ranged from 4.10 ± 0.989 to 6.486 ± 1.618 with maximum during Summer (5.51 ± 1.612 and 6.486 ± 1.618) followed by monsoon (5.12 ± 1.093 and 5.00 ± 0.91), Winter (4.567 ± 1.634 and 4.163 ± 0.966) in both pond and river (Table 3 and Figure 3). Cek *et al*. (2001) and Mitu (2017) reported maximum HSI during the fish breeding season. Jan and Jan (2017), Mojumder *et al*. (2020), Gosavi *et al*. (2020) and Paul *et al*. (2021) observed the minimum HSI during the breeding season. The HSI value in the present study has been supported by the previous work of Jan and Jan (2017), Mojumder *et al*. (2020), Gosavi *et al*. (2020), and Paul *et al*. (2021). The gut of *P. atherinoides* was a maximum of 100 % full from March to May, ¾ full from February to April, ½ full from November to January, and ¼ full and empty from July to October in both habitats in the study area (Figures 6-7). The feeding intensity may be highest before breeding season and lowest during breeding season (Mojumder *et al*., 2020). The present feeding intensity result is supported by research done by Gupta (2015). Begum *et al*. (2008) stated that fish started to feed heavily after spawning in July, with more than 60% of *M. gulio* having full stomachs; the current data contradicts this. Pearson correlations represent GiL's high positive significant correlation (> 0.01) with GiWt, GaSI with very low positive significant correlation with Giwt, Gil and RGL, HSI with low positive significant correlation (> 0.01) with GiL, GIWt, GaSI, RGL and Liv Wt in both aquatic ecosystems (Tables 4-5 and Figures 8-9). There is a significant difference between the groups in the feeding parameters related to the seasonal and ecosystem (lentic and lotic), according to the multivariate test of *P. atherinoides* in both districts, according to Wilk's lambda (Tables 6 and 9). When studied individually for the six variables, except RGL in Jhargram district, there are no significant differences between the other five parameters towards the lentic and lotic ecosystems in both districts; RGL varied due to food availability in the aquat-

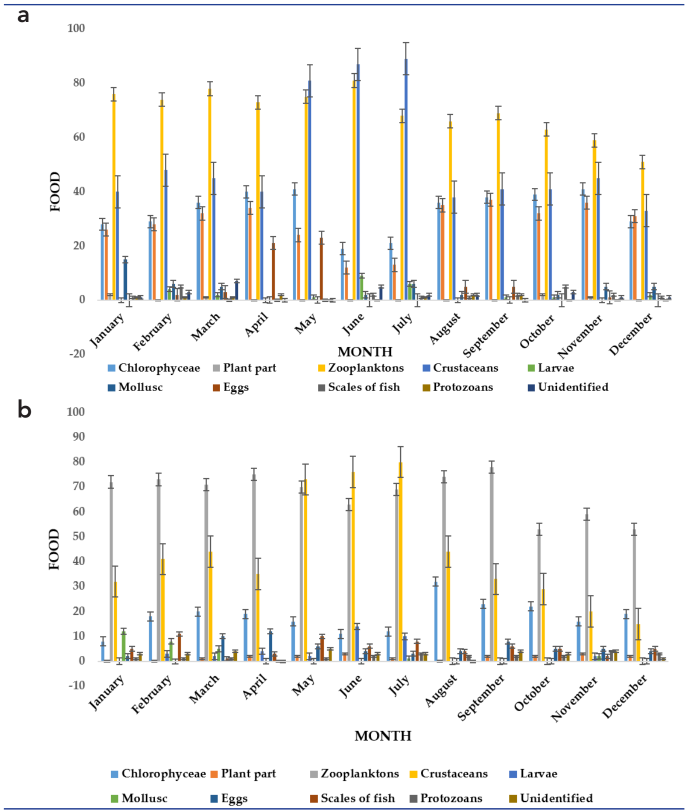

Figure 4. Monthly food item found in gut content of *P. atherinoides* in Jharghram district. a. pond; b. river.

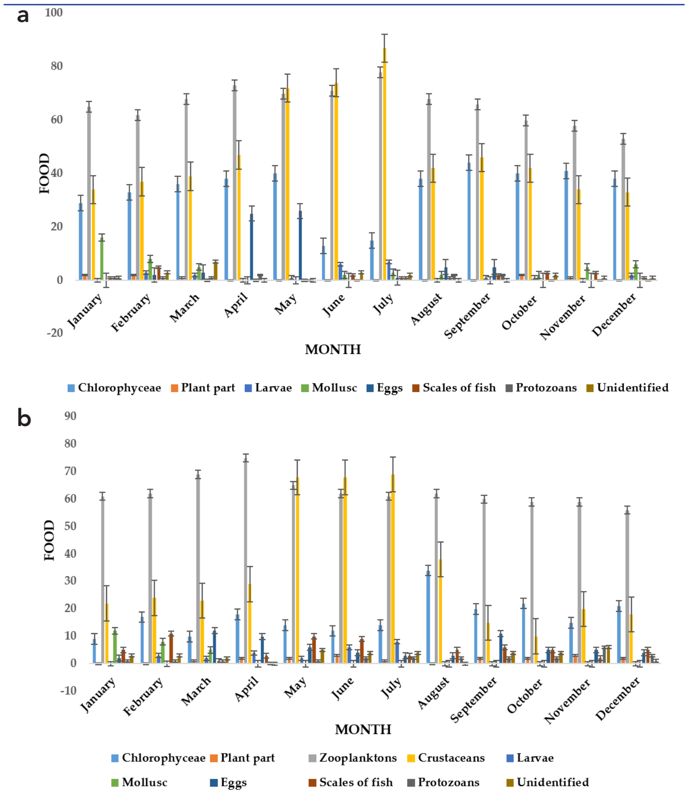

Figure 5. Monthly food item found in gut content of *P. atherinoides* in Paschim Medinipur. a. pond; b. river.

Table 3. Seasonally liver weight and Hepatosomatic index (HSI) of *P. atherinoides* in both ecosystem.

Habitat	Season	Sex	LvWt (g)				HSI			
			Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
POND	PREMONSOON	*Female*	0.02	0.35	0.1859	0.08904	1.90	8.44	5.6362	1.62325
		Male	0.03	0.26	0.1169	0.06104	1.89	8.34	5.5162	1.61245
	MONSOON	*Female*	0.12	0.41	0.2531	0.09413	2.34	7.38	5.2297	1.09303
		Male	0.07	0.41	0.2097	0.11338	2.34	7.28	5.1297	1.08304
	POSTMONSOON	*Female*	0.02	0.24	0.1281	0.06114	1.33	9.61	4.6555	1.62502
		Male	0.02	0.29	0.1513	0.07052	1.33	9.91	4.5675	1.63401
RIVER	PREMONSOON	*Female*	0.02	0.31	0.1575	0.08673	4.08	12.92	6.9707	3.32530
		Male	0.04	0.39	0.2172	0.09085	4.08	12.42	6.4868	1.61879
	MONSOON	*Female*	0.14	0.41	0.2653	0.08048	2.62	6.27	5.1103	0.93395
		Male	0.08	0.43	0.2241	0.03422	2.39	6.17	5.0021	0.91013
	POSTMONSOON	*Female*	0.04	0.24	0.1225	0.05691	2.31	6.22	4.2036	0.97960
		Male	0.02	0.27	0.1591	0.05943	2.21	6.02	4.1063	0.96600

N=128 Min=Minimum; Max=Maximum; SD=Standard Deviation

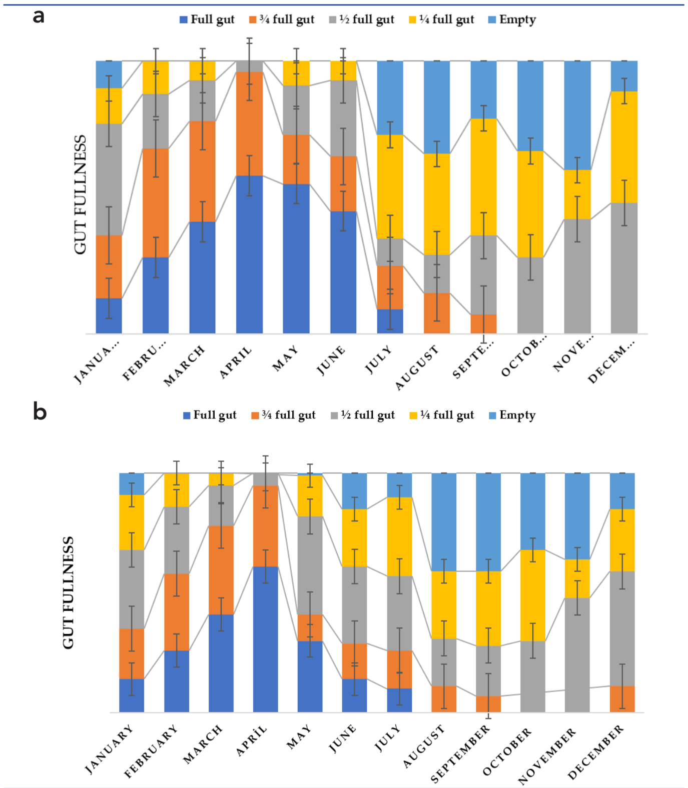

Figure 6. Monthly gut fullness of *P. atherinoides* in Jhargram district. a. pond; b. river.

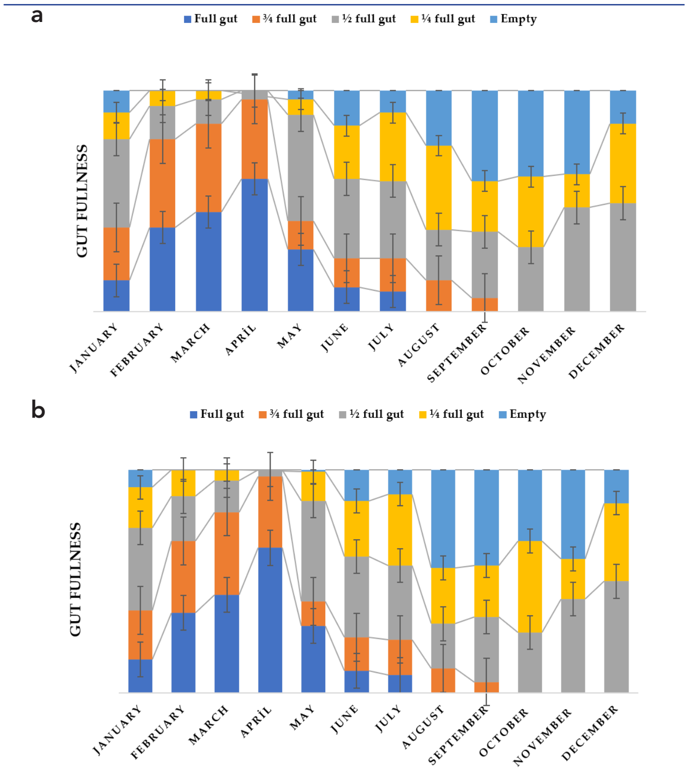

Figure 7. Monthly gut fullness of *P. atherinoides* in Paschim Medinipur district. a. pond; b. river.

ic ecosystem (Tables 7 and 10). Gogoi et al. (2020) stated that Season-wise, GaSI, and RGL were not significant statistically (p>0.05) of *P. atherinoides* in floodplain wetlands of Northeast India; this is not related to the present study. But the seasonal variation of GaSI, and RGL is supported by the study of Kurbah and Bhuyan (2018), Dinh *et al.* (2018), Gosavi *et al.*, (2020), and Mojumder *et al.* (2020).

CONCLUSION

The results of the current study make it clear that zooplankton is a vital source of natural food for *P. atherinoides*, although these fish also favored other foods with a plant origin. This finding raises questions about the conventional wisdom that the catfish species only display carnivorous feeding behaviors. On the other hand, it shows a distinct preference for carnivory over herbivory. The availability of such a diverse and partial preference for Phy-

Table 4. Pearson's correlation among feeding biological parameters in Riverine ecosystem for both district.

	GIL	GiWT	LivWt	GaSI	RGL	HSI
GIL	1	.918**	.112	.227**	.436**	.317**
GiWT	.918**	1	.080	.316**	.433**	.351**
LivWt	.112	.080	1	-.050	-.111	.165*
GaSI	.227**	.316**	-.050	1	.330**	.562**
RGL	.436**	.433**	-.111	.330**	1	.437**
HSI	.317**	.351**	.165*	.562**	.437**	1

*: 0.05 level of significance (2-tailed). **: 0.01 level of significance (2-tailed).

Table 5. Pearson's correlation among feeding biological parameters in Pond ecosystem for both district.

	GIL	GiWT	LivWt	GaSI	RGL	HSI
GIL	1	.909**	.037	.244**	.415**	.348**
GiWT	.909**	1	.105	.303**	.457**	.346**
LivWt	.037	.105	1	.115	.154*	.466**
GaSI	.244**	.303**	.115	1	.483**	.412**
RGL	.415**	.457**	.154*	.483**	1	.315**
HSI	.348**	.346**	.466**	.412**	.315**	1

*: 0.05 level of significance (2-tailed). **: 0.01 level of significance (2-tailed).

to-planktivorous resources suggests that the species' eating behaviors are dynamic. This alternate choice of feeding may result from transient adaptation or a phenomenon related to increased resource accessibility. Therefore, this finding represents that the species culture in any aquatic system like a pond, aquarium, or any aquatic body, does not depend on a definite ecosystem for their good growth and development, and it does not require pricey animal protein in its feed, its diverse diet suggests that it may be a suitable species for aquaculture. Additionally, the fact that these little fish species feed could be a crucial evolutionary factor. The study's findings would be valuable tools for developing management and protection strategies for conservation and

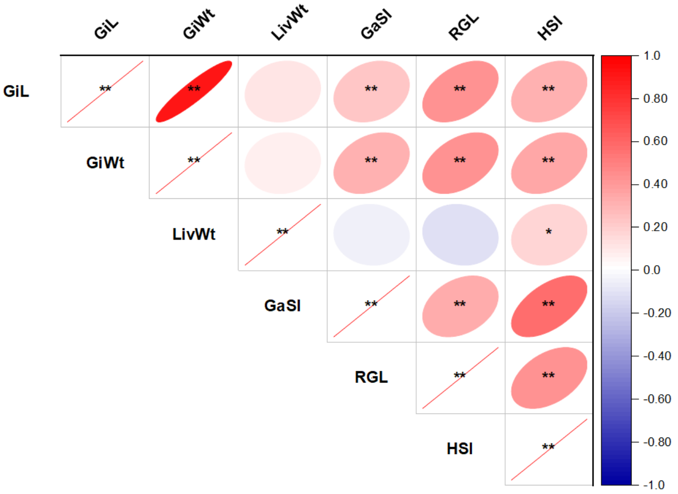

Figure 8. Pearson's correlation of feeding parameters in rivers of both district: *0.05 level of significance, ** 0.01 level of significance.

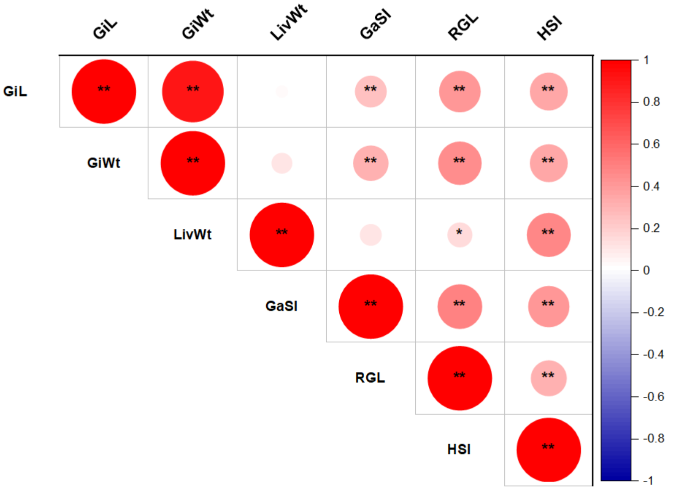

Figure 9. Pearson's correlation of feeding parameters in ponds of both district: *0.05 level of significance, ** 0.01 level of significance.

Table 6. Multivariate Tests among feeding biological parameters in Jhargram district.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.999	16493.140[b]	13.000	174.000	.000
	Wilks' Lambda	.001	16493.140[b]	13.000	174.000	.000
	Hotelling's Trace	1232.246	16493.140[b]	13.000	174.000	.000
	Roy's Largest Root	1232.246	16493.140[b]	13.000	174.000	.000
HABITAT	Pillai's Trace	.241	4.247[b]	13.000	174.000	.000
	Wilks' Lambda	**.759**	**4.247[b]**	**13.000**	**174.000**	**.001**
	Hotelling's Trace	.317	4.247[b]	13.000	174.000	.000
	Roy's Largest Root	.317	4.247[b]	13.000	174.000	.000
SEASON	Pillai's Trace	1.599	53.753	26.000	350.000	.000
	Wilks' Lambda	**.033**	**59.787[b]**	**26.000**	**348.000**	**.001**
	Hotelling's Trace	9.971	66.347	26.000	346.000	.000
	Roy's Largest Root	7.423	99.924[c]	13.000	175.000	.000

b. Exact statistic; c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table 7. Tests of Between-Subjects Effects of *P. atherinodes* in Jhargram district.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
HABITAT	GaSI	1.594	1	1.594	.903	.343
	RGL	.027	1	.027	6.947	**.001**
	HSI	3.974	1	3.974	1.232	.269
	GiL	3.910	1	3.910	5.097	.025
	GiWt	.013	1	.013	1.777	.184
	LvWt	.008	1	.008	1.189	.277
SEASON	GaSI	72.574	2	36.287	20.543	**.001**
	RGL	.253	2	.126	32.690	**.001**
	HSI	114.963	2	57.482	17.815	**.001**
	GiL	42.242	2	21.121	27.532	**.001**
	GiWt	.539	2	.269	36.355	**.001**
	LvWt	.577	2	.289	45.002	**.001**

N=256

Table 8. Post Hoc Tests seasonally of *P. atherinodes* in Jhargram district.

Dependent Variable	(I) SEASON	(J) SEASON	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
GaSI	SUMMER	MONSOON	1.45692*	.234945	**.001**	.90183	2.01201
		WINTER	1.05858*	.234945	**.001**	.50350	1.61367
	MONSOON	SUMMER	-1.45692*	.234945	**.001**	-2.01201	-.90183
		WINTER	-.39833	.234945	.210	-.95342	.15675
	WINTER	SUMMER	-1.05858*	.234945	**.001**	-1.61367	-.50350
		MONSOON	.39833	.234945	.210	-.15675	.95342
RGL	SUMMER	MONSOON	.08580*	.010992	**.001**	.05983	.11177
		WINTER	.02284	.010992	.097	-.00313	.04881
	MONSOON	SUMMER	-.08580*	.010992	**.001**	-.11177	-.05983
		WINTER	-.06297*	.010992	**.001**	-.08894	-.03700
	WINTER	SUMMER	-.02284	.010992	.097	-.04881	.00313
		MONSOON	.06297*	.010992	**.001**	.03700	.08894
HSI	SUMMER	MONSOON	1.1834*	.31754	**.001**	.4332	1.9337
		WINTER	1.8739*	.31754	**.001**	1.1237	2.6242
	MONSOON	SUMMER	-1.1834*	.31754	**.001**	-1.9337	-.4332
		WINTER	.6905	.31754	.078	-.0598	1.4407
	WINTER	SUMMER	-1.8739*	.31754	**.001**	-2.6242	-1.1237
		MONSOON	-.6905	.31754	.078	-1.4407	.0598
GiL	SUMMER	MONSOON	1.0938*	.15483	**.001**	.7279	1.4596
		WINTER	.8516*	.15483	**.001**	.4857	1.2174
	MONSOON	SUMMER	-1.0938*	.15483	**.001**	-1.4596	-.7279
		WINTER	-.2422	.15483	.264	-.6080	.1236
	WINTER	SUMMER	-.8516*	.15483	**.001**	-1.2174	-.4857
		MONSOON	.2422	.15483	.264	-.1236	.6080
GiWt	SUMMER	MONSOON	.1297*	.01522	**.001**	.0937	.1656
		WINTER	.0611*	.01522	**.001**	.0251	.0970
	MONSOON	SUMMER	-.1297*	.01522	**.001**	-.1656	-.0937
		WINTER	-.0686*	.01522	**.001**	-.1045	-.0326
	WINTER	SUMMER	-.0611*	.01522	**.001**	-.0970	-.0251
		MONSOON	.0686*	.01522	**.001**	.0326	.1045
LvWt	SUMMER	MONSOON	-.0577*	.01416	**.001**	-.0911	-.0242
		WINTER	.0763*	.01416	**.001**	.0428	.1097
	MONSOON	SUMMER	.0577*	.01416	**.001**	.0242	.0911
		WINTER	.1339*	.01416	**.001**	.1005	.1674
	WINTER	SUMMER	-.0763*	.01416	**.001**	-.1097	-.0428
		MONSOON	-.1339*	.01416	**.001**	-.1674	-.1005

N=256; *. The mean difference is significant at 0.05 level.

Table 9. Multivariate tests of *P. atherinodes* in Paschim Medinipur district.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.999	20952.388[b]	13.000	174.000	.000
	Wilks' Lambda	.001	20952.388[b]	13.000	174.000	.000
	Hotelling's Trace	1565.408	20952.388[b]	13.000	174.000	.000
	Roy's Largest Root	1565.408	20952.388[b]	13.000	174.000	.000
HABITAT	Pillai's Trace	.272	4.994[b]	13.000	174.000	.000
	Wilks' Lambda	**.728**	**4.994[b]**	**13.000**	**174.000**	**.001**
	Hotelling's Trace	.373	4.994[b]	13.000	174.000	.000
	Roy's Largest Root	.373	4.994[b]	13.000	174.000	.000
SEASON	Pillai's Trace	1.585	51.491	26.000	350.000	.000
	Wilks' Lambda	**.039**	**54.783[b]**	**26.000**	**348.000**	**.001**
	Hotelling's Trace	8.751	58.230	26.000	346.000	.000
	Roy's Largest Root	6.096	82.063[c]	13.000	175.000	.000
	Roy's Largest Root	.314	4.221[c]	13.000	175.000	.000

b. Exact statistic; c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table 10. Tests of Between-Subjects Effects of *P. atherinodes* in Paschim Medinipur district.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
HABITAT	GaSI	17.461	1	17.461	10.818	.347
	RGL	.021	1	.021	9.701	.001
	HSI	11.295	1	11.295	3.598	.059
	GiL	3.658	1	3.658	4.746	.031
	GiWt	.070	1	.070	8.858	.178
	LvWt	6.024	1	6.024	1.223	.270
SEASON	GaSI	112.068	2	56.034	34.716	**.001**
	RGL	.288	2	.144	66.387	**.001**
	HSI	196.681	2	98.340	31.325	**.001**
	GiL	39.883	2	19.941	25.877	**.001**
	GiWt	.387	2	.194	24.459	**.001**
	LvWt	13.907	2	6.953	1.411	.246

N=256

Table 11. Post Hoc Test seasonally of *P. atherinodes* in Paschim Medinipur district.

Dependent Variable	(I) SEASON	(J) SEASON	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
GaSI	SUMMER	MONSOON	1.86032*	.224588	**.001**	1.32970	2.39094
		WINTER	.75404*	.224588	**.003**	.22343	1.28466
	MONSOON	SUMMER	-1.86032*	.224588	**.001**	-2.39094	-1.32970
		WINTER	-1.10627*	.224588	**.001**	-1.63689	-.57566
	WINTER	SUMMER	-.75404*	.224588	**.003**	-1.28466	-.22343
		MONSOON	1.10627*	.224588	**.001**	.57566	1.63689
RGL	SUMMER	MONSOON	.09457*	.008233	**.001**	.07512	.11402
		WINTER	.04085*	.008233	**.001**	.02140	.06030
	MONSOON	SUMMER	-.09457*	.008233	**.001**	-.11402	-.07512
		WINTER	-.05372*	.008233	**.001**	-.07317	-.03427
	WINTER	SUMMER	-.04085*	.008233	**.001**	-.06030	-.02140
		MONSOON	.05372*	.008233	**.001**	.03427	.07317

Table 11. Continue.

Dependent Variable	(I) SEASON	(J) SEASON	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HSI	SUMMER	MONSOON	2.3427*	.31321	**.001**	1.6027	3.0827
		WINTER	1.8739*	.31321	**.001**	1.1339	2.6139
	MONSOON	SUMMER	-2.3427*	.31321	**.001**	-3.0827	-1.6027
		WINTER	-.4687	.31321	.295	-1.2088	.2713
	WINTER	SUMMER	-1.8739*	.31321	**.001**	-2.6139	-1.1339
		MONSOON	.4687	.31321	.295	-.2713	1.2088
GiL	SUMMER	MONSOON	1.0719*	.15518	**001**	.7052	1.4385
		WINTER	.8063*	.15518	**001**	.4396	1.1729
	MONSOON	SUMMER	-1.0719*	.15518	**001**	-1.4385	-.7052
		WINTER	-.2656	.15518	.204	-.6323	.1010
	WINTER	SUMMER	-.8063*	.15518	**001**	-1.1729	-.4396
		MONSOON	.2656	.15518	.204	-.1010	.6323
GiWt	SUMMER	MONSOON	.0827*	.01573	**001**	.0455	.1198
		WINTER	.1042*	.01573	**001**	.0671	.1414
	MONSOON	SUMMER	-.0827*	.01573	**001**	-.1198	-.0455
		WINTER	.0216	.01573	.358	-.0156	.0587
	WINTER	SUMMER	-.1042*	.01573	**001**	-.1414	-.0671
		MONSOON	-.0216	.01573	.358	-.0587	.0156
LvWt	SUMMER	MONSOON	-.5797	.39240	.304	-1.5068	.3474
		WINTER	-.0180	.39240	.999	-.9451	.9091
	MONSOON	SUMMER	.5797	.39240	.304	-.3474	1.5068
		WINTER	.5617	.39240	.327	-.3654	1.4888
	WINTER	SUMMER	.0180	.39240	.999	-.9091	.9451
		MONSOON	-.5617	.39240	.327	-1.4888	.3654

N=256; *. The mean difference is significant at 0.05 level.

captive propagation. The knowledge from the current study may be applied to better manage *P. atherinoides* in India and to future conservation strategies and adoption of these species as possible candidates for commercial aquaculture.

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REFERENCES

Alam, A., Gopinath, V., Jha, D. N., Joshi, K. D., Kumar, J., Das, S. C. S. & Das, B. K. (2020). Food and Feeding Biology of Commercially Important Freshwater Eel, *Mastacembelus armatus* (LACEPÈDE, 1800) from the Ganga River, India. *Oceanography & Fisheries, 11*(4), 0069-0073. http://dx.doi.org/10.19080/OFOAJ.2020.11.555819

Al-Hussain, A.H. (1949). On the functional morphology of alimentary tract of some fishes in relation to differences in their feeding habits; anatomy and histology. *Qualitative Journal of Microscopic Science, 90*, 109-139.

Victor Raj, M., Sivakumar, R., & Mathialagan, R. (2014). Food and feeding habit and length-weight relationship of the Asian striped catfish *Mystus vittatus* (Bloch, 1794) (Siluriformes: Bagridae) in the Vadavar River, Lower Anicut, Tamil Nadu. *Indian Journal of Science*, *8*(20), 55-64.

Begum, M., Alam, M. J., Islam, M. A., & Pal, H. K. (2008). On the food and feeding habit of an estuarine catfish (Mystus gulio Hamilton) in the south-west coast of Bangladesh. *University journal of zoology, Rajshahi University*, *27*, 91-94.

Chanda, A. & Jana, A. (2021). A comparative review on freshwater fish fauna between West Bengal and Odisha, two middle-east Indian states. *Journal of Fisheries*, *9*(3), 93302-93302.

Chattopadhyay, S., Nandi, S., & Saikia, S. K. (2014). Mouth morphometry and architecture of freshwater cat fish *Mystus vittatus* Bloch (1974) (Siluriformes, Bagridae) in relation to its feeding habit. *Journal of Scientific Research*, *6*(1), 169-174. http://dx.doi.org/10.3329/jsr.v6i1.16369

Chaturvedi, J., & Saksena, D. N. (2013). Diet composition, feeding intensity, gastrosomatic index and hepatosomatic index of a catfish, *Mystus cavasius* from Chambal river (near, Rajghat) Morena, Madhya Pradesh. *International Journal of Recent Scientific Research*, *4*, 1350-6.

Dasgupta, M. (2004). Relative length of the gut of some freshwater fishes of West Bengal in relation to food and feeding habits. *Indian Journal of Fisheries, 51*, 381-384.

Desai, V. R. (1970). Studies on the fishery and biology of *Tor tor* (Hamilton, 1822) from river Narmada. *Journal of Inland Fishery Society in India*, *2*(5), 101-112.

Dinh, M. Q., Tran, T. L., & Nguyen, T. K. T. (2018). The relative gut length and gastro-somatic indices of the mudskipper Periophthalmodon septemradiatus (Hamilton, 1822) from the Hau River. *VNU Journal of Science: Natural Sciences and Technology*, *34*(3), 75-83. https://doi.org/10.25073/2588-1140/vnunst.4775

Gogoi, B., Das, D. N., & Saikia, S. K. (2020). Feeding Ecology of *Pachypterus atherinoides* (Actinopterygii; Siluriformes; Schilbeidae): A Small Freshwater Fish from Floodplain Wetlands of Northeast India. *Croatian Journal of Fisheries: Ribarstvo*, *78*(3), 105-120. https://doi.org/10.2478/cjf-2020-0011

Gopalan, C., Rama Sastry, B.V. & Bala Subramanian, S.C. (2004). Nutritive value of Indian foods, *National Institute of Nutrition*, ICMR, Hyderabad, 45-67.

Gosavi, S. M., Tapkir, S. D., Kumkar, P., & Kharat, S. S. (2020). The reproductive biology of *Pachypterus khavalchor* (Kulkarni, 1952) in the Panchaganga River of the Western Ghats, a biodiversity hotspot in India. *Asian Fisheries Science*, *33*, 79-90. https://doi.org/10.33997/j.afs.2020.33.2.001

Gupta, S., & Banerjee, S. (2014). Food and feeding habit of a freshwater catfish, *Mystus tengara* (Siluriformes: Bagridae). *Journal of Ichthyology*, *54*(9), 742-748. https://doi.org/10.1134/S0032945214060071

Gupta, S. (2015). An Overview on Feeding and Breeding Biology of *Mystus tengara*, A Freshwater Catfish of Indian Subcontinent. *World*, *7*(3), 195-197. https://doi.org/10.5829/idosi.wjfms.2015.7.3.94217

Hynes, H. B. N. (1950). The food of fresh-water sticklebacks (Gasterosteus aculeatus and Pygosteus pungitius), with a review of methods used in studies of the food of fishes. *The journal of animal ecology*, 19(1), F36-58. https://doi.org/10.2307/1570

Jan, M., & Jan, N. (2017). Studies on the fecundity (F), gonadosomatic index (GSI) and hepatosomatic index (HSI) of Salmo trutta fario (Brown trout) at Kokernag trout fish farm, Anantnag, Jammu and Kashmir. *International Journal of Fisheries and Aquatic Studies*, *5*(6), 170-173.

Jana, A., Sit, G., & Chanda, A. (2021). Ichthyofaunal diversity of River Kapaleswari at Paschim Medinipur district of West Bengal, India. *Flora and Fauna*, *27*(1), 113-124. https://doi.org/10.33451/florafauna.v27i1pp113-124

Jana, A., Sit, G., Das, P., Chanda, A. & Sahu, S. K. (2022A). Seasonal Length-Weight Relationships and Condition Factors of *Mystus tengara* (Hamilton, 1822) in Two Habitats. *Aquatic Sciences and Engineering*, 37(4), 205-211. https://doi.org/10.26650/ASE202221159748

Jana, A., Sit, G., Chanda, A., Nayak, S. & Sahu, S. K. (2022B). Seasonal length-weight relationships and condition factors of *Pachypterus atherinoides* (Bloch, 1794) in two habitats. *Ecology, Environment and Conservation*; 28, S515-S521. http://doi.org/10.53550/EEC.2022.v28i06s.0083

Koundal, S., Dhanze, R., Koundal, A., & Sharma, I. (2013). Relative gut length and gastro-somatic index of six hill stream fishes, Himachal Pradesh, India. *Journal of Environment and Biosciences*, *27*(1), 11-18.

Kurbah, B. M., & Bhuyan, R. N. (2018). Analysis of feeding behaviour and gastro-somatic index (GaSI) during different phases of breeding cycle of *Monopterus cuchia* (Hamilton, 1822) from Meghalaya, India. *Journal of Applied and Natural Science*, *10*(4), 1187-1191. https://doi.org/10.31018/jans.v10i4.1907

Lanthaimeilu, K., & Bhattacharjee, P. (2018). Relative gut length and gastro-somatic index of *Pethia conchonius* (Hamilton, 1822) and *Trichogaster fasciata* Bloch & Schneider 1801, Tripura. *Journal of Entomology and Zoology Studies*, *6*(2), 2403-2407.

Menon, A.G.K. (1999). Check list - fresh water fishes of India. *Record in Zoological Survey of India*, Miscellaneous Publication, Occasional Paper No. 175.

Mitu, N. R. (2017). Reproductive biology of the female Asian striped dwarf catfish *Mystus tengara* (Hamilton, 1822) (Siluriformes: Bagridae) in the Ganges River of Rajshahi, northwestern Bangladesh. *Turkish Journal of Fisheries and Aquatic Sciences*, *17*(3), 589-598. https://doi.org/10.4194/1303-2712-v17_3_15

Mojumder, N., Saha, D., Utsa, S. S., Maruf, K. K., & Paul, S. K. (2020). Biology of the endangered queen loach (*Botia dario*) collected from wild sources in Bangladesh. *Aquaculture, Aquarium, Conservation & Legislation*, *13*(5), 2599-2609.

Nagar, K. C., & Sharma, M. S. (2016). Food and feeding habit of two hill stream fishes ie *Rasbora daniconius* and *Noemachielus botia* in selected stream of Southern Rajasthan, India. *Research journal of Animal Veterinary and Fishery Science*, *4*(6), 7-14.

Rajaguru, A. (1992). Biology of two co-occurring tonguefishes, *Cynoglossus arel* and *C. lida* (Pleuronectiformes: Cynoglossidae), from Indian waters. *Fishery Bulletin*, *90*(2), 328-367.

Sahil, Mogalekar, H.S., Chandran, S., Nayak, .S.K., Kumar, S. & Singh, S.K. (2023). Length-weight relationship and condition factor of selected small Cyprinid fishes from river Burhi Gandak in North Bihar, India. *Journal of the Inland Fisheries Society of India*, *54*(1), 27-32. https://epubs.icar.org.in/index.php/JIFSI/article/view/132388

Sharma, A. K., Malik, D. S., & Bargali, H. (2018). Food and Feeding Habits of *Schizothorox Richardsonii* (Gray, 1832) Inhibiting Bhagirathi River, Tehri Garhwal, India. *International Journal of Recent Scientific Research*, *9*(4), 25562-2556. https://doi.org/10.24327/IJRSR

Sit, G., Jana, A. & Chanda, A. (2020). Diversity of small indigenous freshwater ornamental fish under genus Puntius from Purba Medinipur, Paschim Medinipur and Jhargram districts of West Bengal, India. *Advances in Zoology and Botany*, *8*(4), 334-341. https://doi.org/10.13189/azb.2020.080405

Sit, G., Jana, A., Das, P., Chanda, A. & Sahu, S. K. (2022A). Induced breeding and embryonic development of indigenous ornamental fish *Puntius terio* (Hamilton, 1822). *Indian Hydrobiology*, 21(2), 43-51.

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2022B). Record of zipper loach *Paracanthocobitis botia* (Hamilton 1822), an ornamental fish from Paschim Medinipur, West Bengal, India. *Journal of Fisheries*, *10*(3), 103401-103401. https://doi.org/10.17017/j.fish.355

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2023A). Dose Selection for Induced Breeding and Larval Development of Indigenous Ornamental Fish *Puntius chola* (Hamilton, 1822). *Aquatic Sciences and Engineering*, 38(1), 62-67. https://doi.org/10.26650/ASE20221177106

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2023B). Record of zipper loach *Paracanthocobitis mackenziei* (Chaudhuri, 1910), an indigenous ornamental fish from West Bengal, India. *Journal of Fisheries*, *11*(2), 112402-112402. https://doi.org/10.17017/j.fish.477

Srivastava, S. C., Verma, P., Verma, A. K., & Singh, A. K. (2014). Assessment for possible metal contamination and human health risk of *Pangasianodon hypophthalmus* (Sauvage, 1878) farming, India. *International Journal of Fisheries and Aquatic Studies*, *1*, 176-181.

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Research Article

Revealing Mucilage Event-Linked Community Composition in the Sea of Marmara from eDNA Metabarcoding Data

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ABSTRACT

Mucilage events are among the most conspicuous phenomena in marine ecosystems and present numerous challenges in determining the composition of communities associated with them. To overcome this problem, we used environmental DNA (eDNA) metabarcoding approaches to reveal the species-level resolution of community composition. Mucilaginous aggregates were sampled at six collecting sites during a novel mucilage event (autumn 2021–summer 2022) in the Sea of Marmara, Türkiye. A wide range of plankton community compositions was detected in mucilage samples. eDNA metabarcoding was effective in predicting the community composition of mucilage, which is composed of a wide variety of organisms from mucilaginous aggregates.

Keywords: eDNA, metabarcoding, mucilage, the Sea of Marmara, molecular ecology, biomonitoring

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INTRODUCTION

Marine ecosystems have been facing a greater impact from human activities than that at any other time in history (Berry et al., 2019), driven by a combination of natural factors and hydrological conditions (Mecozzi et al., 2012). These activities, particularly industrialization (Balint et al., 2018), have had negative consequences such as exploitation, pollution, and habitat loss in marine ecosystems (Lotze et al., 2006). Furthermore, intense industrial activities have caused unprecedented changes in ecosystem functions (Cardinale et al., 2012; Smith, 2003) across the world in this era of anthropogenic degradation (Boussarie et al., 2018; Danovaro, Umani & Pusceddu, 2009). As a result of these impacts, one remarkable event is the occurrence of marine mucilage formation that has been reported over the past 200 years, with an increasing trend in frequency for over the past 40 years (Kovač et al., 2023, Faganeli et al., 2010; Turk et al., 2010; Danovaro et al., 2009). Recently in Türkiye, a eutrophication-linked event was also captured by the Landsat 8 – OLI satellite and shared on the official website of NASA with the title "Blooms in the Sea of Marmara" in the "images day" category in May 2015 (NASA Earth Observatory, 2015) in the Sea of Marmara (SoM). Subsequently, between autumn 2020 and summer 2021, a significant event of mucilage formation was captured by the satellites of Sentinel-2 and Worldview-3 (Tuzcu Kokal, Olgun, & Musaoglu, 2022), which was unprecedented in terms of the amount and duration of observation for the SoM.

Several studies (Fuks et al., 2005; MacKenzie, Sims, Beuzenberg & Gillespie, 2002) have indicated that mucilage phenomenon often begins in regions with stratified water columns. The SoM has also a two-layered water column, where the upper layer is low-salinity Black Sea water, and the lower layer is high-salinity Mediterranean Sea water (Ünlülata, Oğuz, Latif & Özsoy, 1990) with a boundary between them known as a pycnocline (Beşiktepe et al., 1994). The ecosystem of the SoM, which is composed of biological components from these seas, is unique (İşinibilir Okyar, Üstün & Orun, 2015).

Subsequently, the Istanbul (Bosporus) and Çanakkale (Dardanelles) straits have been established as a result of bidirectional dual water-mass exchange events, and studies have indicated that the last occurrence of this event was during the Holocene period at the end of the Würm Glaciation (Çağatay et al., 2009).

In addition to the stratified water columns of the SoM (Çağatay et al., 2015), the effects of anthropogenic activities on the coastal cities of the SoM are of significant concern (Aksu, Balkis, Taşkin & Erşan, 2011) and should be thoroughly investigated (Burak et al., 2009) in terms of mucilage formation. As a nearly enclosed intercontinental basin, the SoM (size ~ 70 × 250 km) (Albayrak, Balkis, Zenetos, Kurun & Erşan, 2006) contains straits that act as biological corridors (Demirel et al., 2023), making it an important region in several manners. To understand the key elements of the SoM that initiate mucilage formations, it is crucial to explore the following points, among others: (i) the SoM is an inland sea between the Anatolian and Thrace peninsulas (Wong, Lüdmann, Ulug & Görür, 1995); (ii) Istanbul, situated on the coast of SoM, is one of the most populous cities in the world with a significant impact on the region's anthropogenic activities (Karaca, 2013); (iii) the region is home to 20% of Türkiye's population and 87% of Türkiye's coastal population (Algan, Balkıs, Çağatay, & Sarı, 2004); (iv) industrial activities in the region have caused significant environmental harm, primarily affecting the coastal and shelf areas of the SoM (Korkmaz et al., 2022); (v) the historical peninsula of Istanbul, including the Yenikapı port and the Golden Horn Estuary, as well as well-delineated polluted coastal inlets such as Erdek (Balkıs & Çağatay, 2001) and Izmit Bay have typically been significant in human settlement (Lotze et al., 2006) throughout history (Onar et al., 2013; Algan et al., 2004); (vi) intense industrial activities responsible for the majority of anthropogenic discharges to the SoM continue to operate in these areas (Demirel et al., 2023); (vii) a high volume of tanker traffic carrying oil, thousands of vessels per day, poses a constant threat to the SoM ecosystem (Albayrak et al., 2006); and (viii) the metropolitan city of Istanbul, with a population of 16 million in 2023 (Türkiye İstatistik Kurumu, 2023), has been an attraction for people for centuries and is facing ongoing challenges related to waste management and pollution (Güneralp et al., 2021). Moreover, Yaşar (2001) reported that Izmit Bay receives untreated domestic waste from two million inhabitants living around its shores, combined with solid and liquid waste discharge from 300 large industrial plants, contributing to pollution in the bay. Furthermore, as emphasized in studies conducted by Okay et al. (Okay et al., 2001; Okay et al., 1998; Okay et al., 1996), Izmit Bay faces challenges in dealing with toxicity, heavy ship traffic, and petroleum refineries, which supply >30% of Turkey's demand, located on the northeastern coast of the bay.

Marine biofilms are colonized primarily by surface-associated (Salta et al., 2013) marine organisms (Dang & Lovell, 2016), which are determined by source type, planktonic activities (Gram et al., 2002), and competition between organisms (Bosch, 2013). Extracellular particles, such as DNA, are released by marine organisms, and can be utilized as a supply of nutrients (including carbon, nitrogen, and phosphorus), which are essential for growth and biofilm development (Finkel & Kolter, 2001) of bacterial communities (Das et al., 2013). Surface-associated/attached communities of microorganisms (Muhammad et al., 2020), zooplankton feces, or feeding structures contain oil compounds, and mucus-rich particles formed by bacteria, which contribute to the formation of marine mucilage (Burd et al., 2020). Zooplankton (e.g., copepods and amphipods) as consumers of marine mucilage or oil provide for the sinking of oil into the seafloor (Almeda, Connelly, & Buskey, 2016; Schwing et al., 2015; Almeda et al., 2014; Fisher et al., 2014; Montagna et al., 2013; Mitra et al., 2012; Conover 1971) and also contribute to the production of marine oil snow with their fecal pellets (Burd et al., 2020). The dynamic and heterogeneous community structure of marine biofilms makes it challenging to model and investigate. Fungi secrete extracellular polymeric substances (Metzger et al., 2009), similar to those secreted by bacteria and phytoplankton, and particularly diatoms (Wotton, 2004). These substances act as a "glue" that holds the different components of marine mucilage together (Burd et al., 2020). Furthermore, the reflection of key environmental factors of a substratum by biofilms has vital implications for larval settlement of marine invertebrates (Dobretsov, 2010).

Despite numerous studies having emphasized the presence of mucilage in the SoM through traditional marine surveillance programs (Toklu-Alicli, Ozdelice & Durmus et al., 2021; Balkıs-Ozdelice, Durmuş, & Balci, 2021; Toklu-Alicli, Polat & Balkıs-Ozdelice et al., 2020; Tas, Kus & Yilmaz, 2020; Ergul et al., 2021, İşinibilir-Okyar et al., 2015; Yilmaz, 2015; Altiok & Kayişoğlu, 2015; Balkis et al., 2011; Tüfekçi et al., 2010) such as "continuous plankton recording" and/or "trawl," (Zingone et al., 2021), these methods are not always easy to use or cost-effective (Zaiko et al., 2015; Barbour, 1999; Jennings & Kaiser, 1998) to monitor the biodiversity (Thomsen et al., 2016). Nevertheless, emerging DNA-based tools, such as environmental DNA (eDNA) metabarcoding, are cost-effective, nature-friendly, and widely adopted and promising for future applications (Zaiko, Samuiloviene & Ardura, 2015; Pochon et al., 2013; Wood et al., 2013; Darling & Mahon, 2011). Mucilage phenomena have been one of the most conspicuous in terms of characterization (Pompei et al., 2003), and their characterization involves several difficulties. However, eDNA analysis (Ogram et al.,1987) is a molecular tool that can overcome some of the abovementioned difficulties (Genitsaris, Stefanidou & Sommer, 2019, Zaiko et al., 2015; Del Campo et al., 2014; Mächler et al., 2014; Bik et al., 2012) to ensure effective biomonitoring. The emerging science of eDNA (Kelly et al., 2014) refers to the genetic remnants of life that can be obtained from a wide range of environmental samples (Taberlet, 2012). This method can provide detailed taxonomic resolution even when the samples are bulk mixtures of organisms obtained via plankton tows (Berry et al., 2019; Taberlet et al., 2018; Thomsen & Willerslev, 2015).

In this study, we applied eDNA metabarcoding approaches to explore the poorly studied biodiversity of marine mucilage/snow using a molecular approach. We built on the study of Doğan et al. (in press) and going to the species-level characterization, we attempt to help understand the possible linkages between the components, including eukaryotic organisms (algae, fungi, and animalia), and community composition of mucilage samples, using the CO1 gene, collected from selected stations in the SoM during the novel 2020–2021 mucilage event, as mentioned earlier.

MATERIALS AND METHODS

During the Marmara Sea and North Aegean Sea Expedition (in June 2021), three replicates of mucilage samples were obtained from six stations (M1–M6) (Figure 1), targeting 0–1 m of the surface seawater layer in the water column, via Yunus-S Research/Vessel (R/V). We followed and modified the protocol outlined by Buxton et al. (2021) and collected three samples of mucilage using negative field controls, according to Keskin (2014), at each station.

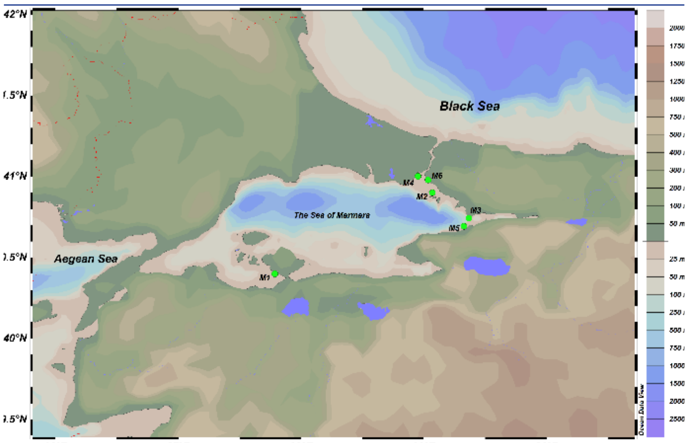

Figure 1. Study area in the Sea of Marmara, (M) indicating the location of the six sampling sites: M1, M2, M3, M4, M5, and M6, as the Bay of Erdek, Kınalıada, Bay of İzmit, Yenikapı, Çınarcık, and Kalamış, respectively.

DNA extraction was performed using the DNeasy Plant Pro Kit-Qiagen (Germany) according to the manufacturer's instructions. The primer pairs of CO1, as described elsewhere (Leray, et al., 2013), were used for the initial PCR assay. The index primers were incorporated into the second PCR assay based on their specific locations and genes. The PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA). The concentration of the pool was determined and validated using the KAPA Library Quantification qPCR kit (Roche, Germany). The pool was then sequenced on an Illumina platform (Illumina, USA) with paired reads (2 × 150 bp) (Gen Era Diagnostics Inc., Türkiye).

Following the sequencing of NovaSeq, S4, 2 × 150 bp read length service, the raw sequences were received as demultiplexed fastq files and processed using The Advanced Pipeline for Simple yet Comprehensive Analyses of DNA metabarcoding data (the APSCALE graphical user interface) "apscale_gui" pipeline v1.2.0 (https://github.com/TillMacher/apscale_gui) (Buchner, Macher & Lesse 2022), which is based on VSEARCH (Rognes, Flouri, Nichols, Quince & Mahe, 2016) and cutadapt (Martin, 2011). This module consisted of demultiplexing, paired-end merging, primer trimming, quality & length filtering, dereplication & pooling, Operational Taxonomic Units (OTU) clustering, denoising (ESVs), chimera removal, LULU filtering, and remapping steps.

The analysis commenced with the adapter primer trimming stage, as the raw data had already been demultiplexed by the sequencing company. The early stages of the analysis pipeline comprised the deletion of primer sequences and tags by cutadapt (Martin, 2011) and the evaluation of the quality of each read based on specific per-base quality and read length thresholds. The pooled and dereplicated reads were clustered into OTUs based on the similarity threshold (97%) and denoised into "Exact Sequence Variants" (ESVs). Chimeras were automatically detected and removed from the OTUs and ESVs using the vsearch -uchime_denovo command. OTUs and ESVs were mapped against the dereplicated files. The LULU filtering algorithm (Frøslev et al., 2017) was used to reduce the number of erroneous OTUs/ESVs and achieve more realistic biodiversity metrics. Finally, the OTUs and ESVs were remapped to the sequences of each sample, and read tables were generated. A taxonomic assignment was performed using the BOLD system with the BOLDigger module (Buchner & Leese, 2020) and Midori2 (MIDORI2_UNIQ_NUC_GB257_CO1_BLAST) in the local blast module. The final taxonomy table was generated using the "JAMP filter" option (Elbrecht, 2022). During this process, ambiguous records were flagged along with their respective situation.

The TaxonTableTools (TTT) v1.4.8 (Macher, Beermann & Leese, 2021) was used for downstream analyses. A taxon table was generated using the taxon table converter module, with the taxonomy and read tables generated in previous analyses serving as input. The replicates were merged, considering of the consistency of the OTUs present in each replicate. Subsequently, progress was made in the taxon table filtering module, where taxa exhibiting <85% similarity were eliminated. The read threshold was set at 0.01%. Negative control subtraction was performed as the final step.

The assigned taxonomy of each OTU in the taxon table was validated against the Global Biodiversity Information Facility (GBIF) database (https://www.gbif.org/). Consequently, any spelling errors were corrected, and the synonyms for each respective taxon were examined and updated automatically. The reads were processed by normalization and metadata were generated to facilitate the execution of downstream analyses. Basic statistics (read count, OTU number, taxonomic resolution, and richness) were generated. Venn diagrams were generated by comparing the BOLD and Midori taxon tables at each taxonomic level, ranging from OTU numbers to phyla. Rarefaction analyses were performed using both all-in-one and per-taxon approaches with the aim of determining the number of species and/or OTUs that separated among each respective taxonomic group. The species-level site occupancy was calculated using a heatmap representation of presence/absence of data. Per-taxon statistical analyses were conducted to retrieve the count of reads, number of OTUs, and species. Parallel categorical analyses were performed to determine the samples that were represented by species and/or higher taxonomic levels. The distribution of designated taxa was confirmed by cross-referencing with the GBIF using an Application Programming Interface (API). At the phylum and species levels, read proportions were calculated and plotted using heat maps, bar charts, and pie charts. Krona charts were generated to display both single sample and combined data points (Supplementary Data).

Diversity analyses were conducted by computing both the alpha and beta diversity measures. Specifically, we determined alpha diversity by calculating the number of OTUs per sample, and for beta diversity, we computed Jaccard distances between samples. The taxon table was used to generate a taxon list that incorporated all recognized taxa and diminished redundancy. In cases where numerous OTUs were assigned to the species level, the maximum, average, and minimum genetic distances between OTUs were calculated to discern highly diverse or cryptic species.

RESULTS AND DISCUSSION

Analyzing the CO1-based community composition within mucilage samples obtained from collection sites

Following the sequencing process, the raw reads underwent processing, resulting in the trimming of 3,078,342 M reads, with an average of 1,669,203 and 1,284,649 reads that successfully passed the quality-filtering step. Subsequently, the preprocessing steps were clustered into 1657 OTUs for further analysis. Of these, 429 records were found as "No Match," 172 records were found as "blank," and the remaining 1056 records were assigned to OTUs. After curation (merging of replicates and subtraction of negative controls), 431 OTUs (629,535 reads) with a similarity of ≥85% to the reference sequence remained and were selected for downstream analyses. Among the records of the 431 OTUs, 22 were ambiguous. The average sequence length was 118 bp, with a minimum length of 100 bp and a maximum length of 147 bp. A total of 34 phyla, 78 classes, 157 orders, 106 families, 65 genera, and 57 species, all of which were unique to each taxonomic level, were observed against the Midori2 database.

When we compared our basic statistics in both Midori2 and BOLD databases, 22 phyla were detected as shared between the two databases, including Amoebozoa, Arthropoda, Ascomycota, Bacillariophyta, Bryozoa, Cnidaria, Echinodermata, Mollusca, Porifera, Rhodophyta, and Rotifera. In addition, Midori2 identified 10 phyla, including Bigyra, Cercozoa, Choanoflagellata, and Evosea, and BOLD identified 5 phyla, including Heterokontophyta, Onychophora, and Zygomycota. At the class level, the 39 taxonomic groups that were detected by both Midori2 and BOLD databases and were the most abundant based on OTUs and species were Anthozoa, Bivalvia, Bacillariophyceae, Copepoda, Dinophyceae, Gastropoda, Hydrozoa, Rhodophyta, Scyphozoa, and Staurozoa. The two databases under consideration shared 214 OTUs (Figure 2a) and 23 species (Figure 2b), whereas Midori2 detected an additional 32 species not found in BOLD, and BOLD detected 16 species not found in Midori2. Moreover, Midori2 detected 217 OTUs not present in BOLD, whereas BOLD detected 143 OTUs not present in Midori2 (Figure 2). The analysis of the two databases revealed that a significant overlap exists between the two, with approximately 20% of OTUs (Figure 3a) and species (Figure 3b) being shared between them.

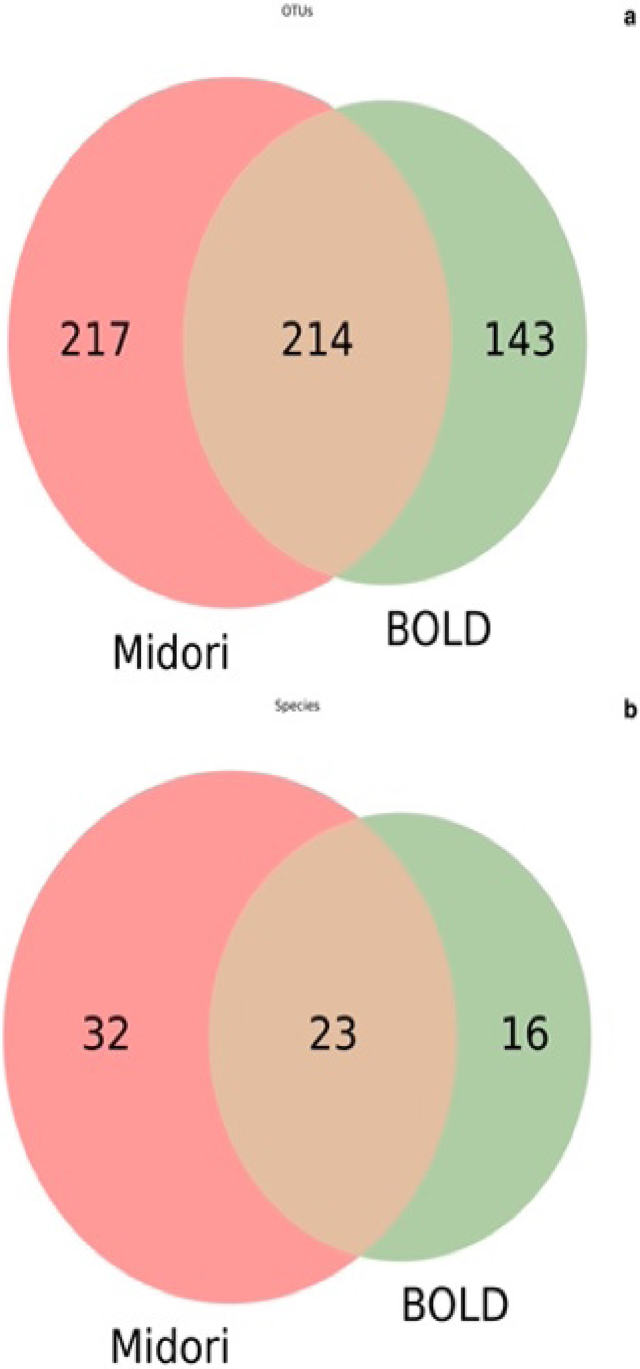

Figure 2. A comparison of Midori2 and BOLD databases focusing on (a) OTU and (b) species in each database.

The relative abundance and distribution proportions of the orders across different samples were investigated by comparing the BOLD and Midori2 databases. Amphipoda, Caenogastropoda, Ceramiales, Cheliostomatida, Corallinales, Diplostraca, Gastrochaenida, Perciformes, Sessila, Stauromedusae, and Thalassiosirales correlated in both databases (Figure 4).

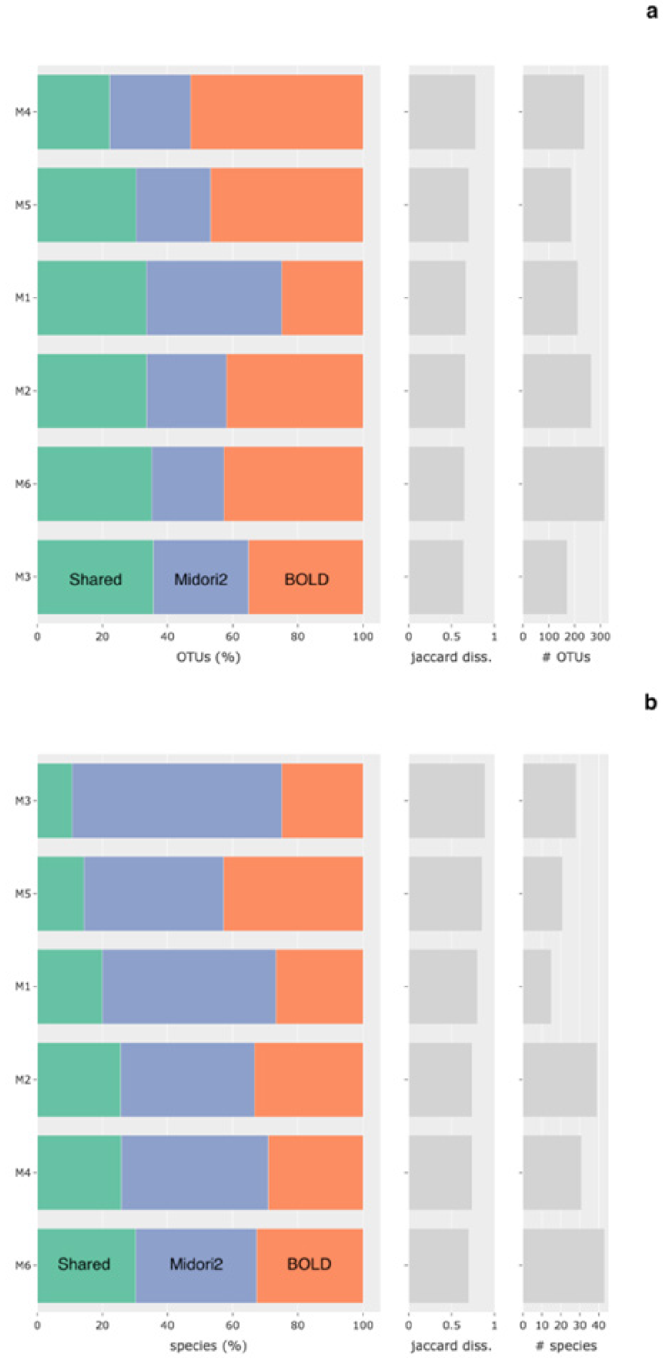

Figure 3. (a) OTUs and (b) species sources between Midori2 and BOLD databases.

At the species level, taxonomic richness retrieved from BOLD and Midori2 databases correlated per phylum. Arthropoda, Rhodophyta, Mollusca, and Ochrophyta were the most prominent phyla (Figure 5).

By selecting the top 10 phyla containing the most OTUs, the sample-based rarefaction analysis demonstrated that 15/52 species observed in the first sample subsequently increased to 41/52 in the all-in-one method (Figure 6a). Testing the species and OTUs to separate among phyla (Figure 6b) revealed that the phylum Ochrophyta reached eight species (8/8) in the second sample, which flattened in subsequent samples. The phylum Mollusca reached five species in the third sample and ended with seven (7/7) species in the second sample. The phylum Cnidaria reached two species (2/2) in the third sample and remained constant throughout the samples (Figure 6b).

The M6 sample exhibited the greatest degree of alpha diversity among all samples, whereas the M3 and M5 samples were limited to fewer than 115 OTUs/30 species each (Figure 7). The Kalamış (M6) sample had 181 OTUs (Figure 7a) (29 OTUs at species level) and 23 species (Figure 7b), following the M1 sample with 159 OTUs. The M3 and M5 samples exhibited the lowest degree of alpha diversity among all samples, as evidenced by the presence of 111 and 100 OTUs, respectively. Despite the comparatively small number of OTUs in M1 and M5 samples, the number of reads at these samples was substantial, with 140 k reads recorded in the M1 sample and 108 k reads in the M5 sample. Compared with the M1 sample, which had a low number of OTUs at the total (111) and species level (13) and 33 k reads, the M2 sample exhibited a higher number of OTUs of 26 at the species level and 121 k reads (Figure 7).

A total of 87 OTUs were identified in the phylum Arthropoda, 73 OTUs were identified in the phylum Ochrophyta, 46 OTUs were identified in the in the phylum Rhodophyta, and 41 OTUs were identified in the in the phylum Mollusca. Conversely, the remaining 27 phyla were represented by fewer than three species each. Various taxonomic groups such as Amoebozoa (27), Chlorophyta (7), Dinoflagellata (4) from Protozoa, Cnidaria (14), Porifera (6), and Echinodermata (3) from Animalia showed the presence of OTUs. Overall, 87 OTUs of the phylum Arthropoda were assigned to 15 distinct species, 73 OTUs of the phylum Ochrophyta were assigned to species, 41 OTUs of the phylum Mollusca were assigned to six species, and 46 OTUs of the phylum Rhodophyta were assigned to five species. Despite comprising the most numbers of OTUs, the phylum Arthropoda exhibited a relatively low proportion of reads by <4%. In contrast, members of the phylum Cnidaria (Figure 8a), including *Aurelia aurita*, exhibited a dominant presence, accounting for >40% of all reads (for samples with >97% similarity) (Figure 8b). Members of Ascomycota, including *Cladosporium allicinum*, accounted for 9% of the reads, followed by the members of Mollusca, such as *Bittium reticulatum*, accounting for 5% of the reads, whereas the coccolithophore *Emiliania huxleyi* (3%) was also represented.

Among the identified Arthropoda OTUs, the class Insecta consisted of the most abundant OTUs, with 56 OTUs comprising >60% of the OTUs. Despite this, only seven species were assigned to this class. In contrast, the class Copepoda that included five OTUs was well represented by three distinct species and comprised >80% of Arthropoda reads. The calanoids *Paracalanus parvus* (83% reads of Copepoda) and *Pseudocalanus elongatus* (13% reads of Copepoda) were dominant among copepod species, with low read representation by the cyclopoid *Oithona similis* (1% reads of Copepoda and 56% of Cyclopoida), with 100% similarity. Furthermore, the maxillopods *Amphibalanus amphitrite* (96% reads of Maxillopoda) and *Amphibalanus impro-*

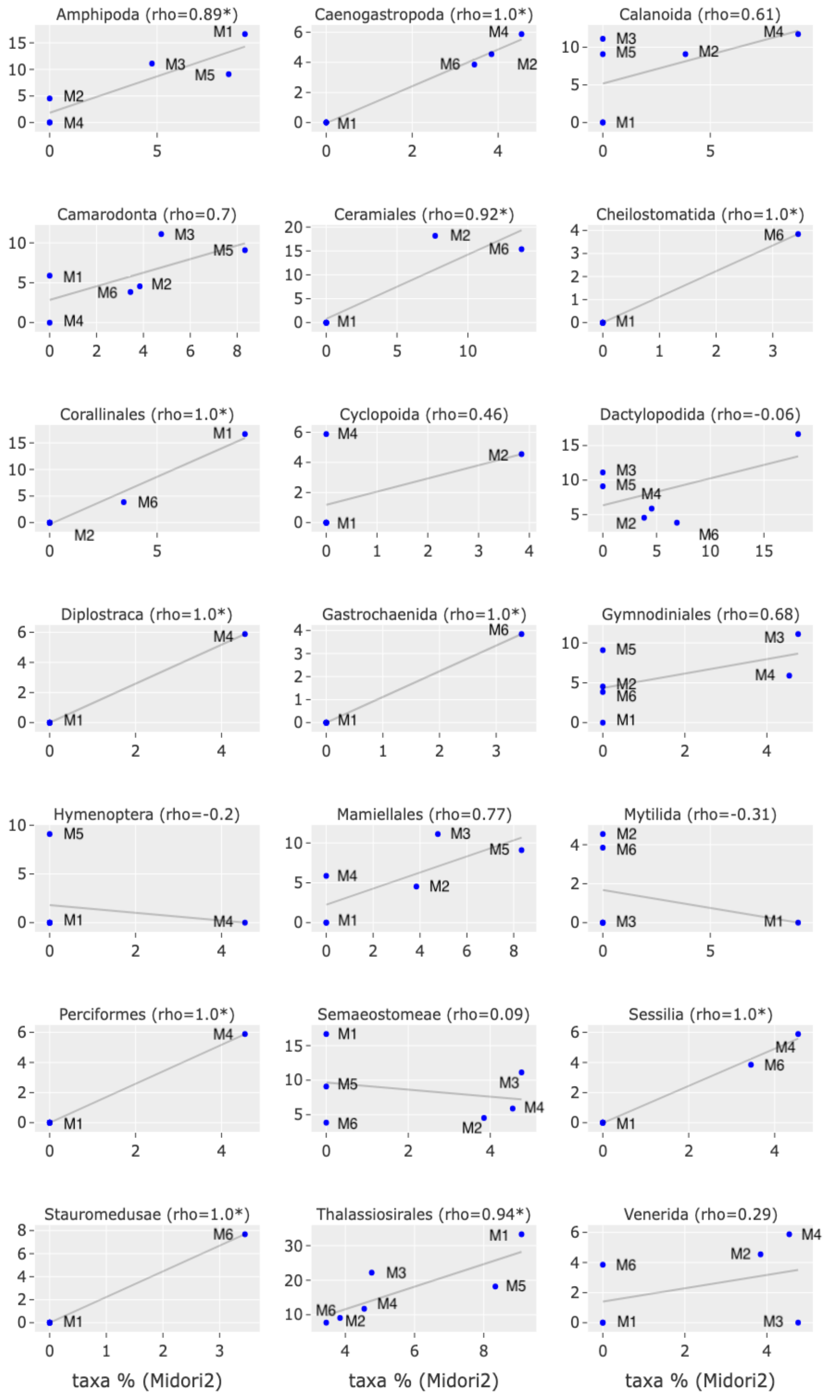

Figure 4. Taxon proportions, pairwise analysis of samples (including >98% similarity): rho (Spearman's rank correlation coefficient), samples marked with an asterisk indicate a positive monotonic relationship (they display consistency in the same direction).

visus (4% reads of Maxillopoda) and the branchiopod *Pleopsis polyphemoides* were represented by three OTUs with 100% similarity and comprised 10% reads of arthropods. Remarkably, nine OTUs were classified as ambiguous and subsequently assigned to the same species, viz., *Lucilia caesar*, which belongs to the order Diptera.

A total of 73 OTUs were detected within the phylum Ochrophyta. In particular, 12 OTUs belonged to the order Ectocarpales, and 16 OTUs were assigned to the order Naviculales within the phylum Ochrophyta. Among the phylum Rhodophyta, 25 OTUs were identified within the order Ceramiales, and the species-level match was well represented by the following species: *Apoglos-*

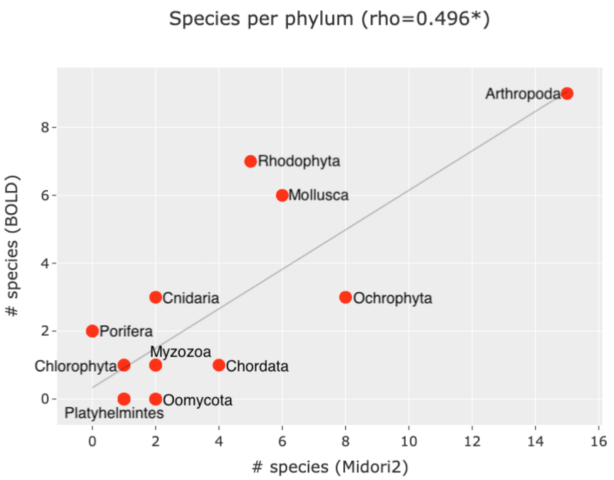

Figure 5. Comparisons of taxonomic richness across each database (BOLD and Midori2) based on phylum level.

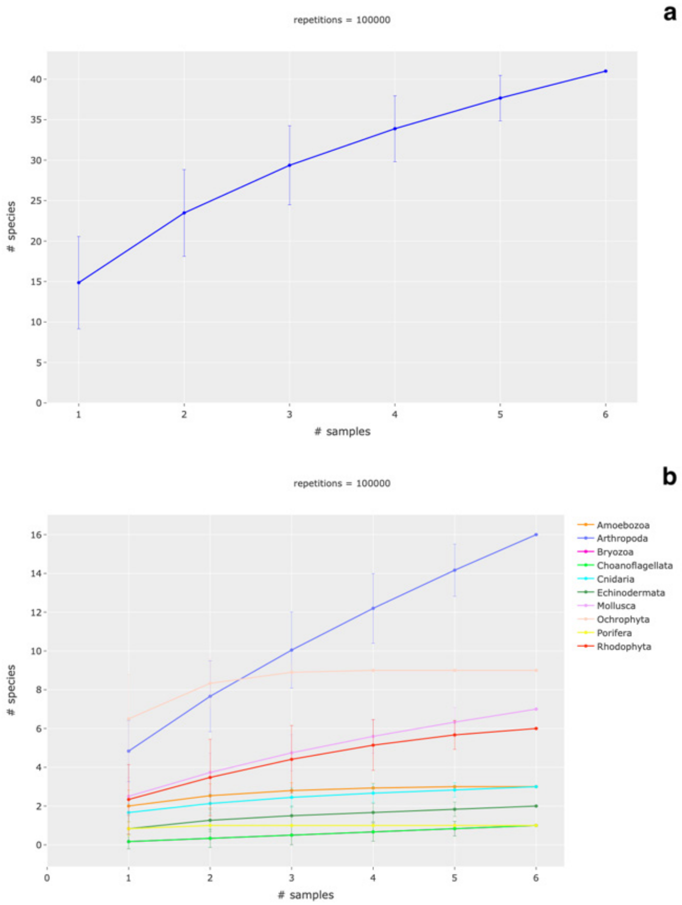

Figure 6. The use of sample-based rarefaction was examined through the application of both all-in-one (a) and per-taxon (b) approaches (100,000 repetitions) in top 10 phyla.

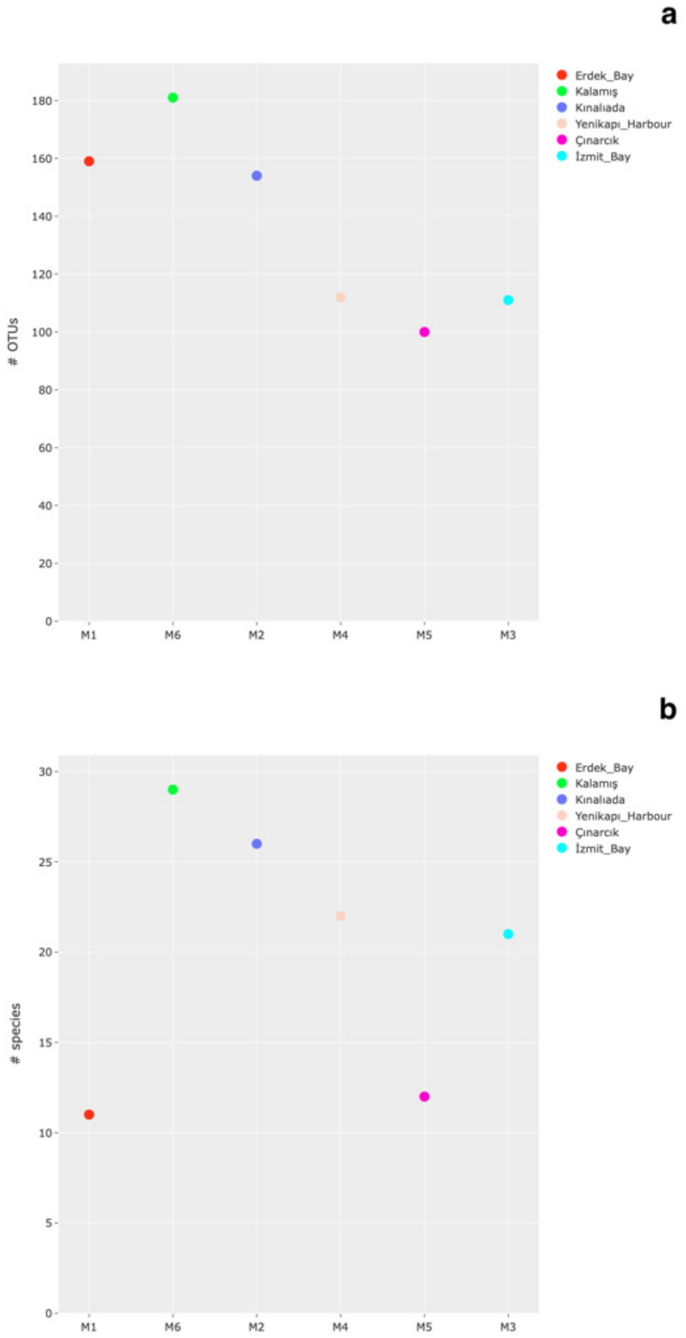

Figure 7. Alpha diversity, comprising (a) the number of OTUs and (b) the number of species per sample.

sum ruscifolium (19% of Rhodophyta reads), *Polysiphonia morrowii*, *Dasysiphonia japonica*, and *Pterothamnion crispum*, all of which exhibited 100% similarity. Furthermore, 11 OTUs were identified within the order Bacillariales that exhibited a remarkable abundance of reads within the phylum Ochrophyta, which comprised three OTUs identified for Cylindrotheca and two OTUs identified for the genus *Nitzschia*. The order Thalassiosirales comprised three OTUs, which included a two-species-level representation of *Skeletonema pseudocostatum* (1% of all reads)

Figure 8. The proportions of reads for (a) each phylum (including all data) and (b) species (for samples with >97% similarity) in a heat map and bar graph among samples.

(observed among all mucilage samples) and *Skeletonema* sp., with 100% similarity. Although taxonomic assignment was 100% similar to the genus *Skeletonema* due to the multiple species hits (*Skeletonema dohrnii*, *Skeletonema marinoi*, and *Skeletonema costatum*), these OTUs corresponded to a single genus (*Skeletonema*). Nevertheless, despite the presence of 12 OTUs in the order Ectocarpales, no OTUs were assigned to species-level representation. The order Lithodesmiales was represented by *Ditylum brightwellii*. The order Chaetocerotales comprised two OTUs that were assigned to *Chaetoceros socialis* with 100% similarity. The order Rhizosoleniales was represented by two OTUs, both of which were assigned to the family Rhizosoleniaceae.

Among the protists, 27 OTUs were identified within the phylum Amoebozoa, exhibiting a high similarity of 85%–93% to the class Discosea. In addition, two OTUs and one taxa *Squamamoeba japonica* were well represented at the species level, with 100% similarity. Four OTUs and two species were identified within Dinoflagellata as follows: *Pfiesteria piscicida* belonged to the order Peridiniales, and *Cochlodinium polykrikoides* and *Gyrodinium instriatum* belonged to the order Gymnodiniales. Although five OTUs were assigned to the Bigyra class, no species were identified within this assignment. The coccolithophore *Emiliania huxleyi* (3% of all reads) that belonged to the phylum Haptophyta was represented by a single OTU. A total of 49 OTUs were identified within Fungi, of which 42 were present in both Oomycota and Basidiomycota, with 22 and 18, respectively, and 9 OTUs were exclusive to Ascomycota. Although Fungi exhibited a relatively high representation, only three OTUs could match at the species level, with their similarity exceeding 100%. These OTUs belonged to the following species: *Cladosporium allicinum* (9% of all reads and 95% of Ascomycota reads), *Aspergillus puulaauensis*, *Globisporangium spinosum* (Oomycota), and *Anisolpidium ectocarpii* (Basidiomycota).

An analysis of the phylum Porifera revealed six OTUs, with five of these belonging to the Demospongiae class and the remaining one belonging to the Homoscleromorpha class. The Demospongiae class was represented by four different orders in Midori2 and *Halisarca desqueyrouxae* with 99% similarity, which belongs to the Halisarcidae family in the BOLD database. A total of 12 OTUs were identified in the phylum Cnidaria, of which 5 were assigned to the Hydrozoa class, and two species were identified. One of the OTUs matched with the Hydractiniidae family with 93% similarity, and three of them were assigned to the Staurozoa class, with one matched with the Lucernariidae family at 91% similarity and another matched with the order Stauromedusae at

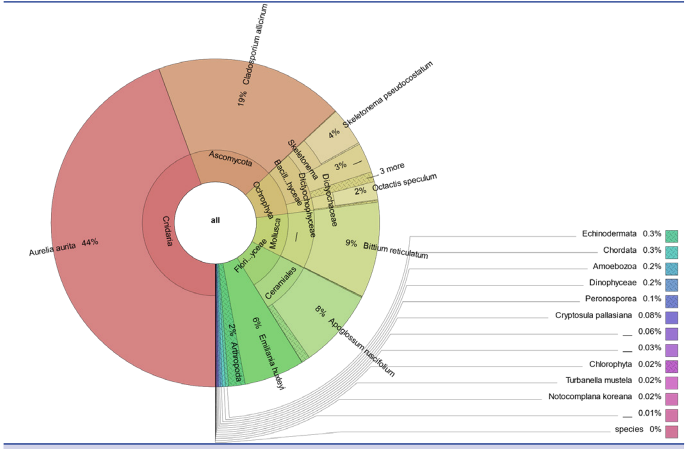

Figure 9. Krona charts based on read proportions (>97% similarity) when sampling locations were merged.

85% similarity. Furthermore, two of them matched with *Haliclystus inabai* and *Calvadosia cruciformis* species with 100% similarity. OTU_2 has been categorized as a member of the class Scyphozoa with 100% similarity to the moon jellyfish *Aurelia aurita*, along with the remarkable high number of reads (99% of Cnidaria) (Figure 9) assigned to this taxon. An analysis of the phylum Mollusca revealed the presence of 41 OTUs. The classes Bivalvia and Gastropoda comprised eight and 32 OTUs, respectively. Multiple species, including *Chamelea gallina* (18% of Bivalvia), *Mytilus edulis* (16% of Bivalvia), *Rocellaria dubia* (3% of Bivalvia), and *Spisula subtruncata* (2% of Bivalvia), exhibited 100% similarity to their corresponding OTUs belonging to the Bivalvia class. Remarkably, two distinct OTUs (OTUs_470 and OTUs_1926) were identified in the same species, *Chamelea gallina*. Due to the possibility of assigning multiple genera in both *Venus verrucosa* and *Chamelea gallina* of OTU_1926, this situation of OTU was considered ambiguous. Despite this ambiguity, the OTU was assigned as *Chamelea gallina* because of its dominance during the taxonomic assignment process. In addition, six of these species matched with the order Littorinimorpha within the class Gastropoda (6% of all and 98% of Mollusca) and had a high number of reads. The classes Neogastropoda and Stylommatophora each contained three and two OTUs, respectively. Both the green sea slug *Elysia viridis* and the sea snail *Bittium reticulatum* (>5% of all, and 85% of Gastropoda reads) (Figure 9) were assigned to their respective OTUs with 100% similarity. The phylum Echinodermata yielded three OTUs, with two of these belonging to the class Echinoidea. In particular, two of these OTUs (OTU_131 and OTU_852) were assigned to the same species (*Paracentrotus lividus*), exhibiting 100% similarity.

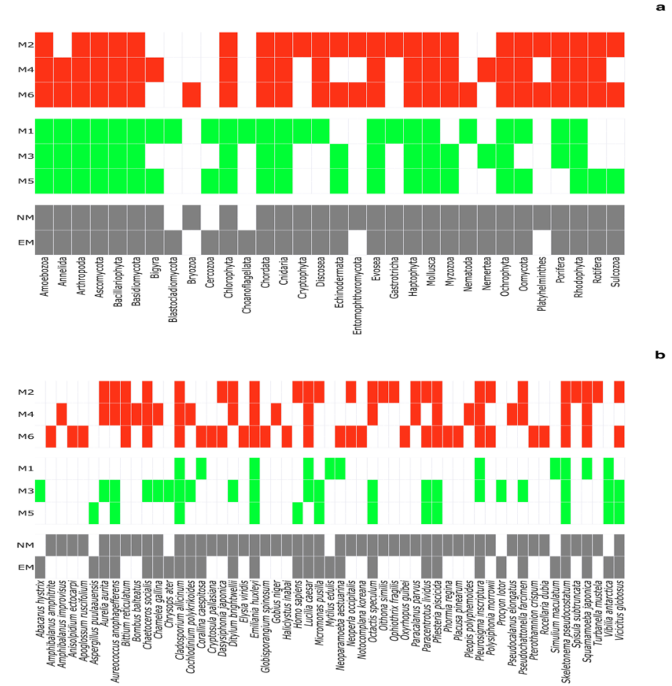

Figure 10. Site occupancy between both phylum (a) and species (b) levels (NM: Northeastern Marmara and EM: Eastern Marmara).

In most locations and regions of northeastern and eastern Marmara, all phyla were well represented, except for Bryozoa, Cercozoa, and Choanoflagellata (Figure 10a). Although Bryozoa were found only at the Kalamış sample in northeastern Marmara, members of Cercozoa were identified in Erdek Bay and Çınarcık in eastern Marmara, and Choanoflagellata members were found only in Erdek Bay (Figure 10b).

In our analysis of similarity test (Anosim), we detected a slight dissimilarity in the composition of OTUs among the regions (R = −0.3, indicating a mild dissimilarity) (Figure 11).

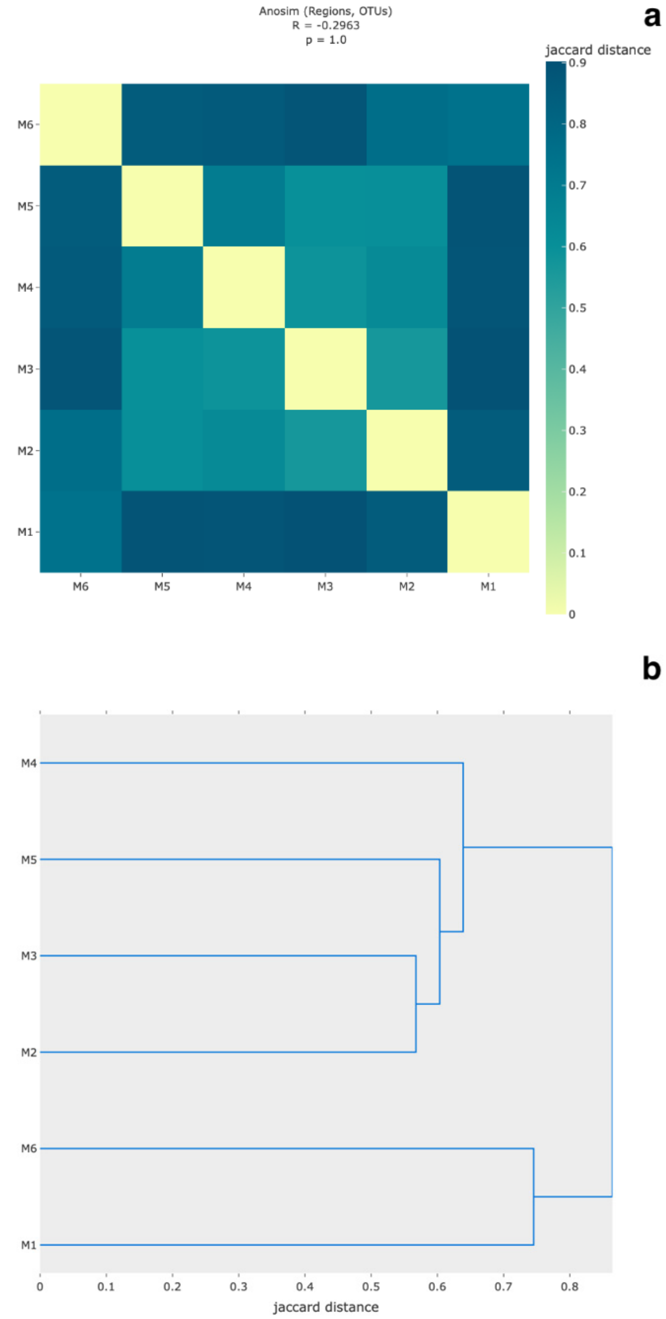

Figure 11. The beta diversity of samples, as determined by the Anosim, Jaccard distance.

In contrast to the representative phyla (Figure 12a), the absence/presence of species was not uniform across all regions (NM and EM), except for a few cosmopolitan organisms such as *Aurelia aurita*, *Aureococcus anophagefferens*, *Cladosporium allicinum*, *Corallina caespitosa*, *Emiliania huxleyi*, *Micromonas pusilla*, *Neoparamoeba aestuarina*, *Octactis speculum*, *Paracentrotus lividus*, *Cochlodinium polykrikoides*, *Gyrodinium instriatum*, *Pfiesteria piscicida*, *Pseudochattonella farcimen*, *Skeletonema pseudocostatum*, and *Vicicitus globosus* (Figure 12b).

This study represents one of the first extensive investigations to utilize metabarcoding approaches to analyze community composition, including protists, fungi, and animalia, associated with the mucilage events that occurred during the novel 2020–2021 mucilage event in the SoM. Results revealed the community composition of the mucilage and identified the predominant microorganisms thriving among samples.

Taxonomic assignment revealed that the mucilage samples were categorized into three orders of the main category and were rep-

Figure 12. An examination of categories, particularly the taxonomic structure of mucilage samples, exploring the phyla (a) and (b) species compositions.

resented by unicellular eukaryotes (protists), fungi, and other multicellular eukaryotes. We detected a variety of groups, including harmful dinoflagellates such as *Cochlodinium polykrikoides* (Gobler, 2008), *Gyrodinium instriatum* (Nagasoe et al., 2006), and *Pfiesteria piscicida* (Burkholder & Glasgow Jr, 1997) that are implicated in the formation of red tides, which have the potential to cause fish death and fishery losses. Within the class Bacillariophyceae, some important groups that could have also contributed to the 2007–2008 mucilage event in the SoM, such as the genera *Cylindrotheca*, *Ditylum* (*Ditylum brightwellii*), and *Skeletonema* (*Skeletonema pseudocostatum*), were also detected.

The coccolithophores *Emiliania huxleyi* and *Dictyocha speculum*, which contribute to carbon and silica cycles, especially in fundamental mineral fluxes within the global ecosystems (Turley, 1991), were observed in our analyses. In the early 2000s, researchers recorded the occurrence of some *D. speculum* species for the first time in the SoM at relatively close stations and at depths with our sampling regime (Deniz, Taş & Koray, 2006), corroborating the findings of the two studies. The dataset has also revealed the presence of filamentous fungal species such as *Cladosporium allicinum* and *Aspergillus puulaauensis* as well as *Globisporangium spinosum* and *Anisolpidium ectocarpii* within the realm of Fungi, which release extracellular polymeric compounds similar to those released by bacteria and phytoplankton that serve as a glue in mucilage (Burd et al., 2020).

Some red algal species, including *Apoglossum ruscifolium*, *Corallina caespitosa*, and *Polysiphonia morrowii*, known for having orders that produce polysaccharides (Duarte, et al., 2004; Usov, 2011), were also observed. We also observed the presence of the bryozoan *Cryptosula* that depends on phytoplankton as a major food source, as well as some microspecies such as bivalves (*Chamelea gallina*, *Mytilus edulis*, *Rocellaria dubia*, and *Spisula subtruncata*) as consumers of mesozooplankton (Davenport, Smith, & Packer, 2000), gastropods (*Bittium reticulatum* and *Elysia viridis*), copepods (Calanoids: *Paracalanus parvus*, *Pseudocalanus elongatus*, and Cyclopoid: *Oithona similis*), the cladoceran *Pleopsis polyphemoides*, and the moon jellyfish *Aurelia aurita*. Among these, some taxa, including the abovementioned ones, were the most abundant during the 2018 mucilage event in the area (Okyar et al., 2015), and their (copepods and cladocerans) prevalence and dominance among zooplankton have been recorded in the northeastern SoM (Isinibilir et al., 2008). Chitinous zooplankton, such as copepods and their fecal pellets are recognized as hotspots for microbial activity. Specifically, copepods, such as *Paracalanus parvus*, *Pseudocalanus elongatus*, and *Oithona similis*, which play vital roles in the pelagic food web (Turner, 2004), and their documented mucilage-consuming habits, were also part of the animal composition. Moreover, the scyphozoan *Aurelia aurita*, a species associated with the major bacterial groups (Kos Kramar et al., 2019) and widely acknowledged as a significant player in marine ecosystems (Weiland-Bräuer et al., 2015), was observed. Studies (Brodeur et al., 2002; Sommer & Lengfellner, 2008) have demonstrated that *Aurelia aurita* can significantly influence ecosystem dynamics by affecting planktonic food web structure. By consuming ichthyoplankton, jellyfish exhibit predatory behavior and can potentially compete with fish (Purcell, 2005).

The phylum Mollusca has been well represented in animals that comprised certain groups such as Gastropoda (*Bittium reticulatum*) and Bivalvia, which are commonly documented in marine snow (Shanks & Walters, 1997). Specifically, the gastropod species *B. reticulatum* (12% reads of Animalia) was also previously demonstrated to be associated with eutrophication (Gacia et al., 2009) and biofilm formation (Castejón-Silvo & Terrados, 2017) (D'alelio et al., 2011). Moreover, some gastropod species are affected by the toxicity of algal species (Díaz, 2006) that also comprise nutrient sources (e.g., *Apoglossum ruscifolium* and *Dasysiphonia japonica*); these two algal species were also detected in our dataset.

CONCLUSION

The application of eDNA metabarcoding tools may provide a snapshot of the community composition of the factors that trigger mucilage formation events. Continuous attempts to gather data at a larger and more continuous timescale are crucial to improve our understanding of this phenomenon.

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Author contributions: Mİ, OD, and RB were responsible for devising the study's design. OD conducted the environmental DNA (eDNA) sampling, while ADÖ and OD managed the laboratory workflow and wet-lab process, respectively. OD performed bioinformatic analyses, visualization, and writing, whereas RB supervised the dry laboratory process. Mİ, OD, and RB composed the initial draft of the manuscript. Mİ supervised the project, administered it, and secured funding, while all authors contributed to the manuscript and approved the final version for publication.

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Conflict of interest: The authors have no conflicts of interest to declare.

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REFERENCES

Aksu, A., Balkis, N., Taşkin, Ö. S., & Erşan, M. S. (2011). Toxic metal (Pb, Cd, As and Hg) and organochlorine residue levels in hake (Merluccius

merluccius) from the Marmara Sea, Turkey. *Environmental monitoring and assessment*, *182*, 509-521. https://doi.org/10.1007/s10661-011-1893-1

Albayrak, S., Balkis, H., Zenetos, A., Kurun, A., & Kubanç, C. (2006). Ecological quality status of coastal benthic ecosystems in the Sea of Marmara. *Marine Pollution Bulletin*, *52*(7), 790-799. https://doi.org/10.1016/j.marpolbul.2005.11.022

Algan, O., Balkıs, N., Çağatay, M. N., & Sarı, E. (2004). The sources of metal contents in the shelf sediments from the Marmara Sea, Turkey. *Environmental Geology*, *46*, 932-950. https://doi.org/10.1007/s00254-004-1104-2

Almeda, R., Baca, S., Hyatt, C., & Buskey, E. J. (2014). Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods. *Ecotoxicology*, *23*, 988-1003. https://doi.org/10.1007/s10646-014-1242-6

Almeda, R., Connelly, T. L., & Buskey, E. J. (2016). How much crude oil can zooplankton ingest? Estimating the quantity of dispersed crude oil defecated by planktonic copepods. *Environmental pollution*, *208*, 645-654. https://doi.org/10.1016/j.envpol.2015.10.041

Altiok, H., & Kayişoğlu, M. (2015). Seasonal and interannual variability of water exchange in the Strait of Istanbul. *Mediterranean Marine Science*, *16*(3), 644-655. https://doi.org/10.12681/mms.1225

Amsler, C. D., Iken, K., McClintock, J. B., Amsler, M. O., Peters, K. J., Hubbard, J. M., ... & Baker, B. J. (2005). Comprehensive evaluation of the palatability and chemical defenses of subtidal macroalgae from the Antarctic Peninsula. *Marine Ecology Progress Series*, *294*, 141-159. https://doi.org/10.3354/meps294141

Balint, M., Pfenninger, M., Grossart, H. P., Taberlet, P., Vellend, M., Leibold, M. A., ... & Bowler, D. (2018). Environmental DNA time series in ecology. *Trends in Ecology & Evolution*, *33*(12), 945-957. https://doi.org/10.1016/j.tree.2018.09.003

Balkıs, N., & Çağatay, M. N. (2001). Factors controlling metal distributions in the surface sediments of the Erdek Bay, Sea of Marmara, Turkey. *Environment international*, *27*(1), 1-13. https://doi.org/10.1016/s0160-4120(01)00044-7

Balkis, N., Atabay, H., Türetgen, I., Albayrak, S., Balkis, H., & Tüfekçi, V. (2011). Role of single-celled organisms in mucilage formation on the shores of Büyükada Island (the Marmara Sea). *Journal of the Marine Biological Association of the United Kingdom*, *91*(4), 771-781. https://doi.org/10.1017/s0025315410000081

Balkis-Ozdelice, N., Durmuş, T., & Balci, M. (2021). A preliminary study on the intense pelagic and benthic mucilage phenomenon observed in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, *8*(4), 414-422. https://doi.org/10.30897/ijegeo.954787

Barbour, M. T. (1999). *Rapid bioassessment protocols for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates and fish*. US Environmental Protection Agency, Office of Water.

Berry, T. E., Saunders, B. J., Coghlan, M. L., Stat, M., Jarman, S., Richardson, A. J., ... & Bunce, M. (2019). Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. *PLoS genetics*, *15*(2), e1007943. https://doi.org/10.1371/journal.pgen.1007943

Beşiktepe, Ş. T., Sur, H. I., Özsoy, E., Latif, M. A., Oğuz, T., & Ünlüata, Ü. (1994). The circulation and hydrography of the Marmara Sea. *Progress in Oceanography*, *34*(4), 285-334. https://doi.org/10.1016/0079-6611(94)90018-3

Bik, H. M., Porazinska, D. L., Creer, S., Caporaso, J. G., Knight, R., & Thomas, W. K. (2012). Sequencing our way towards understanding global eukaryotic biodiversity. *Trends in ecology & evolution*, *27*(4), 233-243. https://doi.org/10.1016/j.tree.2011.11.010

Bosch, T. C. (2013). Cnidarian-microbe interactions and the origin of innate immunity in metazoans. *Annual review of microbiology*, *67*, 499-518. https://doi.org/10.1146/annurev-micro-092412-155626

Brodeur, R. D., Sugisaki, H., & Hunt Jr, G. L. (2002). Increases in jellyfish biomass in the Bering Sea: implications for the ecosystem. *Marine Ecology Progress Series*, *233*, 89-103. https://doi.org/10.3354/meps233089

Buchner, D., & Leese, F. (2020). BOLDigger–a Python package to identify and organise sequences with the Barcode of Life Data systems. *Metabarcoding and Metagenomics*, *4*, e53535. https://doi.org/10.3897/mbmg.4.53535

Buchner, D., Macher, T. H., & Leese, F. (2022). APSCALE: advanced pipeline for simple yet comprehensive analyses of DNA metabarcoding data. *Bioinformatics*, *38*(20), 4817-4819. https://doi.org/10.3897/mbmg.4.53535

Burak, S., Unlu, S., & Gazıoglu, C. (2009). Environmental stress created by chemical pollution in the Marmara sea (Turkey). *Asian Journal of Chemistry*, *21*(4), 3166-3174. https://doi.org/10.1016/j.envpol.2020.115801

Burd, A. B., Chanton, J. P., Daly, K. L., Gilbert, S., Passow, U., & Quigg, A. (2020). The science behind marine-oil snow and MOSSFA: past, present, and future. *Progress in Oceanography*, *187*, 102398. https://doi.org/10.1016/j.pocean.2020.102398

Burkholder, J. M., & Glasgow Jr, H. B. (1997). Pfiesteria piscicida and other Pfiesreria-like dinoflagellates: Behavior, impacts, and environmental controls. *Limnology and Oceanography*, *42*(5part2), 1052-1075. https://doi.org/10.4319/lo.1997.42.5_part_2.1052

Buxton, A., Matechou, E., Griffin, J., Diana, A., & Griffiths, R. A. (2021). Optimising sampling and analysis protocols in environmental DNA studies. *Scientific Reports*, *11*(1), 11637. https://doi.org/10.1038/s41598-021-91166-7

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., ... & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59-67. https://doi.org/10.1038/nature11148

Conover, R. J. (1971). Some relations between zooplankton and bunker C oil in Chedabucto Bay following the wreck of the tanker Arrow. *Journal of the Fisheries Board of Canada*, *28*(9), 1327-1330.

Çağatay, M. N., Eriş, K., Ryan, W. B. F., Sancar, Ü., Polonia, A., Akçer, S., ... & Bard, E. (2009). Late Pleistocene–Holocene evolution of the northern shelf of the Sea of Marmara. *Marine Geology*, *265*(3-4), 87-100. https://doi.org/10.1016/j.margeo.2009.06.011

Çağatay, M. N., Wulf, S., Sancar, Ü., Özmaral, A., Vidal, L., Henry, P., ... & Gasperini, L. (2015). The tephra record from the Sea of Marmara for the last ca. 70 ka and its palaeoceanographic implications. *Marine Geology*, *361*, 96-110. https://doi.org/10.1016/j.margeo.2015.01.005

Castejón-Silvo, I., & Terrados, J. (2017). Experimental assessment of Posidonia oceanica-associated gastropods grazing on an early-successional biofilm community: nutrient availability and species-specific effects. *Marine Ecology*, *38*(1), e12381. https://doi.org/10.1111/maec.12381

D'alelio, D., Cante, M. T., Russo, G. F., Totti, C., & De Stefano, M. (2011). Epizoic diatoms on gastropod shells: when substrate complexity selects for microcommunity complexity. *All flesh is grass: Plant-animal interrelationships*, 345-364. https://doi.org/10.1007/978-90-481-9316-5_16

Dahms, H. U., & Dobretsov, S. (2017). Antifouling compounds from marine macroalgae. *Marine Drugs*, *15*(9), 265. https://doi.org/10.3390/md15090265

Dang, H., & Lovell, C. R. (2016). Microbial surface colonization and biofilm development in marine environments. *Microbiology and molecular biology reviews*, *80*(1), 91-138. https://doi.org/10.1128/mmbr.00037-15

Danovaro, R., Fonda Umani, S., & Pusceddu, A. (2009). Climate change and the potential spreading of marine mucilage and microbial pathogens in the Mediterranean Sea. *PLoS One*, *4*(9), e7006. https://

doi.org/10.1371/journal.pone.0007006

Darling, J. A., & Mahon, A. R. (2011). From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental research*, *111*(7), 978-988. https://doi.org/10.1016/j.envres.2011.02.001

Das, T., Sehar, S., & Manefield, M. (2013). The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. *Environmental microbiology reports*, *5*(6), 778-786. https://doi.org/10.1111/1758-2229.12085

Davenport, J., Smith, R. J., & Packer, M. (2000). Mussels Mytilus edulis: significant consumers and destroyers of mesozooplankton. *Marine Ecology Progress Series*, *198*, 131-137. https://doi.org/10.3354/meps198131

Del Campo, J., Sieracki, M. E., Molestina, R., Keeling, P., Massana, R., & Ruiz-Trillo, I. (2014). The others: our biased perspective of eukaryotic genomes. *Trends in ecology & evolution*, *29*(5), 252-259. https://doi.org/10.1016/j.tree.2014.03.006

Demirel, N., Akoglu, E., Ulman, A., Ertor-Akyazi, P., Gül, G., Bedikoğlu, D., ... & Yilmaz, I. N. (2023). Uncovering ecological regime shifts in the Sea of Marmara and reconsidering management strategies. *Marine Environmental Research*, *183*, 105794. https://doi.org/10.1016/j.marenvres.2022.105794

Deniz, N., Taş, S., & Koray, T. (2006). New records of the Dictyocha antarctica Lohmann, Dictyocha crux Ehrenberg and Nitzschia rectilonga Takano species from the Sea of Marmara. *Turkish Journal of Botany*, *30*(3), 213-216.

Díaz, E., Güldenzoph, C., Molis, M., McQuaid, C., & Wahl, M. (2006). Variability in grazer-mediated defensive responses of green and red macroalgae on the south coast of South Africa. *Marine Biology*, *149*, 1301-1311. https://doi.org/10.1007/s00227-006-0320-6

Dobretsov, S. (2010). Marine biofilms. *Biofouling*, 123-136. https://doi.org/10.1002/9781444315462.ch9

Dogan, O., Örün, D. A., Bilgin, R., İşinibilir, M., Using the metabarcoding approach for characterization of community diversity in the Sea of Marmara. *Ecological Changes in the Sea of Marmara*. (In press).

Duarte, M. E., Cauduro, J. P., Noseda, D. G., Noseda, M. D., Gonçalves, A. G., Pujol, C. A., ... & Cerezo, A. S. (2004). The structure of the agaran sulfate from Acanthophora spicifera (Rhodomelaceae, Ceramiales) and its antiviral activity. Relation between structure and antiviral activity in agarans. *Carbohydrate Research*, *339*(2), 335-347. https://doi.org/10.1016/j.carres.2003.09.028

Elbrecht, V. (2022, January 7). *Just Another Metabarcoding Pipeline*. Retrieved from Github: http://github.com/VascoElbrecht/JAMP

Ergul, H. A., Balkis-Ozdelice, N., Koral, M., Aksan, S., Durmus, T., Kaya, M., ... & Canli, O. (2021). The early stage of mucilage formation in the Marmara Sea during spring 2021. *Journal of the Black Sea/Mediterranean Environment*, *27*(2).

Faganeli, J., Mohar, B., Kofol, R., Pavlica, V., Marinšek, T., Rozman, A., ... & Vuk, A. Š. (2010). Nature and lability of Northern Adriatic macroaggregates. *Marine drugs*, *8*(9), 2480-2492. https://doi.org/10.3390/md8092480

Finkel, S. E., & Kolter, R. (2001). DNA as a nutrient: novel role for bacterial competence gene homologs. *Journal of bacteriology*, *183*(21), 6288-6293. https://doi.org/10.1128/jb.183.21.6288-6293.2001

Fisher, C. R., Demopoulos, A. W., Cordes, E. E., Baums, I. B., White, H. K., & Bourque, J. R. (2014). Coral communities as indicators of ecosystem-level impacts of the Deepwater Horizon spill. *Bioscience*, *64*(9), 796-807. https://doi.org/10.1093/biosci/biu129

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature communications*, *8*(1), 1188. https://doi.org/10.1038/s41467-017-01312-x

Fuks, D., Radić, J., Radić, T., Najdek, M., Blažina, M., Degobbis, D., & Smodlaka, N. (2005). Relationships between heterotrophic bacteria and cyanobacteria in the northern Adriatic in relation to the mucilage phenomenon. *Science of the total environment*, *353*(1-3), 178-188. https://doi.org/10.1016/j.scitotenv.2005.09.015

Gacia, E., Costalago, D., Prado, P., Piorno, D., & Tomas, F. (2009). Mesograzers in Posidonia oceanica meadows: an update of data on gastropod-epiphyte-seagrass interactions. https://doi.org/10.1515/bot.2009.054

Genitsaris, S., Stefanidou, N., Sommer, U., & Moustaka-Gouni, M. (2019). Phytoplankton blooms, red tides and mucilaginous aggregates in the urban Thessaloniki Bay, Eastern Mediterranean. *Diversity*, *11*(8), 136. https://doi.org/10.3390/d11080136

Gobler, C. J., Berry, D. L., Anderson, O. R., Burson, A., Koch, F., Rodgers, B. S., ... & Nuzzi, R. (2008). Characterization, dynamics, and ecological impacts of harmful Cochlodinium polykrikoides blooms on eastern Long Island, NY, USA. *Harmful Algae*, *7*(3), 293-307. https://doi.org/10.1016/j.hal.2007.12.006

Gram, L., Grossart, H. P., Schlingloff, A., & Kiørboe, T. (2002). Possible quorum sensing in marine snow bacteria: production of acylated homoserine lactones by Roseobacter strains isolated from marine snow. *Applied and Environmental Microbiology*, *68*(8), 4111-4116. https://doi.org/10.1128/aem.68.8.4111-4116.2002

Güneralp, B., Xu, X., & Lin, W. (2021). Infrastructure development with (out) ecological conservation: the Northern Forests in İstanbul. *Regional Environmental Change*, *21*(3), 86. https://doi.org/10.1007/s10113-021-01807-w

Haavisto, F., Välikangas, T., & Jormalainen, V. (2010). Induced resistance in a brown alga: phlorotannins, genotypic variation and fitness costs for the crustacean herbivore. *Oecologia*, *162*, 685-695. https://doi.org/10.1007/s00442-009-1494-7

Isinibilir, M., Kideys, A. E., Tarkan, A. N., & Yilmaz, I. N. (2008). Annual cycle of zooplankton abundance and species composition in Izmit Bay (the northeastern Marmara Sea). *Estuarine, Coastal and Shelf Science*, *78*(4), 739-747. https://doi.org/10.1016/j.ecss.2008.02.013

Jennings, S., & Kaiser, M. J. (1998). The effects of fishing on marine ecosystems. In *Advances in marine biology* (Vol. 34, pp. 201-352). Academic Press. https://doi.org/10.1016/s0065-2881(08)60212-6

Karaca, F. (2013). Mapping the corrosion impact of air pollution on the historical peninsula of Istanbul. *Journal of Cultural Heritage*, *14*(2), 129-137. https://doi.org/10.1016/j.culher.2012.04.011

Kelly, R. P., Port, J. A., Yamahara, K. M., Martone, R. G., Lowell, N., Thomsen, P. F., ... & Crowder, L. B. (2014). Harnessing DNA to improve environmental management. *Science*, *344*(6191), 1455-1456. https://doi.org/10.1126/science.1251156

Keskin, E. (2014). Detection of invasive freshwater fish species using environmental DNA survey. *Biochemical Systematics and Ecology*, *56*, 68-74. https://doi.org/10.1016/j.bse.2014.05.003

Korkmaz, N. E., Savun-Hekimoğlu, B., Aksu, A., Burak, S., & Caglar, N. B. (2022). Occurrence, sources and environmental risk assessment of pharmaceuticals in the Sea of Marmara, Turkey. *Science of The Total Environment*, *819*, 152996. https://doi.org/10.1016/j.scitotenv.2022.152996

Kos Kramar, M., Tinta, T., Lučić, D., Malej, A., & Turk, V. (2019). Bacteria associated with moon jellyfish during bloom and post-bloom periods in the Gulf of Trieste (northern Adriatic). *PloS one*, *14*(1), e0198056. https://doi.org/10.1371/journal.pone.0198056

Kovač, N., Viers, J., Faganeli, J., Bajt, O., & Pokrovsky, O. S. (2023). Elemental Composition of Plankton Exometabolites (Mucous Macroaggregates): Control by Biogenic and Lithogenic Components. *Metabolites*, *13*(6), 726. https://doi.org/10.3390/metabo13060726

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., ... & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in zoology*, *10*(1), 1-14. https://doi.org/10.1186/1742-9994-10-34

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., ... & Jackson, J. B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 1806-1809. https://doi.org/10.1126/science.1128035

Macher, T. H., Beermann, A. J., & Leese, F. (2021). TaxonTableTools: A comprehensive, platform-independent graphical user interface software to explore and visualise DNA metabarcoding data. *Molecular Ecology Resources*, *21*(5), 1705-1714. https://doi.org/10.1111/1755-0998.13358

Mächler, E., Deiner, K., Steinmann, P., & Altermatt, F. (2014). Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science*, *33*(4), 1174-1183. https://doi.org/10.1086/678128

MacKenzie, L., Sims, I., Beuzenberg, V., & Gillespie, P. (2002). Mass accumulation of mucilage caused by dinoflagellate polysaccharide exudates in Tasman Bay, New Zealand. *Harmful algae*, *1*(1), 69-83. https://doi.org/10.1016/s1568-9883(02)00006-9

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12. https://doi.org/10.14806/ej.17.1.200

Mecozzi, M., Pietroletti, M., Scarpiniti, M., Acquistucci, R., & Conti, M. E. (2012). Monitoring of marine mucilage formation in Italian seas investigated by infrared spectroscopy and independent component analysis. *Environmental Monitoring and Assessment*, *184*, 6025-6036. https://doi.org/10.1007/s10661-011-2400-4

Metzger, U., Lankes, U., Fischpera, K., & Frimmel, F. H. (2009). The concentration of polysaccharides and proteins in EPS of Pseudomonas putida and Aureobasidum pullulans as revealed by 13 C CPMAS NMR spectroscopy. *Applied microbiology and biotechnology*, *85*, 197-206. https://doi.org/10.1007/s00253-009-2218-y

Mitra, S., Kimmel, D. G., Snyder, J., Scalise, K., McGlaughon, B. D., Roman, M. R., ... & Campbell, P. L. (2012). Macondo‐1 well oil‐derived polycyclic aromatic hydrocarbons in mesozooplankton from the northern Gulf of Mexico. *Geophysical Research Letters*, *39*(1). https://doi.org/10.1029/2011gl049505

Montagna, P. A., Baguley, J. G., Cooksey, C., Hartwell, I., Hyde, L. J., Hyland, J. L., ... & Rhodes, A. C. (2013). Deep-sea benthic footprint of the Deepwater Horizon blowout. *PloS one*, *8*(8), e70540. https://doi.org/10.1371/journal.pone.0070540

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., ... & Huang, T. (2020). Beyond risk: bacterial biofilms and their regulating approaches. *Frontiers in microbiology*, *11*, 928. https://doi.org/10.3389/fmicb.2020.00928

Nagasoe, S., Toda, S., Shimasaki, Y., Oshima, Y., Uchida, T., & Honjo, T. (2006). Growth inhibition of Gyrodinium instriatum (Dinophyceae) by Skeletonema costatum (Bacillariophyceae). *African Journal of Marine Science*, *28*(2), 325-329. https://doi.org/10.2989/18142320609504171

NASA Earth Observatory. (2015, May 17). *Blooms in the Sea of Marmara.* Retrieved from NASA Earth Observatory: https://earthobservatory.nasa.gov/images/85947/blooms-in-the-sea-of-marmara

Ogram, A., Sayler, G. S., & Barkay, T. (1987). The extraction and purification of microbial DNA from sediments. *Journal of microbiological methods*, *7*(2-3), 57-66. https://doi.org/10.1016/0167-7012(87)90025-x

Okay, O. S., Legović, T., Tüfekçi, V., Egesel, L., & Morkoç, E. (1996). Environmental impact of land-based pollutants on Izmit Bay (Turkey): short-term algal bioassays and simulation of toxicity distribution in the marine environment. *Archives of Environmental Contamination and Toxicology*, *31*, 459-465. https://doi.org/10.1007/bf00212428

Okay, O. S. (1998). Investigation of three wastewaters entering Izmit Bay (Turkey) by means of batch and chemostat culture algal bioassays. *Marine environmental research, 46*(1-5), 283-288. https://doi.org/10.1016/s0141-1136(97)00115-3

Okay, O. S., Egesel, L., Tüfekçi, V., Morkoç, E., & Gaines, A. (1998). Investigation of three wastewaters entering Izmit Bay (Turkey) by means of batch and chemostat culture algal bioassays. *Marine environmental research*, *46*(1-5), 283-288. https://doi.org/10.1016/s0141-1136(97)00115-3

İşinibilir-Okyar, M., Üstün, F., & Orun, D. A. (2015). Changes in abundance and community structure of the zooplankton population during the 2008 mucilage event in the northeastern Marmara Sea. *Turkish Journal of Zoology*, *39*(1), 28-38.

Onar, V., Alpak, H., Pazvant, G., Armutak, A., Gezer İnce, N. A. Z. A. N., & Kızıltan, Z. (2013). A bridge from Byzantium to modern day Istanbul: An overview of animal skeleton remains found during Metro and Marmaray excavations. *Istanbul Univ Vet Fak Derg*, *39*, 1-8. https://doi.org/10.9775/kvfd.2014.11767

Pochon, X., Bott, N. J., Smith, K. F., & Wood, S. A. (2013). Evaluating detection limits of next-generation sequencing for the surveillance and monitoring of international marine pests. *PloS one*, *8*(9), e73935. https://doi.org/10.1371/journal.pone.0073935

Pompei, M., Mazziotti, C., Guerrini, F., Cangini, M., Pigozzi, S., Benzi, M., ... & Pistocchi, R. (2003). Correlation between the presence of Gonyaulax fragilis (Dinophyceae) and the mucilage phenomena of the Emilia-Romagna coast (northern Adriatic Sea). *Harmful algae*, *2*(4), 301-316. https://doi.org/10.1016/s1568-9883(03)00059-3

Purcell, J. E. (2005). Climate effects on formation of jellyfish and ctenophore blooms: a review. *Journal of the Marine Biological Association of the United kingdom*, *85*(3), 461-476. https://doi.org/10.1017/s0025315405011409

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R., & Briand, J. F. (2013). Marine biofilms on artificial surfaces: structure and dynamics. *Environmental microbiology*, *15*(11), 2879-2893. https://doi.org/10.1111/1462-2920.12186

Schwing, P. T., Romero, I. C., Brooks, G. R., Hastings, D. W., Larson, R. A., & Hollander, D. J. (2015). A decline in benthic foraminifera following the Deepwater Horizon event in the northeastern Gulf of Mexico. *PloS one*, *10*(3), e0120565. https://doi.org/10.1371/journal.pone.0120565

Shanks, A. L., & Walters, K. (1997). Holoplankton, meroplankton, and meiofauna associated with marine snow. *Marine Ecology Progress Series*, *156*, 75-86. https://doi.org/10.3354/meps156075

Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research*, *10*, 126-139. https://doi.org/10.1065/espr2002.12.142

Sommer, U., & Lengfellner, K. (2008). Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. *Global Change Biology*, *14*(6), 1199-1208. https://doi.org/10.1111/j.1365-2486.2008.01571.x

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

Taberlet, P. C. (2012). Environmental dna. *Molecular ecology, 21*(8), 1789-1793. https://doi.org/10.1111/j.1365-294x.2012.05542.x

Tas, S., Kus, D., & Yilmaz, I. N. (2020). Temporal variations in phytoplankton composition in the northeastern Sea of Marmara: potentially toxic species and mucilage event. *Mediterranean Marine Science*, *21*(3), 668-683. https://doi.org/10.12681/mms.22562

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. *Biological conservation*, *183*, 4-18. https://doi.org/10.1016/j.biocon.2014.11.019

Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., & Willerslev, E. (2016). Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PloS one*, *11*(11), e0165252. https://doi.org/10.1371/journal.pone.0165252

Toklu-Alicli, B., Balkis-Ozdelice, N., Durmus, T., & Balci, M. (2021). Relationship between environmental factors and zooplankton diversity in the Gulf of Bandırma (the Sea of Marmara). *Biologia*, *76*(6), 1727-1736. https://doi.org/10.2478/s11756-020-00668-8

Toklu-Alicli, B., Polat, S., & Balkis-Ozdelice, N. (2020). Temporal variations in the abundance of picoplanktonic Synechococcus (Cyanobacteria) during a mucilage event in the Gulfs of Bandırma and Erdek. *Estuarine, Coastal and Shelf Science*, *233*, 106513. https://doi.org/10.1016/j.ecss.2019.106513

Tüfekçi, V., Balkis, N., Beken, C. P., Ediger, D., & Mantikci, M. (2010). Phytoplankton composition and environmental conditions of the mucilage event in the Sea of Marmara. *Turkish Journal of Biology*, *34*(2), 199-210. https://doi.org/10.3906/biy-0812-1

Turk, V., Hagström, Å., Kovač, N., & Faganeli, J. (2010). Composition and function of mucilage macroaggregates in the northern Adriatic. *Aquatic microbial ecology*, *61*(3), 279-289. https://doi.org/10.3354/ame01447

Türkiye İstatistik Kurumu. (2023). Retrieved from https://cip.tuik.gov.tr/

Turley, C. M. (1991). Protozoa Associated with Marine 'Snow'and 'Fluff'—Session Summary. In *Protozoa and their role in marine processes* (pp. 309-326). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-73181-5_19

Turner, J. T. (2004). The importance of small planktonic copepods and their roles in pelagic marine food webs. *Zool. Stud*, *43*(2), 255-266. https://doi.org/10.1021/acs.est.2c04660.s001

Tuzcu Kokal, A., Olgun, N., & Musaoğlu, N. (2022). Detection of mucilage phenomenon in the Sea of Marmara by using multi-scale satellite data. *Environmental Monitoring and Assessment*, *194*(8), 585. https://doi.org/10.1007/s10661-022-10267-6

Ünlülata, Ü., Oğuz, T., Latif, M. A., & Özsoy, E. (1990). On the physical oceanography of the Turkish Straits. *The physical oceanography of sea straits*, 25-60. https://doi.org/10.1007/978-94-009-0677-8_2

Usov, A. I. (2011). Polysaccharides of the red algae. In *Advances in carbohydrate chemistry and biochemistry* (Vol. 65, pp. 115-217). Academic Press. https://doi.org/10.1016/b978-0-12-385520-6.00004-2

Weiland-Bräuer, N., Neulinger, S. C., Pinnow, N., Künzel, S., Baines, J. F., & Schmitz, R. A. (2015). Composition of bacterial communities associated with Aurelia aurita changes with compartment, life stage, and population. *Applied and environmental microbiology*, *81*(17), 6038-6052. https://doi.org/10.1128/aem.01601-15

Wong, H. K., Lüdmann, T., Ulug, A., & Görür, N. (1995). The Sea of Marmara: a plate boundary sea in an escape tectonic regime. *Tectonophysics*, *244*(4), 231-250. https://doi.org/10.1016/0040-1951(94)00245-5

Wood, S. A., Smith, K. F., Banks, J. C., Tremblay, L. A., Rhodes, L., Mountfort, D., ... & Pochon, X. (2013). Molecular genetic tools for environmental monitoring of New Zealand's aquatic habitats, past, present and the future. *New Zealand Journal of Marine and Freshwater Research*, *47*(1), 90-119. https://doi.org/10.1080/00288330.2012.745885

Wotton, R. S. (2004). The utiquity and many roles of exopolymers (EPS) in aquatic systems. *Scientia marina*, *68*(S1), 13-21. https://doi.org/10.3989/scimar.2004.68s113

Yaşar, D. (2001). Anthropogenic pollution in Izmit Bay: heavy metal concentrations in surface sediments. *Turkish Journal of Engineering and Environmental Sciences*, *25*(4), 299-313.

Yilmaz, I. N. (2015). Collapse of zooplankton stocks during Liriope tetraphylla (Hydromedusa) blooms and dense mucilaginous aggregations in a thermohaline stratified basin. *Marine Ecology*, *36*(3), 595-610. https://doi.org/10.1111/maec.12166

Zaiko, A., Samuiloviene, A., Ardura, A., & Garcia-Vazquez, E. (2015). Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. *Marine Pollution Bulletin*, *100*(1), 53-59. https://doi.org/10.1016/j.marpolbul.2015.09.030

Zingone, A., Escalera, L., Aligizaki, K., Fernández-Tejedor, M., Ismael, A., Montresor, M., ... & Totti, C. (2021). Toxic marine microalgae and noxious blooms in the Mediterranean Sea: A contribution to the Global HAB Status Report. *Harmful Algae*, *102*, 101843. https://doi.org/10.1016/j.hal.2020.101843

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Review Article

A Review on the Impact of Thermal Stress on Fish Biochemistry

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ABSTRACT

Fish are an important resource for humans, providing food, economic support, and ecological services. However, rising global temperatures and subsequent increases in their habitat water temperature, pose a significant challenge. We conducted a systematic review to understand the biochemical responses of thermal stress on fish. Stress can be acute (rapid exposure for a short duration) or chronic (repetitive long-term exposure). Stress responses occur at neurotransmitter and hormonal levels, progressing to peripheral and organism-wide effects. Prolonged stress leads to reduced growth, reproductive impairments, heightened infection susceptibility, and mortality. Elevated temperatures serve as abiotic stressors, triggering biotic stress responses. Fish employ strategies to cope with thermal stress, including altering gene expression, metabolite profiles, cellular signaling, and enzyme activity. Cumulative effects of thermal stress induce oxidative stress, causing cell death, organ failure, and mortality. Stressors increase the energy demand, prompting changes in hormonal, enzymatic, and biomolecular responses. Cortisol alters gene expression, stimulating glucose synthesis (gluconeogenesis). Other hormones (thyroid hormones, epinephrine, norepinephrine, insulin, glucagon) also play roles in the thermal stress response. Enzymes involved in metabolic pathways have optimal temperature and pH ranges altered by thermal stress. Heat shock proteins and warm acclimation proteins act as protective mechanisms by preserving the structural integrity of proteins, which is crucial for maintaining proper functionality and cellular responses. Further research is needed to expand on these molecular mechanisms to evaluate proper mitigation strategies.

Keywords: Fish stress, Global warming, Thermal stress, Biochemical responses, Molecular mechanism

INTRODUCTION

Importance of fishes

Fish are very important aquatic animals to humans as they provide different health benefits, economic support, and ecological services (FAO, 2016). As per the www.fishbase.de website, there are a total of 33,230 fish species in the world (Fishbase, 2020). Direct or indirect beneficial impacts of fish on the human population are numerous and have prevailed from ancient ages; only a few of these are discussed here. Fishes are important nutritional food for humans as well as for other animals. Fish are a low-cost protein source for humans and nearly 3.1 billion humans depend on fish to the tune of at least 20% of their total animal protein intake. Fish are the source of different essential amino acids such as lysine, valine, and functional amino acids, such as leucine and arginine (Ganguly et al., 2018). Fish are also a source of important vitamins, minerals, and other nutrients such as A, D, E, K, B12, sodium, potassium, magnesium, zinc, calcium, iron, copper, iodine, selenium, and fish oils (http://www.fao.org/fileadmin/user_upload/newsroom/docs/BlueGrowthNutritionRev2.pdf). Fish oils are rich in important PUFAs, such as linoleic acid, α-linolenic acid, arachidonic acid, EPA and DHA. Fish oils have different physiological benefits in several pathological conditions such as heart problems, atherosclerosis, rheumatoid arthritis, bipolar disorder, osteoporosis, asthma, etc. (Mohanty et al., 2019). Ornamental fish also are used in pet therapy to reduce mental depression (Gardiánová & Hejrová, 2015). Fish spas are also very popular nowadays (Riyaz & Arakkal, 2011). Fish are also used in the production of feed for other animals including other fish (Tacon & Metian, 2008).

Increment in water temperature

'Climate change' is a nightmare for the whole world and one of the most challenging hazards to be faced on the Earth in the coming future. Climate change results in a rise of the global surface temperature, known as 'Global warming'. The mean temperature of Earth's surface so far has risen by 1.4 °F over the last century and is expected to rise more (2 to 11.5 °F) over the coming hundred years (http://www.epa.gov/climatechange/basics/). Global warming is not only an increase in the temperature but also an alteration of different parameters of water bodies (Ficke et al., 2007, Brander, 2010). The surface temperature of the Earth has already increased (nearly +0.93 °C in the past 150 years) and is predicted to increase a few degrees more (1–4 °C) up to the end of this century (IPCC, 2007). Other than the increment in the Earth's surface temperature, experts have predicted some regional variations in temperatures (IPCC editor, 2012). Thus, impacts will vary in different parts of the globe. More events of extreme, abrupt, and frequent changes in temperature are documented in past decades (1991–2000) compared to earlier decades (1971–1990) (Dash & Mamgain, 2011). Different researchers (Diffenbaugh et al., 2007; Ray et al., 2012) also have found extreme events earlier. A heatwave was observed in Ahmedabad, India when the temperature rose to 46.8 °C during May 2010 (Azhar et al., 2014). Increments in the air temperature also led to a rise in the water temperature. Jurgelėnaitė and Jakimavičius (2014) have established a good positive correlation between air temperature and water temperature. The IPCC (2013) in their fifth assessment report estimated ocean warming by 0.09 to 0.13 °C per decade over the past 40 years. There will be an increment in the temperature of water bodies that will alter the habitat of different aquatic animals. Fishes are temperature-sensitive animals (poikilotherms); therefore, increments in the habitat temperature in the coming future beyond the adaptation capacity may exert abiotic stress among them.

STRESS BIOLOGY

General stress in animals

Cannon (1929) was the pioneer researcher who proposed the concept of stress as an "emotionally stimulation situation". Later, Selye (1936) explained the stress in the biological point of view. He demonstrated stress as a "non-specific pathological" response to different "noxious agents". However, several researchers encountered the concept of "the non-specific nature" of stress and proposed it is as highly specific (Mason, 1971; Pacak & Palkovitis, 2001). Stress is the discomfort state of the body under a threat where coordinated responses of physiological, biochemical, and behavioral processes cumulatively help the organism to overcome the situation (Chrousos & Gold, 1992). The initial response is to adapt to the situation by changing different biological processes of the body to a new normal situation in response to external as well as internal stimuli, which are cumulatively known as "allostatic load" (Schreck, 2001). The term "allostatic" denotes the body's capacity to attain stability amid change, with "load" signifying the toll or expense incurred by the body in adapting to stress (McEwen, 2013). Conceptually, stress can be defined as an alteration of physiological, biochemical (in the lower and higher organism), and mental (only in higher organisms) states due to different external (environmental factors), internal (body imbalance), or emotional stimuli that in the long-run effect performance and create physiological disorders, which every organism tries to avoid although it is nearly impossible.

Stress responses

Under stress, fish initially respond at the neurotransmitter and hormonal level (primary response) then at the peripheral level (secondary response) (Mazeaud et al., 1977), and finally to the whole organism or population level (tertiary responses) (Wodemeyer & McLeay, 1981). The primary responses are the "alarming stages" and two hormonal axes are involved in any kind of stress response in fish. The sympathetic-chromaffin (SC) axis and the hypothalamic-pituitary-interrenal (HPI) axis regulate oxygen uptake, transfer, and energy metabolisms (Wendelaar, 1997, Roychowdhury et al., 2020b). The secondary responses are the stages of "resistance". During that stage, fish try to engage all the available mechanisms to cope with the situation. The tertiary response of an organism is the "stage of exhaustion" where fish are unable to fight against the stress, reflected in their performance, and finally may cause even death (Roychowdhury et al., 2020a, Roychowdhury et al., 2020b). Therefore, the primary responses occur at a systemic level whereas secondary responses occur at the peripheral level, and the tertiary response affects the whole organism (Wodemeyer & McLeay, 1981). Once a fish receives stress stimuli, its sensory neurons in the brain activate the hypothalamus to release different hormones and neurotransmitters. Releasing hormones (RHs) such as CRH and TRH are the most important. These RHs act on the pituitary to release different hormones such as ACTH and α-MSH. ACTH further helps to release cortisol, an important hormone under stress. Cortisol is produced from an internal gland whereas catecholamines are produced in a chromaffin gland and interestingly both of them are found in the kidneys of fish (Milano et al., 1997). These hormones further alter the biochemistry, immunology, and physiology of fish under stress and induce the secondary responses (Weyts et al., 1999). These effects, in the long term, are reflected in fish performance as a reduction in growth, reproduction, susceptibility to infection, and ultimately mortality (Schreck et al., 2001).

Acute stress and chronic stress

Stress responses in fish depend on the types of stressors, the duration of exposure, the rate of introduction to stressors, and the amount of the stress (Barandica & Tort, 2008; Wedemeyer, 1997). Stresses are of two types, acute and chronic, based on the duration of exposure time. Acute stress involves rapid exposure for a short duration of exposure time (hours). Chronic stress is due to constant or repetitive exposure to the stressor for long periods (Barandica & Tort, 2008).

Acute stress is mainly the "fight or flight" response and is initiated mostly in the primary response. Acute stress in Black Sea trout

(*Salmo trutta labrax)* due to thermal exposure may involve an alteration in blood glucose, cortisol, total protein, serum ion concentrations, and lysozyme activity (Dengiz Balta et al., 2017). Acute stress is sometimes helpful for an organism. However, chronic stress can be viewed as repetitive exposure to stressors and the central nervous system (CNS) does not get sufficient time to recover (Sapolsky, 1996). It generally causes secondary and even tertiary responses (health issues). Under any kind of chronic stress, the body changes to 'a new normal' state. Chronic stress impacts nearly all physiological processes, and experimental conditions have been devised to induce such stress, revealing varying durations in different fish species. For instance, it necessitated 15 days in zebrafish (Chakravarty et al., 2013) and Nile tilapia (Volpato & Barreto, 2001), 28 days in rainbow trout (Moltesen et al., 2016), and 8 days in zebrafish (Golla et al., 2020). Intriguingly, chronic stress elicits a more intricate set of responses compared to acute stress. While acute stress predominantly triggers primary and secondary responses, chronic stress encompasses all three types of stress responses. This complexity underscores the multifaceted nature of physiological adaptations in animals under prolonged stress conditions (McEwen & Gianaros, 2011).

THERMAL STRESS IN FISH

In the earlier section, it was discussed that fishes are temperature-sensitive animals, therefore, an increment in the habitat temperature beyond the thermal tolerance capacity causes fish to feel abiotic stress. Thermal tolerance is the range of temperatures encompassing minimum to maximum levels where fish can survive and perform all their physiological functions. The upper-temperature tolerance limit (UTTL) of fish is the highest temperature up to which fish can survive (Daniel et al., 2008, Roychowdhury et al., 2019). The UTTL of fish depends on geographical location (Sorte et al., 2011), previous thermal history (Beitinger et al., 2000), transgenerational acclimatization (Donelson et al., 2012), the type of feed consumed (Kumar et al., 2014), and the rate of temperature increments (Camilo & Maria, 2006). The most commonly employed tools for studying the UTTL in fish are the lethal temperature maximum (LTmax) and critical temperature maximum (CTmax) (Beitinger et al., 2000). Thermal plasticity in fish refers to the capacity of fish species to adjust their physiological and behavioral traits in response to temperature changes (Comte & Olden, 2017). Fish exhibit thermal plasticity through alterations in metabolism, enzyme activity, gill function remodeling, changes in spawning time, and behavioral adaptations (Angiulli et al., 2020; Das et al., 2012; Farrell, 2009; Roychowdhury et al., 2020b). Parameters like warming temperature (WT) and thermal safety margin (TSM) are crucial for studying thermal plasticity in ectothermic animals (Becker & Genoway, 1979; Madeira et al., 2007). In fish physiology, aerobic space refers to the volume of oxygen available for aerobic metabolism, crucial for energy production (Jobling, 1995). Factors influencing this space include dissolved oxygen levels, gill efficiency, and metabolic rate (Farrell, 2009). Elevated water temperature often results in decreased dissolved oxygen (DO), leading to hypoxic conditions. Understanding aerobic space helps assess fish adaptation to environmental conditions and stressors (Eliason et al., 2013). The Temperature Coefficient (Q10) measures the temperature sensitivity of physiological or biochemical processes, indicating the rate of change with a 10-degree Celsius temperature increase. In the context of oxygen consumption in fish, the Q10 value describes how oxygen consumption rates vary with temperature changes. Researchers use Q10 values to understand thermal dependence in fish and other ectothermic organisms, providing insights into the temperature's influence on metabolic processes and aiding predictions of climate change impacts on fish physiology and ecology. Variations are observed among fish species based on their adaptations to specific environmental conditions (Claireaux & Lefrançois, 2007; Farrell, 2009; Pörtner & Farrell, 2008).

'Fight or flight' is the initial strategy for most organisms in a hostile environment. Fish try to move toward the more preferable environment but if they fail to do so, they will feel stress (Kovach et al., 2012). A warmer temperature is abiotic stress that causes different biotic stress in organisms (Nakano et al., 2014). However, data on the impacts of thermal stress on different biochemical pathways are limited in carp fish. Therefore, previous literature on thermal stresses on fish and other organisms needs to be reviewed to understand the thermal stress biology of an organism. Besides, this information can help to design proper methodology and selection of different biomolecules expected to be altered under thermal stress in fish. The process of adaption and stress are difficult to distinguish at the initial stages as fish try to fight the stress by altering their biochemical pathways in higher temperatures. Patterns of the expression of different biomolecules are changed initially due to adaptation and later on due to stress (Bijlsma & Loeschcke, 2005). The consequence of an event depends on the extent of stress, the genetic makeup of the organisms, and previous history of exposure (Beitinger et al., 2000). A higher temperature is known to increase the kinetic properties of molecules causing a higher diffusion rate for micro-molecules (Bag et al., 2014) and denaturation of macromolecules (Wu, 1995). Maintenance of the native three-dimensional structure of a protein is very much essential for proper functioning, and interaction with other biomolecules involved in cellular response, and any randomness beyond certain limits may lead to cellular death (Nakamura & Lipton, 2009). Fish try to cope with the situation by changing gene expression patterns and are reflected in alteration in metabolites (biomolecules), cellular signaling systems (hormones and others), and enzyme activity (Lancaster et al., 2016). The stress is tolerable to a certain extent after the performance of the species drops (La & Cooke, 2011; Pörtner & Knust, 2007). Due to the cumulative impacts of the different effects of thermal stress, oxidative stress begins to occur and results in cellular death, organ failure and ultimately death of the organism (Figure 1).

BIOCHEMICAL MECHANISM

Any kind of stressor increases the requirement of physiological energy that can be fulfilled by alteration of hormonal, enzymes and bimolecular responses. In general, glucose, triglycerides and protein act as energy molecules depending upon the nature and quantity of energy needed (Mergenthaler et al., 2013). Glucose serves as an immediate energy source, while triglycerides provide major energy, and protein provides energy during hun-

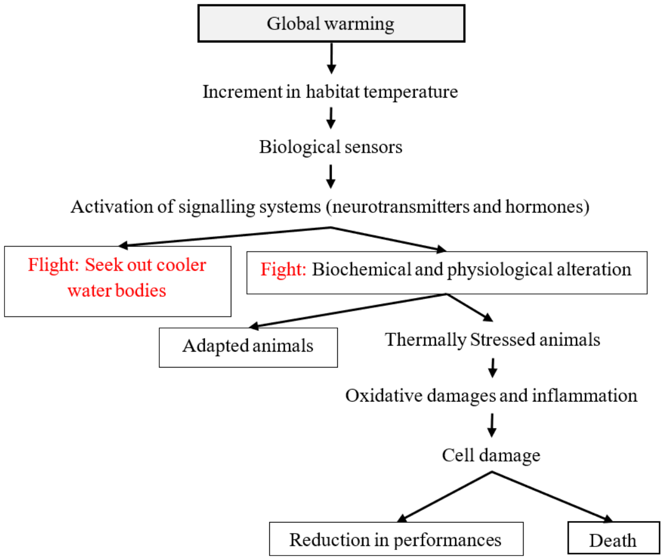

Figure 1. Conceptual representation of the consequences of thermal stress in fish.

ger or in some physiological disorders and stress. In stressful situations, fishes try to increase blood glucose to provide more energy to vital organs including the brain by changing the metabolism of biomolecules under the control of hormones (Mergenthaler et al., 2013). The steroid hormone, cortisol alters the pattern of gene expression (transcription level) and increases the expression of the different enzymes required for the synthesis of new glucose molecules (gluconeogenesis) (Babitha & Peter, 2010; Vijayan et al., 1997) and breakdown of the stored glycogen (glycogenolysis) (Vijayan et al., 1993 and 1996) during stress to increase blood glucose concentration in serum. Cortisol converts the non-essential proteins of muscle to amino acids and transports them through the blood to the liver for gluconeogenesis (Vijayan et al., 1993; Vijayan & Moon, 1994; Freeman & Idler, 1973). Cortisol is also documented to mobilize stored lipids in teleost species (Dave et al., 1979). Thyroid hormones, T3 and T4 are produced in the thyroid gland and normally maintain a positive nitrogen balance, but T3 inhibits protein synthesis in higher concentrations (Murray et al., 1996). Like cortisol, thyroid hormones are also known to act at the transcriptional level and modify its function or 'fine-tune' the actions of cortisol and adrenaline in a stressful situation (Peter, 2011). Epinephrine and norepinephrine, released from chromaffin cells, are recognized by their immediate elevation of plasma glucose levels (Arends et al., 1999; Ruane et al., 2001). Their roles encompass enhancing glucagon secretion, facilitating glycogen breakdown (glycogenolysis), initiating new glucose synthesis (gluconeogenesis), mobilizing fatty acids (beta oxidation), and concurrently suppressing insulin production, glycogen synthesis (glycogenesis), and glucose breakdown (glycolysis) (Nelson & Cox, 2001). Their primary targets are muscle, adipose tissue, and liver, and increase the supply of oxygen to tissue by increasing heart rate, blood pressure, and respiratory passage (Nelson & Cox, 2001). Cortisol plays a role mostly in chronic stress, while epinephrine and norepinephrine act as emergency hormones and are involved in immediate responses. Insulin and glucagon maintain glucose homeostasis in blood in normal conditions but their direct involvement in heat stress is limited. Insulin increases the utilization of glucose by increasing glucose transportation in cells and increasing the rate of glycolysis in extrahepatic tissues and glycogenesis in hepatic tissue but glucagon plays the counter role of insulin by producing glucose through gluconeogenesis and glycogenolysis thus increasing blood glucose (Nelson & Cox, 2001).

Blood glucose is used to monitor general stress for different species. Energy-rich molecules like ATP, GTP, phosphocreatine, succinate, and ketone bodies are also known to play an important role during thermal stress and can be used as possible markers for thermal stress (Dijk et al., 1999). Metabolism of biomolecules is under hormonal control necessitating monitoring of the hormones cortisol, T3, T4, epinephrine, norepinephrine, insulin, and glucagon during stress.

Every enzyme has an optimum temperature and pH and thermal stress is known to change these parameters for cold-blooded fish. Enzymes involved in the metabolism of energy-rich biomolecules alter their activity by changing their concentration (expression) or affinity (modification) towards the substrate under a stressed condition. Enzymes catalyzing the irreversible steps of carbohydrate metabolism (hexokinase, phosphofructokinase1, pyruvate kinase, citrate synthase, glucose 6 phosphatase, fructose-1,6 bis-phosphatase, etc.), protein metabolism (glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase), lipid metabolism (lipase), and creatinine metabolism (creatinine kinase) are important from this perspective (Dhanasiri et al., 2013, Abbaraju & Rees, 2012; Ton et al., 2003).

Thermodynamics explains life as the maintenance of organized cellular structures through energy generated by oxidizing energy-rich nutrients by enzyme(s) that act at an optimum temperature under the control of hormones. Temperature changes directly affect these activities. Proteins are the mediators of diverse types of cellular functions (Alberts et al., 2002) including enzymatic action to scale formation, body growth to reproduction, and digestion to excretion. The function of a protein depends on its native structure that gets denatured due to the rise in temperature causing more vibration in molecules and related disturbance. Heat shock proteins (Hsps), which are highly conserved, are the molecular chaperones required for the proper folding of the protein and are also involved in protein transport to cellular compartments (Alberts et al., 2002; Shi & Thomas, 1992). At higher temperatures Hsps refold the denatured protein to its original native structure and the heat shock factor (Hsf) induces the expression of various Hsps in the process (Wu, 1995). There are various types of Hsps within the cell such as Hsp10, HspB group (Hsp27, hspB1, hspB6), HspA group (Hsp70, Hsp71, Hsp72, Hsp78), HspC group (Hsp90, hsp94), Hsp40, Hsp60, Hsp104, and Hsp110 (Antonova et al., 2007; Benjamin & McMillan, 1998; Li & Srivastava, 2004; McLemore et al., 2005; Salinthone et al., 2008; Schlesinger, 1990). Hsp30, Hsp27, and Hsp47 are identified in goldfish (Wang et al., 2007) while Hsp78 and Hsp100 are found in *Poeciliopsis lucida* (Norris et al., 1995). Warm acclimation protein (wap) is another candidate that plays an important role during heat stress (Kikuchi et al., 1997) (Figure 2).

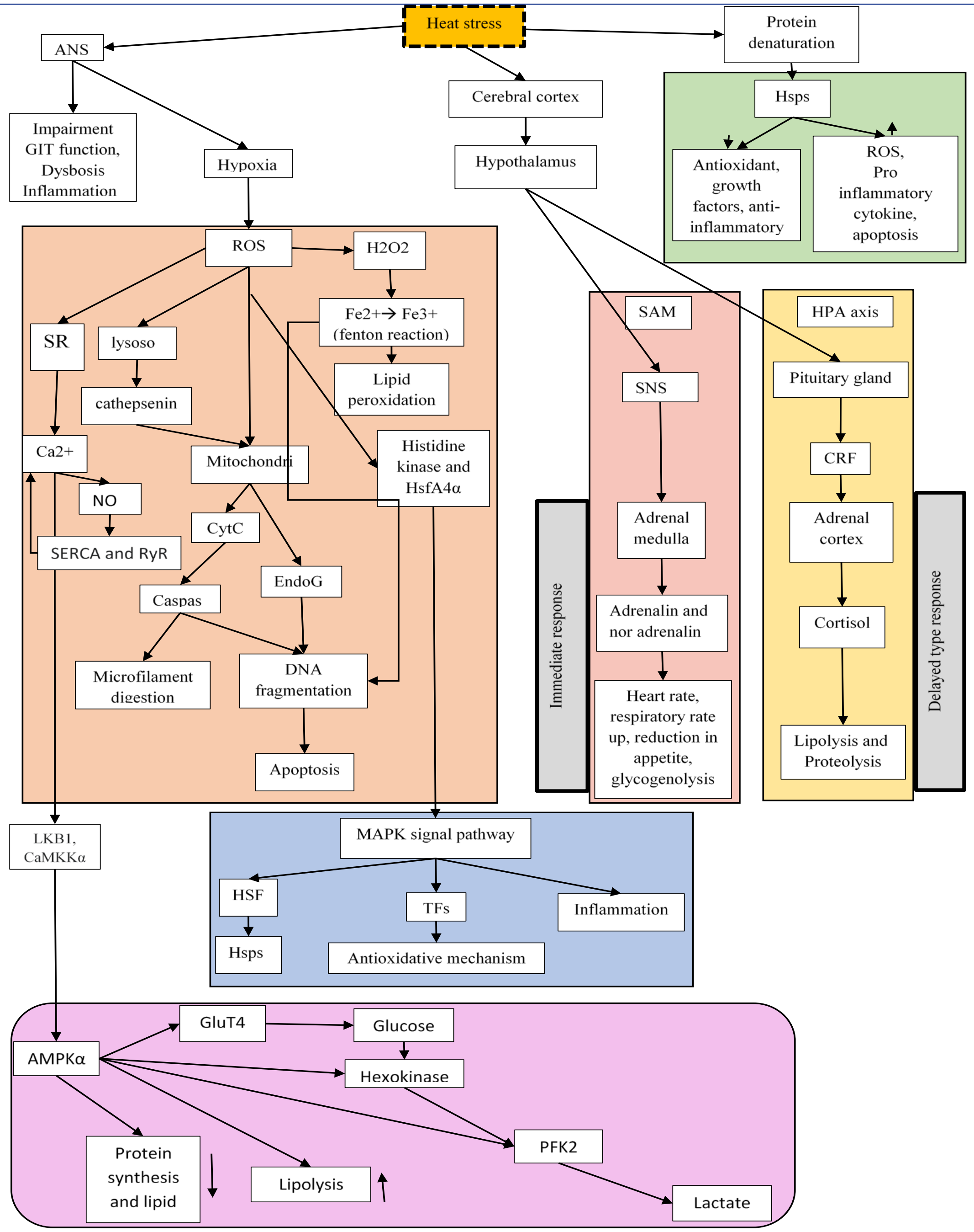

Figure 2. Impact of thermal stress at the molecular biology level (Antoun et al., 2017; Cui et al., 2016; Kourtis and Tavernarakis, 2011; Kültz, 2015; Xing et al., 2019).

Numerous experiments have been conducted to study the impacts of heat stress on fish and a few of them are cited here: rahu (Akhtar et al., 2013; Kumar et al., 2015; Mohapatra et al., 2014), common carp (Ouellet, et al., 2013), rainbow trout (Recsetar et al., 2012), blenny species (Camilo and Maria, 2006), salmon (Dengiz Balta et al., 2017; Nakano et al., 2014), *Channa punctatus (*Kaur et al., 2005), pacific sardine (Kaur et al., 2005), and *Danio dangila* and *Brachydanio rerio* (Majhi & Das, 2013). The selected temperatures for treatments were mostly sub-lethal with no fish death (Das et al., 2002; Das et al., 2005; Nakano et al., 2014). These experiments were mainly focused on the recovery responses (Kumar et al., 2015), adaptation strategies (Das, 2002), gene expression pattern (Ouelle et al., 2013), oxidation-reduction state (Nakano et al., 2014), thermal tolerance, growth and oxygen consumption (Das et al., 2005), and search for bio-markers (Purohit et al., 2014). Different experimental conditions were used for warmer water exposure to induce heat stress in fish. The three main determinants to introduce thermal stress to fish are the amount of temperature, duration of exposure, and rates of temperature increment, and all of them varied in the experiments (Akhtar et al., 2013; Das et al., 2005; Dengiz Balta et al, 2017; Kaur et al., 2005; Kumar et al., 2015; Majhi & Das, 2013; Mohapatra et al., 2014; Nakano et al., 2014; Recsetar et al., 2012). Fish may feel both acute stress and chronic stress based on events that will occur in future (IPCC, 2014). Different researchers have studied the impacts of both acute and chronic thermal stress among other animals including fish (Hernández-López et al., 2018) but data is very limited for IMC. Thermal stress is expected to alter the different physiological and biochemical processes of the body and that may be reflected in different pathways and performance of fish (Figure 3).

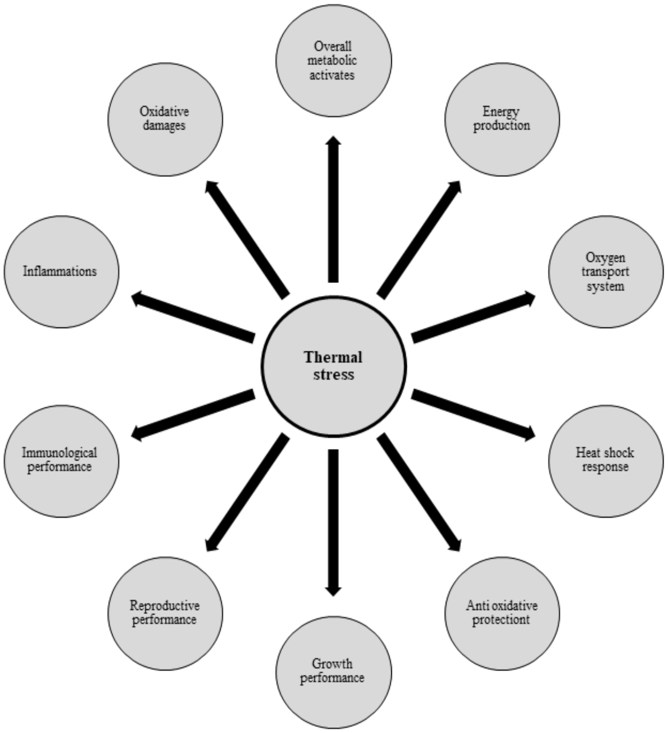

Figure 3. Abiotic thermal stress affects different physiological pathways (Aftabuddin et al., 2017; Aftabuddin and Roychowdhury, 2019).

DISCUSSION

The review revealed that thermal stress has substantial effects on fish, leading to reduced growth, reproductive impairments, increased susceptibility to infections, and even mortality. These consequences can have profound ecological and economic implications, considering the vital role of fish in providing food, supporting economies, and maintaining ecosystem balance. The study highlights the distinction between acute and chronic stressors, with acute stress resulting from short-term rapid exposure and chronic stress arising from long-term repetitive exposure. Understanding this differentiation is crucial for developing effective strategies to mitigate the impacts of thermal stress on fish populations. The elevated water temperatures associated with global warming act as abiotic stressors, triggering various biotic stress responses in fish. It was observed that fish employ a range of mechanisms to cope with thermal stress, including alterations in gene expression patterns, metabolite profiles, cellular signaling pathways, and enzyme activity. These adaptive responses enable fish to maintain their physiological balance and attempt to counteract the detrimental effects of thermal stress. However, the cumulative impact of thermal stress can induce oxidative stress, leading to cellular death, organ failure, and ultimately mortality. This highlights the urgent need to address the challenges posed by rising temperatures and the associated thermal stress on fish populations. The study emphasizes the role of hormonal, enzymatic, and biomolecular responses in the fish's attempt to meet the increased energy demands caused by stressors. Hormones such as cortisol play a significant role in altering gene expression and stimulating glucose synthesis through gluconeogenesis. Other hormones, including thyroid hormones, epinephrine, norepinephrine, insulin, and glucagon, are also involved in the fish's response to thermal stress. Enzymes associated with metabolic pathways in fish have optimal temperature and pH ranges that can be altered by thermal stress. Maintaining the proper structure and functionality of proteins is crucial for cellular responses and overall fish health. Heat shock proteins and warm acclimation proteins act as protective mechanisms, helping to preserve protein integrity and mitigate the adverse effects of thermal stress.

The findings of this systematic review emphasize the importance of further research to enhance our understanding of the molecular mechanisms underlying these responses. Such knowledge can guide the development of targeted conservation and management strategies to safeguard fish populations in the face of a rapidly changing environment.

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Ethical Statement: We certify that this is our original research work and it has neither been submitted or published elsewhere as a whole or in part. The authors are responsible for all the data and content in the Manuscript.

Declaration of Generative Ai in Scientific Writing: During the preparation of this work the author(s) used [https://chat.openai.com/] in order to improve English language such as checking grammar, spelling and for finding some references. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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REFERENCES

Abbaraju, N. V., & Rees, B. B. (2012). Effects of dissolved oxygen on glycolytic enzyme specific activities in liver and skeletal muscle of *Fundulus heteroclitus*. *Fish Physiology and Biochemistry*, 38(3), 615-624.

Aftabuddin, M., & Roychowdhury, P. (2019). Thermal effect on physio-biochemical aspects of fishes and thermal tolerance under changing climatic scenario. *Perspectives on Climate Change and Inland Fisheries in India*, 258.

Aftabuddin, M., Roychowdhury, P., & Sarkar, U. K. (2017). Understanding Thermal Tolerance of Potential Fish in the changing climatic environment. *Indian Farming*, 67(3).

Akhtar, M. S., Pal, A. K., Sahu, N. P., Ciji, A., Meena, D. K., Das, P. (2013). Physiological responses of dietary tryptophan fed *Labeo rohita* to temperature and salinity stress. *Journal of Animal Physiology and Animal Nutrition*, 97(6), 1075-1083.

Alberts, B., Johnson, A., & Lewis, J. (2002). *In Molecular Biology of the Cell* (4th edition). New York: Garland Science

Angiulli, E., Pagliara, V., Cioni, C., Frabetti, F., Pizzetti, F., Alleva, E., & Toni, M. (2020). Increase in environmental temperature affects exploratory behaviour, anxiety and social preference in *Danio rerio*. *Scientific Reports*, 10(1), 5385.

Antonova, G., Lichtenbeld, H., Xia, T., Chatterjee, A., Dimitropoulou, C., & Catravas, J. D. (2007). Functional significance of hsp90 complexes with NOS and sGC in endothelial cells. *Clinical Hemorheology and Microcirculation*, 37(1-2), 19-35.

Antoun, M., Edwards, K. M., Sweeting, J., & Ding, D. (2017). The acute physiological stress response to driving: A systematic review. *PloS One*, 12(10), e0185517.

Arends, R. J., Mancera, J. M., Munoz, J. L., Bonga, S. W., & Flik, G. (1999). The stress response of the gilthead sea bream (*Sparus aurata L.*) to air exposure and confinement. *Journal of Endocrinology*, 163(1), 149.

Azhar, G. S., Mavalankar, D., Nori-Sarma, A., Rajiva, A., Dutta, P., Jaiswal, A., ... & Hess, J. J. (2014). Heat-related mortality in India: Excess all-cause mortality associated with the 2010 Ahmedabad heat wave. *PLoS One*, 9(3), e91831.

Babitha, G. S., & Peter, M. S. (2010). Cortisol promotes and integrates the osmotic competence of the organs in North African catfish (*Clarias gariepinus Burchell*): Evidence from in vivo and in situ approaches. *General and Comparative Endocrinology*, 168(1), 14-21.

Bag, N., Yap, D. H. X., & Wohland, T. (2014). Temperature dependence of diffusion in model and live cell membranes characterized by imaging fluorescence correlation spectroscopy. *Biochimica et Biophysica Acta (BBA) Biomembranes*, 1838(3), 802-813.

Barandica, C. L. M. & Tort, L. (2008). Neuroendocrinología e inmunología de la respuesta al estrés en peces. *Revista de la Academia Colombiana de Ciencias*, 32(123), 267-284.

Becker, D. C., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes*, 4(4), 245–256.

Beitinger, T. L., Bennett, W. A., & McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*, 58(3), 237-275.

Benjamin, I. J., & McMillan, D. R. (1998). Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circulation Research*, 83(2), 117-132.

Bijlsma, R., & Loeschcke, V. (2005). Environmental stress, adaptation and evolution: an overview. *Journal of Evolutionary Biology*, 18(4), 744-749.

Brander, K. (2010). Impacts of climate change on fisheries. *Journal of Marine Systems*, 79(3- 4), 389-402.

Camilo, M., & Maria, F.M. (2006). Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology*, 31(4), 337-44.

Cannon, W. B. (1929). Organization for physiological homeostasis. *Physiological Reviews*, 9(3), 399-431.

Chakravarty, S., Reddy, B. R., Sudhakar, S. R., Saxena, S., Das, T., Meghah, V., ... & Idris, M. M. (2013). Chronic unpredictable stress (CUS)-induced anxiety and related mood disorders in a zebrafish model: altered brain proteome profile implicates mitochondrial dysfunction. *Plos One*, 8(5), e63302.

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *Jama*, 267(9), 1244-1252.

Comte, L., & Olden, J. D. (2017). Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. *Global Change Biology*, 23(2), 728-736.

Cui, Y., Hao, Y., Li, J., Bao, W., Li, G., Gao, Y., & Gu, X. (2016). Chronic Heat Stress Induces Immune Response, Oxidative Stress Response, and Apoptosis of Finishing Pig Liver: A Proteomic Approach. *International Journal of Molecular Sciences*, 17(5), 393.

Daniel, G. B., Derek, P. T., & Boris, W. (2008). Effects of Temperature on Global Patterns of Tuna and Billfish Richness. *Marine Ecology Progress Series*, 355, 267–276.

Das, M. K., Dutta, T., Acharya, S., & Bhowmick, S. (2002). Sublethal temperature stress in juvenile Labeo rohita (Ham-Buch.) and Rita rita (Ham.): some physiological changes. *Indian Journal of Experimental Biology*, 40(5), 589–593.

Das, M. K., Srivastava, P. K., Dey, S., & Rej, A. (2012). Impact of temperature and rainfall alterations on spawning behavior of Indian major carps and consequence on fishers' income in Odisha. *Journal of Inland Fisheries Society of India*, 44(2), 1-11.

Das, T., Pal, A. K., Chakraborty, S. K., Manush, S. M., Sahu, N. P., & Mukherjee, S. C. (2005). Thermal tolerance, growth and oxygen consumption of *Labeo rohita* fry (Hamilton, 1822) acclimated to four temperatures. *Journal of Thermal Biology*, 30(5), 378-383.

Dash, S. K., & Mamgain, A. (2011). Changes in the frequency of different categories of temperature extremes in India. *Journal of Applied Meteorology and Climatology*, 50(9), 1842-1858.

Dave, G., Johansson-Sjöbeck, M. L., Larsson, Å., Lewander, K., & Lidman, U. (1979). Effects of cortisol on the fatty acid composition of the total blood plasma lipids in the European eel, *Anguilla anguilla L. Comparative Biochemistry and Physiology Part A*: Physiology, 64(1), 37-40.

Dengiz Balta, Z., Akhan, S., & Balta, F. (2017).The physiological stress response to acute thermal exposure in Black Sea trout (*Salmo trutta labrax Pallas*, 1814). *Turkish Journal of Veterinary & Animal Sciences*, 41(3), 400-406

Dhanasiri, A. K., Fernandes, J. M., & Kiron, V. (2013). Liver transcriptome changes in zebrafish during acclimation to transport-associated stress. *PLoS One*, 8(6), e65028.

Diffenbaugh, N. S., Pal, J. S., Giorgi, F., & Gao, X. (2007). Heat stress intensification in the Mediterranean climate change hotspot. *Geophysical Research Letters*, 34(11).

Dijk, P. L., Tesch, C., Hardewig, I., & Portner, H. O. (1999). Physiological disturbances at critically high temperatures: a comparison between stenothermal Antarctic and eurythermal temperate eelpouts (Zoarcidae). *Journal of Experimental Biology*, 202(24), 3611-362.

Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, 2(1), 30-32.

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., ... & Hinch, S. G. (2013). Differences in thermal tolerance among sockeye salmon populations. *Science*, 342(6159), 1098-1101.

FAO. (2016). In FAO year book, 2014 Fishery and Aquaculture statistics (pp.105). *Food and Agriculture Organisation of the United Nations*, Rome, Italy.

Farrell, A. P. (2009). *In Encyclopedia of Fish Physiology: From Genome to Environmen*t. Academic Press.

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, 17(4), 581-613.

Fishbase. (2020). https://www.fishbase.se/Country/CountryChecklist.php?what=list&trpp=50&c_code=356&cs ub_code=&cpresence=Reported&sortby=alpha2&ext_CL=on&ext_pic=on&vhabitat=all2

Freeman, H. C., & Idler, D. R. (1973). Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdnerii*) and the brook trout (Salvelinus fontinalis). *General and Comparative Endocrinology*, 20(1), 69-75.

Ganguly, S., Mahanty, A., Mitra, T., Mohanty, S., Das, B. K., & Mohanty, B. P. (2018). Nutrigenomic studies on hilsa to evaluate flesh quality attributes and genes associated with fatty acid metabolism from the rivers Hooghly and Padma. *Food Research International*, 103, 21-29.

Gardiánová, I., & Hejrová, P. (2015). The use of small animals–mammals, birds, fish in zootherapy. *Kontakt*, 17(3), e171-e176.

Golla, A., Østby, H., & Kermen, F. (2020). Chronic unpredictable stress induces anxiety-like behaviors in young zebrafish. *Scientific Reports*, 10(1), 1-10.

Hernández-López, J. R., Hernández-Rodríguez, M., Rivas-Manzano, P., & Bückle-Ramirez, L. F. (2018). Thermal Effect of Acute and Chronic Stress on Hepatic and Renal Tissue of the Pacific Sardine, Sardinops sagax caeruleus (Jenyns, 1842). *International Journal of Morphology*, 36(1), 212-220.

IPCC editor. (2012). Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation: A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press.

IPCC. (2007). Climate Change: The Physical Science Basis. Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press.

IPCC. (2013). Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker, T. F. D., Qin, G. K., Plattner, M., Tignor, S. K., Allen, J., Boschung, A., … Midgley, P. M. (Eds.). Cambridge University Press, Cambridge, United Kingdom, and New York, NY, USA.

IPCC. (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri, R. K., and Meyer, L. A. (Eds.). Geneva, Switzerland: IPCC.

Jobling, M. (1995).*In Environmental Biology of Fishes*. Chapman & Hall.

Jurgelėnaitė, A., & Jakimavičius, D. (2014). Prediction of river water temperature and its dependence on hydro-meteorological factors. *Environmental Research, Engineering and Management*, 68(2).

Kaur, M., Atif, F., Ali, M., Rehman, H., & Raisuddin, S. (2005). Heat stress-induced alterations of antioxidants in the freshwater fish *Channa punctata Bloch*. *Journal of Fish Biology*, 67(6), 1653-1665.

Kikuchi, K., Watabe, S., & Aida, K. (1997). The Wap65 gene expression of goldfish (Carassius auratus) in association with warm water temperature as well as bacterial lipopolysaccharide (LPS). *Fish Physiology and Biochemistry*, 17(1-6), 423-432.

Kourtis, N., & Tavernarakis, N. (2011). Cellular stress response pathways and ageing: intricate molecular relationships. *The EMBO Journal*, 30(13), 2520–2531.

Kovach, R. P., Gharrett, A. J., & Tallmon, D. A. (2012). Genetic change for earlier migration timing in a pink salmon population. *Proceedings of the Royal Society B: Biological Sciences*, 279(1743), 3870-3878.

Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology*, 218(12), 1907-1914.

Kumar, N., Minhas, P. S., Ambasankar, K., Krishnani, K. K., & Rana, R. S. (2014). Dietary lecithin potentiates thermal tolerance and cellular stress protection of milk fish (*Chanos Chanos*) reared under low dose endosulfan-induced stress. *Journal of Thermal Biology*, 46, 40-46.

Kumar, P., Pal, A. K., Sahu, N. P., Jha, A. K., & Priya, P. (2015). Biochemical and physiological stress responses to heat shock and their recovery in *Labeo rohita* fingerlings. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 85(2), 485-490.

La, V. T., & Cooke, S. J. (2011). Advancing the science and practice of fish kill investigations. *Reviews in Fisheries Science*, 19(1), 21-33.

Lancaster, L. T., Dudaniec, R. Y., Chauhan, P., Wellenreuther, M., Svensson, E. I., & Hansson, B. (2016). Gene expression under thermal stress varies across a geographical range expansion front. *Molecular Ecology*, 25(5), 1141-1156.

Li, Z., & Srivastava, P. (2004). In *Heat-shock proteins Current protocols in immunology*, edited by John E Coligan [et al.].

Madeira, C., Mendonça, V., Leal, M. C., Flores, A. A., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2017). Thermal stress, thermal safety margins and acclimation capacity in tropical shallow waters-An experimental approach testing multiple end-points in two common fish. *Ecological Indicators*, 81, 146-158.

Majhi, S. K., & Das, S. K. (2013). Thermal tolerance, oxygen consumption and stress response in *Danio dangila* and *Brachydanio rerio* (Hamilton, 1822) acclimated to four temperatures. *Turkish Journal of Fisheries and Aquatic Sciences*, 13(2), 359-365.

Mason, J. W. (1971). A re-evaluation of the concept of 'non-specificity'in stress theory. *In Principles, Practices, and Positions in Neuropsychiatric Research* (pp. 323-333).

Mazeaud, M. M., Mazeaud, F., & Donaldson, E. M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society*, 106(3), 201-212.

McEwen, B. S. (2013). Allostasis and allostatic load: implications for neuropsychopharmacology. *Stress and the Brain*, 2-18.

McEwen, B. S., & Gianaros, P. J. (2011). Stress- and allostasis-induced brain plasticity. *Annual Review of Medicine*, 62, 431–445.

McLemore, E. C., Tessier, D. J., Thresher, J., Komalavilas, P., & Brophy, C. M. (2005). Role of the small heat shock proteins in regulating vascular smooth muscle tone. *Journal of the American College of Surgeons*, 201(1), 30-36.

Mergenthaler, P., Lindauer, U., Dienel, G. A., & Meisel, A. (2013). Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends in Neurosciences*, 36(10), 587-597.

Milano, E. G., Basari, F., & Chimenti, C. (1997). Adrenocortical and adrenomedullary homologs in eight species of adult and developing teleosts: morphology, histology, and immunohistochemistry. *General and Comparative Endocrinology*, 108(3), 483-496.

Mohanty, B. P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D., & Anandan, R. (2019). Nutritional composition of food fishes and their importance in providing food and nutritional security. *Food Chemistry*, 293, 561-570.

Mohapatra, S., Chakraborty, T., Prusty, A. K., PaniPrasad, K., & Mohanta, K. N. (2014). Beneficial effects of dietary probiotics mixture on hemato-immunology and cell apoptosis of *Labeo rohita* fingerlings reared at higher water temperatures. *Plos One*, 9(6), e100929.

Moltesen, M., Laursen, D. C., Thörnqvist, P. O., Andersson, M. Å., Winberg, S., & Höglund, E. (2016). Effects of acute and chronic stress on telencephalic neurochemistry and gene expression in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, 219(24), 3907-3914.

Murray, R. K., Granner, D. K., Mayes, P. A., & Rodwell, V. W. (1996). *In Harper's Illustrated Biochemistry (24th ed.)* (pp. 537). Appleton & Lange, Stamford, CT. ISBN 0-8385-3612-3.

Nakamura, T., & Lipton, S. A. (2009). Cell death: protein misfolding and neurodegenerative diseases. *Apoptosis*, 14(4), 455-468.

Nakano, T., Kameda, M., Shoji, Y., Hayashi, S., Yamaguchi, T., & Sato, M. (2014). Effect of severe environmental thermal stress on redox state in salmon. *Redox Biology*, 2, 772-776.

Nelson, D. L., & Cox, M. M., (2001). *Lehninger principles of biochemistry*. Macmillan.

Norris, C. E., diIorio, P. J., Schultz, R. J., & Hightower, L. E. (1995). Variation in heat shock proteins within tropical and desert species of poeciliid fishes. *Molecular Biology and Evolution*, 12(6), 1048-1062.

Ouellet, V., Pierron, F., Mingelbier, M., Fournier, M., Fournier, M., & Couture, P. (2013). Thermal Stress Effects on Gene Expression and Phagocytosis in the Common Carp (*Cyprinus Carpio*): a Better Understanding of the Summer 2001 st. Lawrence River Fish Kill. *The Open Fish Science Journal*, 6(1).

Pacak, K., & Palkovits, M. (2001). Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocrine Reviews*, 22(4), 502-548.

Peter, M. S. (2011). The role of thyroid hormones in stress response of fish. *General and Comparative Endocrinology*, 172(2), 198-210.

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315(5808), 95-97.

Purohit, G. K., Mahanty, A., Suar, M. Sharma, A. P., Mohanty, B. P., & Mohanty, S. (2014). Investigating hsp gene expression in liver of *Channa striatus* under heat stress for understanding the upper thermal acclimation. *BioMed Research International*, 381719, 1-10.

Ray, K. A. T., Apte, N. Y., & Chicholikar, J.R. (2012). *In Climate of Ahmedabad.* Meteriological Center Ahmedabad, Ahmedabad.

Recsetar, M. S., Zeigler, M. P., Ward, D. L., Bonar, S. A., & Caldwell, C. A. (2012). Relationship between fish size and upper thermal tolerance. *Transactions of the American Fisheries Society*, 141(6), 1433-1438.

Riyaz, N., & Arakkal, F. R. (2011). Spa therapy in dermatology. *Indian Journal of Dermatology, Venereology and Leprology*, 77, 128.

Roychowdhury, P., Aftabuddin, M., & Pati, M. K. (2019). Studies on biochemical responses of table sized *Labeo rohita* (Hamilton, 1822) to the thermal exposure at critical maximum temperature (CTmax). *Exploratory Animal and Medical Research*, 9(2), 197-203.

Roychowdhury, P., Aftabuddin, M., & Pati, M. K. (2020a). Thermal stress–induced oxidative damages in the liver and associated death in fish, *Labeo rohita. Fish Physiology and Biochemistry*, 1-12.

Roychowdhury, P., Aftabuddin, M., & Pati, M. K. (2020b). Thermal stress altered growth performance and metabolism and induced anaemia and liver disorder in *Labeo rohita. Aquaculture Research*, 51(4), 1406-1414.

Ruane, N. M., Huisman, E. A., & Komen, J. (2001). Plasma cortisol and metabolite level profiles in two isogenic strains of common carp during confinement. *Journal of Fish Biology*, 59(1), 1-12.

Salinthone, S., Tyagi, M., & Gerthoffer, W. T. (2008). Small heat shock proteins in smooth muscle. *Pharmacology & Therapeutics*, 119(1), 44-54.

Sapolsky, R. M. (1996). Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress*, 1(1), 1-19.

Schlesinger, M. J. (1990). Heat shock proteins. *Journal of Biological Chemistry*, 265(21), 12111-12114.

Schreck, C. B., Contreras-Sanchez, W., & Fitzpatrick, M. S. (2001). Effects of stress on fish reproduction, gamete quality, and progeny. *In Reproductive Biotechnology in Finfish Aquaculture* (pp. 3-24).

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature*, 138(3479), 32-32.

Shi, Y. A. N. G. G. U., & Thomas, J. O. (1992). The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. *Molecular and Cellular Biology*, 12(5), 2186-2192.

Sorte, C. J., Jones, S. J., & Miller, L. P. (2011). Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *Journal of Experimental Marine Biology and Ecology*, 400(1-2), 209-217.

Tacon, A. G., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285(1-4), 146-158.

Ton, C., Stamatiou, D., & Liew, C. C. (2003). Gene expression profile of zebrafish exposed to hypoxia during development. *Physiological Genomics*, 13(2), 97-106.

Vijayan, M. M., & Moon, T. W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Canadian Journal of Zoology*, 72(3), 379-386.

Vijayan, M. M., Foster, G. D., & Moon, T. W. (1993). Effects of cortisol on hepatic carbohydrate metabolism and responsiveness to hormones in the sea raven, *Hemitripterus americanus. Fish Physiology and Biochemistry*, 12(4), 327-335.

Vijayan, M. M., Mommsen, T. P., Glémet, H. C., & Moon, T. W. (1996). Metabolic effects of cortisol treatment in a marine teleost, the sea raven. *Journal of Experimental Biology*, 199(7), 1509-1514.

Vijayan, M. M., Pereira, C., Grau, E. G., & Iwama, G. K. (1997). Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 116(1), 89-95.

Volpato, G. L., & Barreto, R. E. (2001). Environmental blue light prevents stress in the fish Nile tilapia. *Brazilian Journal of Medical and Biological Research*, 34(8), 1041-1045.

Wang, J., Wei, Y., Li, X., Cao, H., Xu, M., & Dai, J. (2007). The identification of heat shock protein genes in goldfish (Carassius auratus) and their expression in a complex environment in Gaobeidian Lake, Beijing, China. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 145(3), 350-362.

Wedemeyer, G. A. (1997). Effects of rearing conditions on the health and physiological quality of fish in intensive culture. *Fish Stress and Health In Aquaculture*, 35-71.

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, 77(3), 591- 625.

Weyts, F. A. A., Cohen, N., Flik, G., & Verburg-van Kemenade, B. M. L. (1999). Interactions between the immune system and the hypothalamo-pituitary-interrenal axis in fish. *Fish & Shellfish Immunology*, 9(1), 1-20.

Wodemeyer, G. A., & McLeay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. *In Stress and fish* (pp. 247-275). Academic Press, Western Fisheries Research Center.

Wu, C. (1995). Heat shock transcription factors: structure and regulation. *Annual Review of Cell and Developmental Biology*, 11(1), 441-469.

Xing, T., Gao, F., Tume, R. K., Zhou, G., & Xu, X. (2019). Stress effects on meat quality: A mechanistic perspective. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 380-401.

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Short Communication

The Death of the Beluga Sturgeon (*Huso huso*) Rediscovered in the Küçükçekmece Lagoon: Let's Focus on Causes

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ABSTRACT

Sturgeon, a key species in fisheries and aquaculture due to their high economic and ecological value, face numerous challenges in their natural habitats. The spawning grounds in rivers, essential for sturgeon reproduction, are often compromised by the blockage of migration routes caused by various obstacles. Lagoons, providing favorable conditions for fish growth, are also hunting grounds for predatory fish species. The Küçükçekmece Lagoon, located within İstanbul's borders in Türkiye, stands as one of the most significant lagoons in the region, especially noted for its position on the bird migration route. However, it has been suffering from unplanned urbanization, leading to a notable decline in water quality due to improper urban planning and industrial activities over the years. This decline is aggravated by waste drainage into the lagoon's wetland area. Notably, the last sturgeon sighting in the Küçükçekmece Lagoon was in 1986. After approximately 35 years, the discovery of two deceased beluga sturgeon (*Huso huso*) in the lagoon, which connects to the Marmara Sea in Türkiye, marked a significant event. This study aims to investigate the causes behind the death of these beluga sturgeons and also proposes potential solutions for enhancing the ecosystem's vitality of the lagoon. These solutions include addressing waste drainage, ensuring shoreline compliance, improving the sediment structure laden with organic matter, and artificially introducing fresh water. Such measures are crucial for the restoration and maintenance of the ecological balance in the Küçükçekmece Lagoon, which is vital for the survival of significant species like the sturgeon.

Keywords: Acipenseridae, brackish water, eutrophication fish migration, water pollution

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INTRODUCTION

Over recent decades, there has been a continuous and significant decrease in natural sturgeon populations (Pikitch, et al., 2005; Zhang, et al., 2019). This trend, combined with the ongoing high demand for caviar on the market, has paved the way for the growth of sturgeon farming, with a primary focus on caviar production, as reported by Bronzi & Rosenthal (2014). These fish are typically recognized for their distinctive appearance, massive size, and their renowned product, caviar. They are an ancient species that inhabit only the northern hemisphere (Ustaoğlu & Okumuş, 2004). Currently, all Eurasian sturgeon species are facing a significant threat, while the situation is somewhat better in America (Williot et al., 2011).

Memis (2014) reported that the status of all sturgeon species in Türkiye has undergone a complete transformation in the past 40 years, and many are now on the brink of extinction. This change is believed to be due to various factors, including human-made modifications to rivers, such as the construction of embankments, dams, regulators, and hydroelectric power plants, which have prevented upstream migration to spawning sites (Ustaoğlu Tırıl & Memiş, 2018). Pollution from industries, agriculture, and sewage, as well as overfishing, have also been identified as significant contributors to the drastic decline in sturgeon populations, both along the Black Sea coast and in Türkiye's three largest rivers (Kızılırmak, Yeşilırmak, Sakarya) (Ustaoğlu Tırıl & Memiş, 2018; Memiş et al., 2019).

Due to the significant decline in sturgeon populations, this species has been under protection since the 1980s. Türkiye signed the CITES agreement on 22 December 1996 and the catching and sale of sturgeon was banned by the Turkish authorities on April 1, 1998 (Memiş et al., 2020). In an effort to preserve natural sturgeon populations, sturgeon are being bred through aquaculture methods and released into the wild, contributing to the conservation of these endangered species (Akbulut et al., 2011).

Wetlands are the most productive areas on earth in terms of biological production and are also very rich in biodiversity (Tırıl, 2004). Lagoons have a special place among wetlands due to their connection to the sea. They are formed by the complete or partial closure of river mouths that flow into the sea with a coastal spit and sometimes by the accumulation of sea sand transported by currents in front of bays or gulfs (Joyeux & Ward, 1998; Karataş, 2005). They cover 13% of the continental coastal zone and are of great importance for biochemical processes and high productivity. Also, because of differences in salinity values, they have special ecological conditions that contribute to the richness of lagoon areas in terms of biodiversity (Gilabert, 2001; Çevik et al., 2008; Acarlı et al., 2009). They provide suitable conditions for fish to spawn and grow, as well as being preferred places for predatory fish species to feed. With these features, lagoons are also attractive centers for waterbirds and important feeding and offspring care areas.

Küçükçekmece Lagoon is one of the most important lagoons in Türkiye. Because of urbanization and industrialization, deterioration in water quality affects the ecosystem significantly (Albay et al., 2005; Köker et al., 2021). In the 1980s, various species of commercial fish were detected in the lagoon, but the fish population decreased in the early 2000s (Topcuoğlu et al., 1999). In the study conducted by Meriç (1986) on Küçükçekmece Lagoon, it was reported that 31 fish species inhabit the lagoon. These were *Acipenser gueldenstaedtii*, *Alburnus istanbulensis, Alosa tanaica*, *Anguilla anguilla*, *Aphanius fasciatus*, *Atherina boyeri*, *Belone belone*, *Clupeonella cultriventris*, *Chelon ramada, C. aurata*, *C. saliens*, *Engraulis encrasicolus*, *Esox lucius*, *Gasterosteus aculeatus*, *Huso huso*, *Knipowitschia caucasica*, *Mugil cephalus*, *Mullus surmuletus*, *Neogobius melanostomus*, *Nerophis ophidion*, *Petroleuciscus borysthenicus*, *Platichthys flessus*, *Proterorhinus semilunaris*, *Sander lucioperca*, *Sardina pilchardus*, *Scardinius erythrophthalmus*, *Pomatomus saltatrix*, *Sprattus sprattus*, *Syngnathus abaster*, *Trachurus mediterraneus* and *Vimba vimba* (Meriç, 1986; Deveciyan, 2006). Because of water pollution, many fish species have moved away from this lagoon and its fish population and diversity have decreased in recent decades (Özuluğ & Saç, 2019).

Beluga sturgeon (*Huso huso* Linnaeus, 1758) has not been recorded in Küçükçekmece Lagoon since 1986 (Meriç, 1986). Recently, two beluga sturgeon were found in the lagoon after 35 years. While the original goal of this study was to investigate their existence and causes of death, it has become evident that a broader approach is required. Given the deteriorated condition of the fish, which precludes detailed biochemical or histopathological analysis, the study now emphasizes understanding the lagoon's ecosystem structure and identifying ways to improve it. This approach is crucial, considering the fragile state of species like the beluga sturgeon and the need for a comprehensive strategy to enhance the resilience and health of the entire ecosystem.

MATERIALS AND METHODS

Study area and environmental variables

Küçükçekmece Lagoon, located in the west of Istanbul and connected to the Marmara Sea by a narrow channel, is 18 m deep and has a surface area of 15.22 km^2 (Figure 1). Eşkinoz, Nakkaşdere and Sazlıdere creeks flow into the lagoon and these creeks show seasonal flow (Albay et al., 2005, Topcuoğlu et al., 1999). Sazlıdere Dam, which is a drinking water supply, is located on Sazlıdere, the most important freshwater source of the lagoon. Due to this dam, the freshwater inflow into the lagoon has decreased.

During our field studies, the discovery of two nearly decomposed *H. huso* individuals on July 1, 2020, in the Küçükçekmece Lagoon was initially reported by local residents who frequently visit the lagoon's shores for recreational and fishing activities. This encounter was later confirmed by public officials who monitor the lagoon. The discovery was facilitated by wave movements bringing the fish to the shore (Figure 2). One of the individuals was found to be completely deteriorated with body integrity lost.

The length of the other fish stranded on the shore was measured on the field with a 1 mm calibrated meter, and a sample was taken from the pectoral fin rays to determine its age. The fin ray was prepared for reading by being placed in bleach for 2 min, and all excess tissue and skin were removed using a scalpel and forceps (Parr et al., 2018). The fin ray was sectioned using a Metkon low-speed saw (Metkon Microcut, Bursa) equipped with two diamond-tipped blades (Metkon Microcut 150) and a 0.15 mm spacer between the blades (Deval et al., 2018). Three sections were cut serially, beginning at approximately 25% of the total length of the fin ray, starting at the condyle base (Parr et al., 2018). The method for determining the age of the beluga sturgeons involved analyzing the sectioned fin rays, specifically focusing on the number of translucent bands present. Following the approach outlined by Bakhshalizadeh et al. (2017), we identified both opaque and translucent bands as annual growth rings, or annuli, on the fin ray. This identification is based on the principle that these bands represent distinct periods of growth and development in the beluga sturgeon's life cycle. Fin ray sections were examined and photographed under the reflected light using an Olympus SZX16 stereo microscope (with 2 × 10 magnification) (Deval et al., 2018). The fish species identification was made according to Akşıray (1987) and Kottelat & Freyhof (2007). The current scientific names of the species were verified according to Fricke et al. (2022).

For water quality analysis, water samples were taken from the surface waters where the beluga sturgeon were found. The YSI multiparameter (YSI 650 MDS) was used to measure dissolved oxygen, salinity, pH, and temperature *in situ*. Chlorophyll-*a* (chl-*a*)

Figure 1. Location of Küçükçekmece Lagoon. (Modified from Köker et al., 2021).

was determined using the method of ISO 10260 (1992). Nutrient concentrations: total inorganic nitrogen (TIN: NO_2-N+NO_3-N+NH_4-N) and total phosphorus (TP) were measured according to APHA-AWWA WPCF (2005). The Secchi depth (SD) was measured *in situ*. The trophic state of the Küçükçekmece Lagoon, Carlson Index was used and total phosphorus (TP), chlorophyll-a (Chl-*a*), and Secchi depth (SD) measurements were used to calculate the index (Carlson, 1977).

RESULTS AND DISCUSSION

The subject of this present study is the beluga sturgeon-*Huso huso*, an anadromous fish species that migrates kilometers upstream to the source of rivers from the seas in order to spawn on deep and gravelly-stony river bottoms (Chebanov & Galich, 2013). It is known that beluga sturgeon entered the Küçükçekmece Lagoon in the past (Meriç, 1986). This species would enter the lagoon not for reproduction but for feeding purposes. The fish species known to inhabit the Küçükçekmece Lagoon are among the preferred food sources in the diet of beluga sturgeon.

The beluga sturgeon (*Huso huso*) can reach approximately 6 m in length and up to 1000 kg in weight, with records indicating lengths of 8 m and weights of 3200 kg. Individuals are typically found to measure between 120-260 cm and weigh up to 363 kg (Berg, 1948). In the Küçükçekmece Lagoon, the age of the beluga sturgeon found in July 2020 was determined from the pectoral fin ray and it was around 20 years old. The specimen measured 250 cm in total length, indicating that the beluga sturgeon had reached the reproductive stage.

There are numerous reports that various hydraulic obstacles such as hydroelectric power stations, bridge abutments, and culverts built on rivers affect fish migration (DWA, 2005; Shortnose Sturgeon Status Review Team, 2010; Nyqvist et al., 2017; Nielsen and Szabo-Meszaros, 2022). Küçükçekmece Lagoon is a special wetland due to its location in the middle of Istanbul and it is an important habitat for living organisms from bacteria to fish. Since the 2000s, the lagoon has continued to be polluted, and with the construction of the Sazlıdere dam, the ecosystem has faced change and collapse due to the decrease in freshwater input (Köker et al, 2023). It has been reported that algal blooms have occurred in the Küçükçekmece Lagoon since the 1990s, resulting in the death of some fish (Albay et al. 2005; Köker et al. 2021).

Due to the increase in the salinity of the water, cyanobacterial blooms ceased to occur. The calculated $TSI_{(Chl\text{-}a)}$ and $TSI_{(TP)}$ mean values ($TSI_{(mean)}$=58.21) suggest that the Küçükçekmece Lagoon is in a nutrient-rich, eutrophic condition.

The physicochemical parameters measured during the investigation of the death of the beluga sturgeon individuals are shown in Table 1. The measured water temperature value was within the normal range for the summer season. Although it is known that the salinity level varies between 6-9 ppt in the area of Sazlıdere Dam where the freshwater inflow enters (Köker et al., 2021), the salinity level was determined as 13.5 ppt in the sampling area with currents coming from the channel where the lagoon connects to the Marmara Sea. The most important parameter was dissolved oxygen which measured 0.78 mg L^{-1}.

The trophic state of Küçükçekmece Lagoon was determined by following formulas based on total phosphorus (TP) and chlorophyll-*a* (Chl-*a*) (Carlson, 1977): $TSI_{(Chl\text{-}a)}=9.81\ln(Chl\text{-}a)+30.6$, and $TSI_{(TP)}=14.42\ln(TP)+4.15$. According to Table 1, it was determined that Küçükçekmece Lagoon is eutrophic.

Table 1. Some physical and chemical variables of the Küçükçekmece Lagoon during the sampling.

Environmental Parameters	June 2020
Temperature (°C)	26.35
Dissolved oxygen (mg L^{-1})	0.78
Salinity (ppt)	13.49
pH	8.20
Chl-*a* (µg L^{-1})	1.11
TIN (µg L^{-1})	5559.2
TP (µg L^{-1})	270.1
$TSI_{(TP)}$	84.88
$TSI_{(Chl\text{-}a)}$	31.53
$TSI_{(mean)}$	58.21

In July 2020, two individuals of *H. huso* who had died and whose bodies had washed ashore were found. One individual's tissues had completely decayed and its total length could not be measured. The total length of the other individual, which was in the process of decay, was 250 cm (Figure 2). Based on the overall condition of the fish bodies, it was clear that they had died a few days before our sampling.

The exact age of the *H. huso* individual shown in Figure 2-3, could not be determined due to the deterioration of the fin rays (Figure 4). The first ray of the pectoral fin taken from the 250 cm long fish and used for age determination was sectioned at the base. It was estimated to be approximately 20 years old (Figure 4).

Figure 2. Measurement of the length of the *Huso huso* individual.

Figure 3. Investigation of the species from the mouth and barbels.

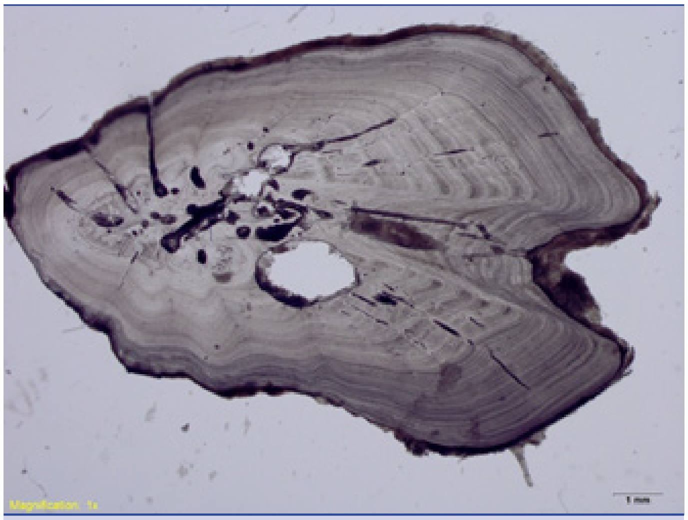

Figure 4. Pectoral fin barb beam section for age determination of beluga sturgeon.

Although the gonad examination of the fish suggested that it was female based on the consistency of the mass of the gonad and its large volume, there are signs similar to a male (M. Chebanov, personal communication, 7 July 2020).

Particularly during the summer season, factors such as increased water temperature and biological decomposition of waste within the lagoon lead to significant decreases in dissolved oxygen levels, even dropping to 0.78 mg O_2 L^{-1} (Table 1). The formation of oxygen-depleted zones throughout the water column, from the surface to the bottom of the lagoon, results in mass mortality events, especially among benthic invertebrates (e.g., crabs) and bottom-dwelling fish species (e.g., Gobiidae, Syngnathidae), as well as pelagic species like *A. boyeri* and *Chelon* spp., which have been observed during field observations. Atlantic sturgeon populations have been found to be declining in the Chesapeake Bay due to increased hypoxia (Secor & Gunderson, 1998; Secor & Niklitschek (2001). Even if the sturgeon has adapted physiologically to the environment, hypoxic conditions impair its respiratory metabolism, foraging activity, and growth (Wilson & Mckinley, 2004). These fish are typically sensitive to dissolved oxygen decreases. Cech & Doroshov (2004) reported that at moderate hypoxia (3 mg O_2 L^{-1}) at 15 °C, the activity of Atlantic sturgeon did not change but gill ventilatory frequency increased by 50%. Also, Jenkins et al. (1993) report that regardless of the life stage, shortnose sturgeon died at oxygen concentrations of 2 mg O_2 L^{-1}. For beluga hybrid sturgeon the critical concentration of dissolved oxygen was determined as 4 mg O_2 L^{-1} at 25 °C and the threshold concentration was 1.5 mg O_2 L^{-1} mg at 25 °C (Secor & Niklitschek, 2002). The natural stocks of this species are classified as Critically Endangered (CR) according to IUCN criteria (Gessner et al., 2022). The conservation of Küçükçekmece Lagoon, which serves as a feeding ground for endangered species such as the beluga sturgeon, is crucial for their continued existence. To this end, improving water quality through appropriate measures is essential. In the short term, immediate action should focus on reducing pollution sources, such as enforcing stricter controls on industrial effluents and urban wastewater discharges into the lagoon. This would quickly alleviate some of the direct pressures on the water quality.

In the medium term, efforts should concentrate on restoring the natural flow and water levels of the lagoon by reevaluating the impact of the Sazlıdere Dam and exploring ways to increase freshwater input. This step will help in balancing the lagoon's ecosystem and reducing the salinity levels, which have been identified as a contributing factor to the deterioration of the habitat. Moreover, the employment of sturgeon culture facilities for conservation purposes, including the restocking of sturgeon into the lagoon or connected river systems, should be considered. This approach would not only aid in the direct replenishment of the sturgeon population but also serve as a vital tool for their conservation and genetic diversity.

For long-term sustainability, a comprehensive management plan should be developed, focusing on habitat restoration, including the revitalization of native vegetation along the shores and the creation of buffer zones to reduce nutrient runoff (Gessner et al., 2014). Additionally, establishing a continuous monitoring system to track water quality and biodiversity changes in the lagoon will provide valuable data for ongoing conservation efforts and help in adapting strategies as needed.

Implementing these measures in a phased approach will not only aid in the recovery and preservation of the Küçükçekmece Lagoon's ecosystem but also ensure the protection of species such as the beluga sturgeon, which rely on this habitat for survival.

CONCLUSIONS

In summary, the deaths of beluga sturgeon individuals and other fish species in the Küçükçekmece Lagoon, primarily attributed to pollution, algal blooms, and oxygen depletion, highlight the urgent need for conservation and rehabilitation efforts, especially during the summer season. Factors such as high temperature and salinity likely contributed to the stress on the beluga sturgeon, exacerbating their vulnerability. The broader challenges facing sturgeon species in Türkiye, including habitat degradation and migration barriers like hydroelectric power plants and bridges, further complicate their survival and reproduction.

While the sturgeon's current situation in Turkish waters is alarming, with their migration routes and spawning grounds severely impacted, there remains a significant opportunity to revitalize the Küçükçekmece Lagoon. This lagoon, situated within one of the world's major metropolises, Istanbul, still holds potential as a living wetland. To enhance the vitality of this critical habitat, specific measures are recommended:

1. Improving waste management and drainage systems to prevent pollution of the lagoon,
2. Ensuring compliance with and respect for the shoreline, thereby reducing habitat destruction and erosion,
3. Enhancing the organic-laden sediment structure, which is vital for the growth and sustenance of various aquatic species,
4. Replenishing Küçükçekmece lagoon with fresh water to balance the salinity levels and improve overall water quality.

Implementing these measures will not only contribute to the vitality of the Küçükçekmece Lagoon but also set a precedent for the conservation of other sturgeon habitats in Türkiye. It is imperative that a 'National Action Plan for the Conservation and Restoration of the Sturgeons of Turkey' be developed and executed by relevant authorities (Anon., 2015). This plan should focus on identifying and protecting the most suitable rivers for sturgeon survival and restoring their natural spawning grounds. Such efforts are crucial for the recovery of these ancient fish species and for preserving the biodiversity and ecological balance of Türkiye's aquatic ecosystems.

The findings of this study underscore the urgent need for a comprehensive and coordinated approach to conservation, highlighting the potential for recovery and revitalization of lagoon life, including the Küçükçekmece Lagoon. It is hoped that these insights will inform and inspire local, national, and international institutions to take decisive actions towards the sustainable management and preservation of these vital ecosystems.

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REFERENCES

Acarlı, D., Kara, A., Bayhan, B. & Çoker, T. (2009). Homa Lagünü'nden (İzmir Körfezi, Ege Denizi) Yakalanan Türlerin Av Kompozisyonu ve Av Verimi. *Ege Üniversitesi Su Ürünleri Dergisi*, *26*(1), 39-47

Akbulut, B., Zengin, M., Çiftçi, Y., Ustaoğlu Tiril, S., Memiş, D., Alkan, A., ... & Serdar, S. (2011). Stimulating sturgeon conservation and rehabilitation measures in Turkey: an overview on major projects (2006–2009). *Journal of Applied Ichthyology*, *27*(2), 415-419.

Akşıray, F. (1987). *Türkiye Deniz Balıkları ve Tayin Anahtarı*. İstanbul Üniversitesi Rektörlüğü Yayınları, No: 3490, İstanbul, 811 pp.

Albay, M., Matthiensen, A. & Codd, G. A. (2005). Occurrence of toxic blue-green algae in Kucukcekmece Lagoon (Istanbul-Turkey). *Environmental Toxicology*, *20*, 277–284. doi: 10.1002/tox.20118

Anonymous (2015). National Action Plan for the Conservation and Restoration of the Sturgeons of Turkey. Published by The Ministry of Food Agriculture and Livestock, DG Fisheries and Aquaculture, Medisan Yayınevi, Ankara, 127 pp.

APHA, AWWA, WPCF. (2005). Standard Methods for the Examination of Water and Wastewater. Washington DC.

Bakhshalizadeh, S., Bani, A., Abdolmalaki, S., & Moltschaniwskyj, N. (2017). Identifying major events in two sturgeons' life using pectoral fin spine ring structure: exploring the use of a non-destructive method. *Environmental Science and Pollution Research*, 24, 18554-18562.

Berg, L. S. (1948). *Fish of fresh waters of the USSR and adjacent countries.* Moscow, Izdatel'stvo Akademii Nauk SSSR. 468 pp. (In Russian).

Bronzi, P. & Rosenthal, H. (2014). Present and future sturgeon and caviar production and marketing: A global market overview. *Journal of Applied Ichthyology*, *30*(6), 1536-1546. doi: 10.1111/jai.12628

Carlson, R. E. (1977). A Trophic State Index for Lakes. *Limnology and Oceanography*, *22*(2):361–369. https://doi.org/10.4319/lo.1977.22.2.0361

Cech, J. J. & Doroshov, S. I. (2004). *Environmental Requirements, Preferences, and Tolerance Limits of North American Sturgeons*. In: LeBreton, G.T.O., Beamish, F.W.H., McKinley, R.S. (eds) *Sturgeons and Paddlefish of North America*. Fish & Fisheries Series, vol 27. Springer, Dordrecht. doi: 10.1007/1-4020-2833-4_4

Chebanov, M. S. & Galich, E. V. (2013). *Sturgeon Hatchery Manual*. FAO Fisheries and Aquaculture Technical Paper No 558. Food and Agriculture Organization of the United Nations, Ankara, ISBN 978-92-5-106823-6, 338 pp.

Çevik, F., Polat, S. & Dural, M. (2008). Akyatan ve Tuzla Lagünlerinin (Adana, Türkiye) Fitoplanktonu ve Mevsimsel Değişimi. *Journal of FisheriesSciences.com*, *2*(1), 19-29. doi: 10.3153/jfscom.2008002

Deval, M. C., Kebapçıoğlu, T., Güven, O. & Olguner, M. T. (2018). Population pattern and dynamics of the Bluemouth *Helicolenus dactylopterus* (Delaroche, 1809) in the eastern Mediterranean Sea. *Journal of Applied Ichthyology. 34*, 568–580. doi: 10.1111/jai.13613

Deveciyan, K. (2006). *Türkiye'de Balık ve Balıkçılık.* Aras Yayıncılık, İstanbul, 574 p. ISBN: 975-7265-75-6.

DWA (2005). *Fish Protection Technologies and Downstream Fishways. Dimensioning, Design, Effectiveness, Inspection*; DWA German Association for Water, Wastewater and Waste: Hennef, Germany.

Fricke, R., Eschmeyer, W. N. & van der Laan, R. (2022). *Eschmeyer›s Catalog of Fishes: Genera, Species, References.* Electronic version accessed: 28.06.2022. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Gessner, J., Chebanov, M. & Freyhof, J. 2022. *Huso huso. The IUCN Red List of Threatened Species* 2022: e.T10269A135087846. https://dx.doi.org/10.2305/IUCN.UK.2022-1.RLTS.T10269A135087846.en Accessed on 15 December 2023.

Gessner, J., Zahn, S., Jaric, I. & Wolter, C. (2014). Estimating the potential for habitat restoration and connectivity effects on European sturgeon (*Acipenser sturio* L. 1758) population rehabilitation in a lowland river – the Havel, Germany. *Journal of Applied Ichthyology*, *30*, 1473-1482. doi: 10.1111/jai.12613

Gilabert, J. (2001). Seasonal phytoplankton dynamics in a Mediterranean hypersaline coastal lagoon: Mar Menor. *Journal of Plankton Research*, *23*(2), 207-217. doi: 10.1093/plankt/23.2.207

ISO 10260 (1992). Water Quality—Measurement of Biochemical Parameters—Spectrometric Determination of the Chlorophyll—A Concentration. International Organization for Standardization, Geneva.

Jenkins, W.E., Smith, T., Heyward, L. & Knott, D. M. (1993). Tolerance of shortnose sturgeon, *Acipenser brevirostrum*, juveniles to different salinity and dissolved oxygen concentrations. *Proc. Ann. Conf. SE Assoc. Fish & Wildl. Agen., 47*, 476-484.

Joyeux, J-C. & Ward, A. B. (1998). Constraints on coastal lagoon fisheries. *Advances in Marine Biology*, *34*, 73-199. doi: 10.1016/S0065-2881(08)60211-4

Karataş, A. (2005). *Oseanoloji Deniz Bilimlerine Giriş.* Ege Üniversitesi Yayınları Su Ürünleri Fakültesi Yayın No:60, VII. Baskı, Bornova-İzmir, ISBN: 975-483-182-1, pp: 357.

Kottelat, M. & Freyhof, J. (2007). *Handbook of European Freshwater Fishes.* Kottelat, Cornol, Switzerland & Freyhof, Berlin, Germany, ISBN: 978-2-8399-0298-4, xiv + 646 pp.

Köker, L., Akçaalan, R., Dittmann, E., & Albay, M. (2021). Depth profiles of protein-bound microcystin in Küçükçekmece Lagoon. *Toxicon, 198*, 156-163.

Köker, L., Ozbayram, E. G., Oğuz Çam, A., Akçaalan, R., & Albay, M. (2023). Variation in Water Quality in an Impacted Coastal Lagoon over the Last Decade (Küçükçekmece Lagoon, Turkey). *Environmental Sciences Proceedings*, *25*(1), 26.

Memis, D. (2014). A short history of sturgeon caviar production in Turkey. *Journal of Applied Ichthyology*, *30*(6), 1552-1556. doi: 10.1111/jai.12602

Memiş, D., Tosun, D. D., Yamaner, G., Tunçelli, G. & Gessner, J. (2019). Present status of sturgeon in the lower Sakarya river in Turkey. *Aquatic Research*, *2*(2), 53-60. doi: 10.3153/AR19007

Memiş, D., Yamaner, G., Tosun, D., Tunçelli, G. & Tınkır, M. (2020). Current status of economically important diadromous fish species of Turkey; European eel, Black Sea trout and sturgeon species. *Aquatic Research*, *3*(4), 188-196. doi: 10.3153/AR20017

Meriç, N. (1986). Fishes Encountered in Küçükmece Lake, Istanbul. *İstanbul Üniversitesi Fen Fakültesi Mecmuası Seri B*, *51*: 33-39.

Nielsen, N. & Szabo-Meszaros, M. (2022). *A Roadmap for Best Practice Management on Hydropower and Fish – IEA Hydro report on Annex XIII Hydropower and Fish*, 170 pp. doi: 10.5281/zenodo.7805685.

Nyqvist, D., Nilsson, P., Alenäs, I., Elghagen, J., Hebrand, M., Karlsson, S., Kläppe, S. & Calles, O. (2017). Upstream and downstream passage of migrating adult Atlantic salmon: Remedial measures improve passage performance at a hydropower dam. *Ecological Engineering*, *102*, 331-343. doi: 10.1016/j.ecoleng.2017.02.055.

Özuluğ, M. & Saç, G. (2019). İstanbul ili (Türkiye) tatlısu balık faunası. *Turkish Journal of Bioscience and Collections*, *3*(1), 19–36. doi: 10.26650/tjbc.201930004.

Parr, R. T., Bringolf, R. B. & Jennings, C. A. (2018). Efficacy of otoliths and first dorsal spines for preliminary age and growth determination in Atlantic Tripletails. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *10*, 71–79. doi: 10.1002/mcf2.10008.

Pikitch, E. K., Doukakis, P., Lauck, L., Chakrabarty, P. & Erickson, D. L. (2005). Status, trends and management of sturgeon and paddlefish fisheries. *Fish and Fisheries*, *6*, 233-265. doi: 10.1111/j.1467-2979.2005.00190.x

Secor, D. H. & Gunderson, T. E. (1998). Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon, *Acipenser oxyrinchus. Fishery Bulletin*, *96*(3), 603-613.

Secor, D. H. & Niklitschek, E. J. (2001). *Hypoxia and sturgeons: Report to the Chesapeake Bay Program dissolved oxygen criteria team.* UMCES Tech. Ser. No. TS-314-01-CBL.

Secor, D. H. & Niklitschek, E. J. (2002). Sensitivity of sturgeons to environmental hypoxia: a review of physiological and ecological evidence. In: Thurston, R.V. (Ed.), *Fish Physiology, Toxicology, and Water Quality.* Proceedings of the Sixth International Symposium, La Paz, Mexico.

Shortnose Sturgeon Status Review Team. (2010). *A Biological Assessment of shortnose sturgeon* (*Acipenser brevirostrum*). Report to National Marine Fisheries Service, Northeast Regional Office. November 1, 2010. 417 pp.

Tırıl, A. (2004). Türkiye'nin Sulak Alanları ve Su Kuşları. *Kebikeç*, *18*, 139-157.

Topcuoğlu, S., Güngör, N. & Kirbaşoğlu, Ç. (1999). Physical and chemical parameters of brackish water lagoon, Küçükçekmece Lake, in northwestern Turkey. *Toxicological & Environmental Chemistry*, *69*(1-2), 101-108. doi: 10.1080/02772249909358691.

Ustaoğlu Tırıl, S. & Memiş, D. (2018). An Overview of the Factors Affecting the Migration of Sturgeons in Yeşilırmak. *Aquatic Sciences and Engineering*, *33*(4), 138-144. doi: 10.26650/ASE20180002

Ustaoğlu, S. & Okumuş, İ. (2004). The Sturgeon: Fragile Species Need Conservation. *Turkish Journal of Fisheries and Aquatic Sciences, 4*, 49-57.

Williot, P., Rochard, E., Desse-Berset, N., Gessner, J. & Kirschbaum, F. (2011). *Brief Introduction to Sturgeon with a Special Focus on the European Sturgeon,* Acipenser sturio *L. 1758*. In: Williot, P., Rochard, E., Desse-Berset, N., Kirschbaum, F., Gessner, J. (eds) *Biology and Conservation of the European Sturgeon* Acipenser sturio *L. 1758*. Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-20611-5_1

Wilson, J. A. & Mckinley, R. S. (2004). *Distribution, Habitat, and Movements*. In: LeBreton, G. T. O., Beamish, F. W. H., McKinley, R.S. (eds) *Sturgeons and Paddlefish of North America.* Fish & Fisheries Series, vol 27. Springer, Dordrecht. doi: 10.1007/1-4020-2833-4_3

Zhang, H., Kang, M., Wu, J., Wang, C., Li, J., Du, H., Yang, H. & Wei, Q. (2019). Increasing River Temperature Shifts Impact the Yangtze Ecosystem: Evidence from the Endangered Chinese Sturgeon. *Animals*, *9*(8), 583. doi: 10.3390/ani9080583.

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Submitted manuscripts that pass preliminary control are scanned for plagiarism using iThenticate software. After plagiarism check, the eligible ones are evaluated by Editor-in-Chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope.

Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, citizenship, religious belief or political philosophy of the authors and ensures a fair double-blind peer review of the selected manuscripts.

The selected manuscripts are sent to at least two national/international external referees for evaluation and publication decision is given by Editor-in-Chief upon modification by the authors in accordance with the referees' claims.

Editor-in-Chief does not allow any conflicts of interest between the authors, editors and reviewers and is responsible for final decision for publication of the manuscripts in the Journal.

Reviewers' judgments must be objective. Reviewers' comments on the following aspects are expected while conducting the review.

- Does the manuscript contain new and significant information?
- Does the abstract clearly and accurately describe the content of the manuscript?
- Is the problem significant and concisely stated?
- Are the methods described comprehensively?
- Are the interpretations and consclusions justified by the results?
- Is adequate references made to other Works in the field?
- Is the language acceptable?

Reviewers must ensure that all the information related to submitted manuscripts is kept as confidential and must report to the editor if they are aware of copyright infringement and plagiarism on the author's side.

A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines for non-randomized studies, and COREQ guidelines for qualitative studies.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at https://dergipark.org.tr/en/journal/507/submission/step/manuscript/new. Manuscripts submitted via any other medium will not be processed.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission. These are available for download at istanbul.dergipark.gov.tr/ase

- Copyright Agreement Form,
- Author Contributions Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, and highest academic degree(s) of the author(s) and ORCID ID (orcid.org)
- Grant information and detailed information on the other sources of financial support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,

- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text should contain Introduction, “Materials and Methods”, “Result and Discussion”, "Conclusion" and "References" sections.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE’s “Guidance for Editors: Research, Audit and Service Evaluations” document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in researches and should guide future studies. The main text should start with Introduction and end with "Conclusion" and "References" sections. Authors may choose to use any subheading in between those sections.

After the Conclusion section and before references list, information regarding conflict of interest and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

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Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers’ attention, particularly educative cases, may also be submitted in the form of a “Short Communication” Readers can also present their comments on the published manuscripts in the form of a “Short Communication”. The main text should contain Introduction, “Materials and Methods”, “Result and Discussion”, "Conclusion" and "References" sections.

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Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤20	250	40
Review Article	≤25	250	60
Short Communication	≤5	250	20

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. List references in alphabetical order. Each listed reference should be cited in text, and each text citation should be listed in the References section. The reference styles for different types of publications are presented in the following examples.

Reference Style and Format

Aquatic Sciences and Engineering complies with APA (American Psychological Association) style 6th Edition for referencing and quoting. For more information:

- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
- http://www.apastyle.org

Accuracy of citation is the author's responsibility. All references should be cited in text. Reference list must be in alphabetical order. Type references in the style shown below.

Citations in the Text

Citations must be indicated with the author surname and publication year within the parenthesis.

If more than one citation is made within the same paranthesis, separate them with (;).

Samples:

More than one citation;
(Esin et al., 2002; Karasar, 1995)
Citation with one author;
(Akyolcu, 2007)
Citation with two authors;
(Sayıner & Demirci, 2007)
Citation with three, four, five authors;
First citation in the text: (Ailen, Ciambrune, & Welch, 2000) Subsequent citations in the text: (Ailen et al., 2000)
Citations with more than six authors;
(Çavdar et al., 2003)

Major Citations for a Reference List

Note: All second and third lines in the APA Bibliography should be indented.

- **A book in print:** Baxter, C. (1997). *Race equality in health care and education*. Philadelphia: Ballière Tindall. ISBN 4546465465
- **A book chapter, print version:** Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17-43). New York, NY: Guilford Press. ISBN 4546469999
- **An eBook:** Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
- **An article in a print journal:** Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy, 14*(1), 38-48.
- **An article with DOI:** Gaudio, J. L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (saguinus oedipus) reversal learning. *Journal of Comparative Psychology*, https://doi.org/10.1037/0735-7036.122.4.441
- **Websites - professional or personal sites:** The World Famous Hot Dog Site. (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.15)
- **Websites - online government publications:** U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15)
- **Photograph (from book, magazine or webpage):** Close, C. (2002). *Ronald*. [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.15)
- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby*. [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
- **Artwork - from website:** Close, C. (2002). *Ronald*. [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.15)

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

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