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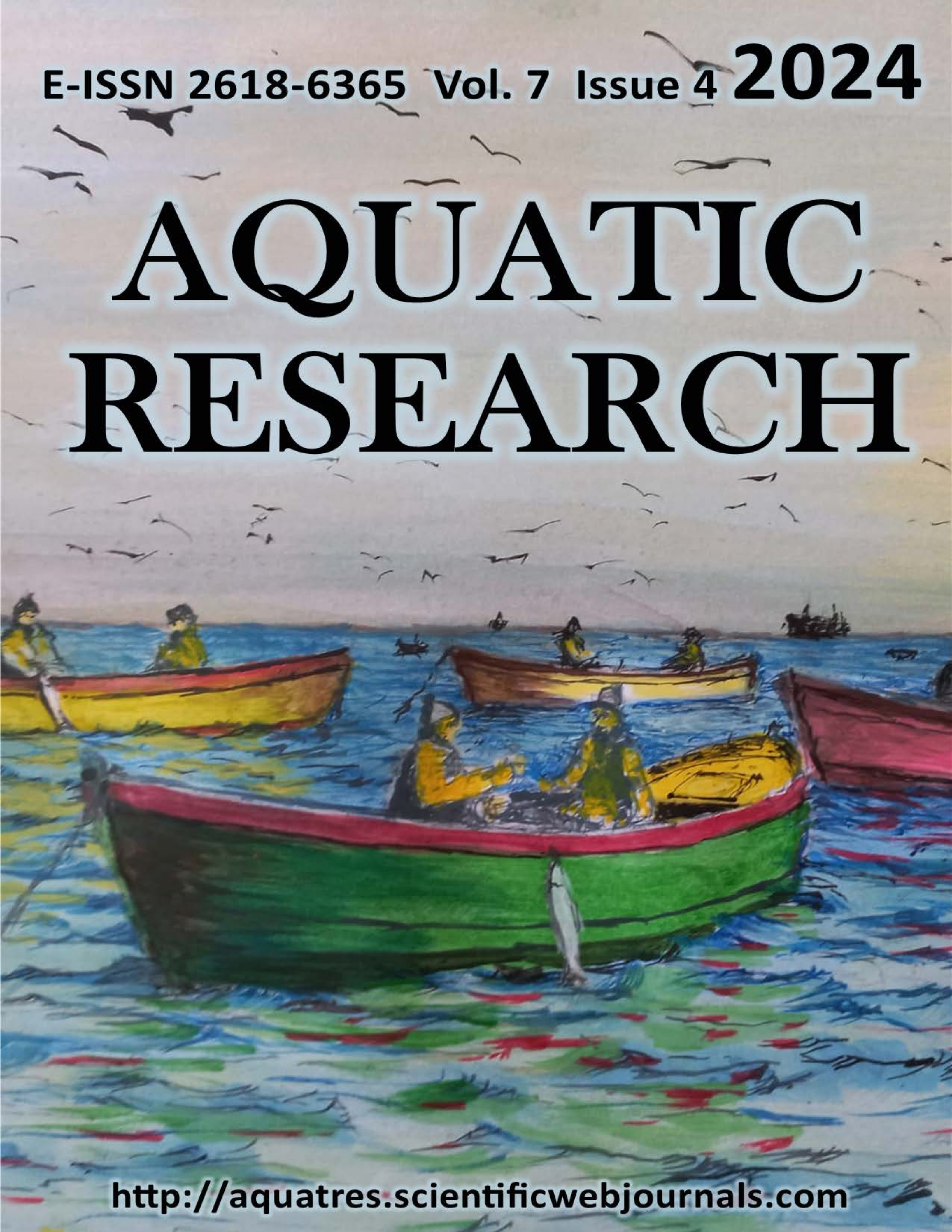
AQUATIC RESEARCH



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Aims and Scope

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Aquatic Research 7(4), 178-188 (2024) • https://doi.org/10.3153/AR24016 *Research Article*

In vitro, amoebicidal activities of submerged plant *Ceratophyllum demersum* L. extract against *Acanthamoeba castellanii* trophozoites

Beyhan TAŞ[1], Zeynep KOLÖREN[1], Onur KOLÖREN[2]

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ABSTRACT

Ceratophyllum demersum L. is a hydrophyte with potential for use as an analgesic, antipyretic and anti-inflammatory drug. It has also been stated that it is a hepatoprotective and anti-diarrheal agent with potential use in stomach disorders and respiratory diseases. Our study investigated the anti-amoebic activity of C. demersum, which became a hydrophyte underwater collected from Samsun River Miliç. Different concentrations of the pathogenic strain of *Acanthamoeba castellanii* (ATCC 30010) and the ethanolic extract of *C. demersum* were used to determine anti-Acanthamoeba activity. *A. castellanii* trophozoites were treated with *C. demersum* extract at different concentrations ranging from 1.9, 3.8, 7.6, 15.2, 30.4, 60.8 µg/mL and incubated at 26°C for 72 h. The 50% inhibitory concentration (IC50) of *C. demersum* extract was approximately 42.5 µg/mL at 72 hours. Trophozoite cell viability decreased to 42% and 58.33% in the presence of 30.4 and 60.8 µg/mL *C. demersum* extract at 72 hours, respectively. These results indicate that the ethanolic extract of *C. demersum* has anti-Acanthamoeba activity against *A. castellanii* trophozoites. The study highlights that this extract can be a potential protective drug source against *Acanthamoeba* trophozoites.

Keywords: *Ceratophyllum demersum, Acanthamoeba castellanii*, Amoebicidal activities, Hydrophyte

Introduction

Ceratophyllum demersum L. (Ceratophyllaceae), one of the important primary producers of aquatic ecosystems, is a cosmopolitan hydrophyte that lives completely under water (submerged), floats freely in the water column, does not produce roots, and can form dense covers just below the surface. The plant has a high ecological tolerance, often occurring in ponds, lakes, ditches, and calm streams with moderate to high nutrient levels. In Turkish waters, *C. demersum* and *C. submersum* are commonly found in the Yeşilırmak Delta (Taş & Topaldemir, 2021). The use of hydrophytes living in freshwater as medicinal plants has received less attention than terrestrial plants. However, phytochemical evaluations reveal the existence of secondary metabolites such as tannins, steroids, glycosides, flavonoids, phenolic compounds, and alkaloids. For this reason, researchers have recently shown interest in hydrophytes (Bhowmik et al., 2013; Roma et al., 2017).

C. demersum has traditionally been used to treat diarrhoea, fever, wounds, haemorrhoids or haemorrhoids, internal bleeding, hyperdipsia, and hematemesis (Li et al., 2020). Some studies have shown that *C. demersum* extracts are antioxidant (Karatas et al., 2015), antifungal (Malathy et al., 2015), insecticidal (Lu et al., 2007), antidiarrheal and wound healing (Taranhalli et al., 2011), antibacterial (especially Effective against *E. coli* and *Bacillus cereus*) and anti-leishmanial (Al-Halbosiy et al., 2020) activity. The literature research found no research on the anti-Acanthamoeba effects of *C. demersum.*

Acanthamoeba are opportunistic pathogens widely distributed throughout the world. *Acanthamoeba* spp. Commonly found in damp soil, freshwater accumulations, sewage, swimming pools, contact lens equipment, lakes, dam lakes, tap water, and air. They have two important diseases, Acanthamoeba keratitis (AK) and Acanthamoeba granulomatous encephalitis (GAE), caused by these parasites. GAE is usually chronic, lasting over a week, sometimes even months (Lass et al., 2014).

There are two forms in the life cycle of Acanthamoeba. It has an active trophozoite form with a dormant cyst under stressful conditions. Depending on the conditions, these two forms can transform into each other (Khan, 2006). The cyst wall of Acanthamoeba makes it tolerant to drugs. Changes in physiological and radiological conditions, chlorination and biocides do not prevent the survival of the cyst. Cysts are primarily responsible for the long-term treatment of Acanthamoeba infections. Cysts also resist the drugs used (Elsheikha et al., 2020).

Acanthamoeba cysts are difficult to treat because they are highly resistant to antibiotics and other agents. Therefore, studies are ongoing to find an effective treatment against Acanthamoeba infections (Chiboub et al.,2017). This study investigated the antiparasitic effect of *C. demersum,* a submerged hydrophyte collected from Samsun River Miliç. The present study aimed to survey and evaluated in vitro antiamoebic activities of C. demersum, a submerged hydrophyte collected from Samsun River Miliç.

Materials and Methods

Sample Collection

Aquatic plant species (*C. demersum*) were collected from Miliç River, Terme, Samsun (Figure 1). Collected samples were washed with water to remove epiphytes and other freshwater organisms. The plants were transported to the laboratory in sterile polythene bags.

Extraction of Macrophytes

The collected *C. demersum* samples were moved in a cool container to the laboratory. Firstly, the samples were washed with distilled water. Then, the samples were shade-dried, cut into small pieces, and finely powdered in a mixer grinder. An organic solvent (ethanol) was used for extraction. Ethanol has proven to be the best solvent for extracting compounds with antimicrobial activity and antioxidant capacity (regardless of the extraction method used) (Borges et al., 2020). They were homogenized with a blender. The samples (30 g) were extracted in 250 mL of ethanol for 48 h at room temperature in a shaking incubator. The solvent was then removed from the aquatic plant extract by evaporation. The final concentration was adjusted to 60.8 µg/mL with distilled water from the residue. Different dilutions of *C. demersum* extract (1.9, 3.8, 7.6, 15.2, 30.4, and 60.8 µg/mL) were made by serial dilution with distilled water.

Amoebicidal Activity

Acanthamoeba castellanii (ATCC 30010 from the American Type Culture Collection) was used in this study. The A. castellani strain was cultured on Ringer agar plates seeded with Gram-negative bacteria (*E. coli*) as a food source. The plates were incubated at 26 ºC in the incubator, and three days later, they were microscopically examined for the presence of *Acanthamoeba* trophozoites (Tepe et al., 2012; Koloren et al., 2019).

The pathogenic strain of *A. castellanii* (ATCC 30010) and the different concentrations of *C. demersum* ethanolic extract were used to determine the anti-amoebic activity assays. *A. castellanii* trophozoites were treated with different concentrations of *C. demersum* extract in the range of 1.9, 3.8, 7.6, 15.2, 30.4, and 60.8 µg/mL and incubated for different hours at 26 ºC. The increase or decrease in amoebae was checked at 1, 3, 6, 8, 24, 48, and 72 h intervals using a Thoma haemocytometric chamber. Approximately 100 *A. castellanii* trophozoites were examined each time, and all the tests were repeated three times. The control group was a culture of amoebae without *C. demersum* extract and statistical analyses were done to show the cell viability percentage.

Figure 1. *Ceratophyllum demersum* collected from Miliç River (Terme, Samsun)

Statistical Analysis

A one-way test of variance (ANOVA) with the SPSS software package for Windows was applied to complete all statistical analogies was used for all results. The results were expressed as Mean ± standard error (SE). We found differences in the means by performing a Tukey multiple comparison analysis to determine which means are similar or different. Analysis of variance was determined by A one-way test of variance (ANOVA) with the SPSS software package for Windows. Differences at $p < 0.01$ were statistically significant.

Moreover, Principal Components Analysis (PCA) was carried out with the Jamovi 2.4.11 program.

Results and Discussion

This study examined different ethanolic extracts of C. demersum for their anti-amoebic activity against *A. castellanii* trophozoites at different hours at 26 °C.

The amoebicidial activity of *C. demersum*'s ethanolic extracts on A. castellanii trophozoites is shown in Table 1 and Figure 1.

The trophozoite growing stopped in ethanolic extracts of *C. demersum* with IC50/72h at 42.5 µg/mL. The ethanolic extracts of *C. demersum* showed strong inhibitory effects at 60.8, 30.4, and 15.2 µg/mL concentrations at 72 h against Acanthamoeba trophozoites. The seaweed extract (1.9 µg/mL) on trophozoites with IC50/72 h out of the different concentrations of ethanolic extracts of C. demersum used in the study showed the most vital anti-amoebic activity.

The results were expressed as percentage inhibition relative to control cells (Figure 2).

The results are the mean standard errors, as in Table 1. Tables 2 and 3 show which means are similar and different.

The data were expressed as mean ± SD. The statistical difference between values marked with different letters, such as a and b, is important.

It was determined that the effect of the application times of the doses on viability was statistically significant, $p<0.001$. When the effects of application times on viability were examined, it was determined that viability decreased as the waiting time increased. The highest viability was measured at the end of 24 hours, while the lowest viability was measured at the end of the 72nd hour.

Explanatory variables were visualised with PCA to summarize the results for all doses. Figure 3 shows the PCA biplot of the values of different doses at different times. Both the first (F1, 2.31%) and second (F2, 97.31%) principal components collectively contributed to the largest variation (99.6%) in the dataset.

The % cell viability values in the 24 hours of all applied doses show a significant negative correlation with the 72-hour values, p (0.05). The 48th-hour cell viability data are located in the middle of the axis, and it has been observed that cell viability decreases after this time. In this context, the 48th hour can be considered the threshold value for all doses (Figure 4).

Table 4 shows that all axes explain 99.6% of the variances.

Table 1. Percentages of cell viability in the Acanthamoeba trophozoites when exposed to the different concentrations of Ceratophyllum demersum, extracts at 72h.

The stage of A. castellanii	The concentrations of C. demersum leaf extracts	Percentage of cell viability ±SE
Trophozoites	60.8 µg/mL	41.67 ±1.20
	30.4 µg/mL	58 ±1.53
	15.2 µg/mL	63 ±0.58

Table 2. Mean ± SD values for the percentages of cell viability of Acanthamoeba trophozoites when exposed to various concentrations of *Ceratophyllum demersum* L. ethanolic extract at varied hours

Hour	Dose	Percentages of cell viability
24	Control	99,33±1,15[a]
	1.9 µg/mL	99±1,73[ab]
	3.8 µg/mL	96,33±1,53[abc]
	7.4 µg/mL	94,33±0,58[bc]
	15.2 µg/mL	93±1[cd]
	30.4 µg/mL	89±1[de]
	60.8 µg/mL	79±1[f]
48	Control	99±1[ab]
	1.9 µg/mL	97,67±2,52[abc]
	3.8 µg/mL	89±1[de]
	7.4 µg/mL	85±1[e]
	15.2 µg/mL	80±1[f]
	30.4 µg/mL	73±1[g]
	60.8 µg/mL	59±1[hi]
72	Control	97,33±0,58[abc]
	1.9 µg/mL	87±1[e]
	3.8 µg/mL	79,33±3,06[f]
	7.4 µg/mL	71±1,73[g]
	15.2 µg/mL	63±1[h]
	30.4 µg/mL	58±2,65[i]
	60.8 µg/mL	41,67±2,08[j]
p	<0,001	

Table 3. Summary of the effect of Ceratophyllum demersum L. ethanol extract on cell viability of Acanthamoeba trophozoites at various doses and durations (Mean±SD)

	24	48	72	Σ
Control	99,33±1,15[a]	99±1[ab]	97,33±0,58[abc]	98,56±1,24[a]
1.9 µg/mL	99±1,73[ab]	97,67±2,52[abc]	87±1[e]	94,56±5,92[b]
3.8 µg/mL	96,33±1,53[abc]	89±1[de]	79,33±3,06[f]	88,22±7,6[c]
7.4 µg/mL	94,33±0,58[bc]	85±1[e]	71±1,73[g]	83,44±10,22[d]
15.2 µg/mL	93±1[cd]	80±1[f]	63±1[h]	78,67±13,06[e]
30.4 µg/mL	89±1[de]	73±1[g]	58±2,65[i]	73,33±13,51[f]
60.8 µg/mL	79±1[f]	59±1[hi]	41,67±2,08[j]	59,89±16,23[g]
Σ	92,86±6,79[a]	83,24±13,44[b]	71,05±17,87[c]	

Data were expressed as mean ± SD
A statistical difference exists between values marked with letters such as a and b.

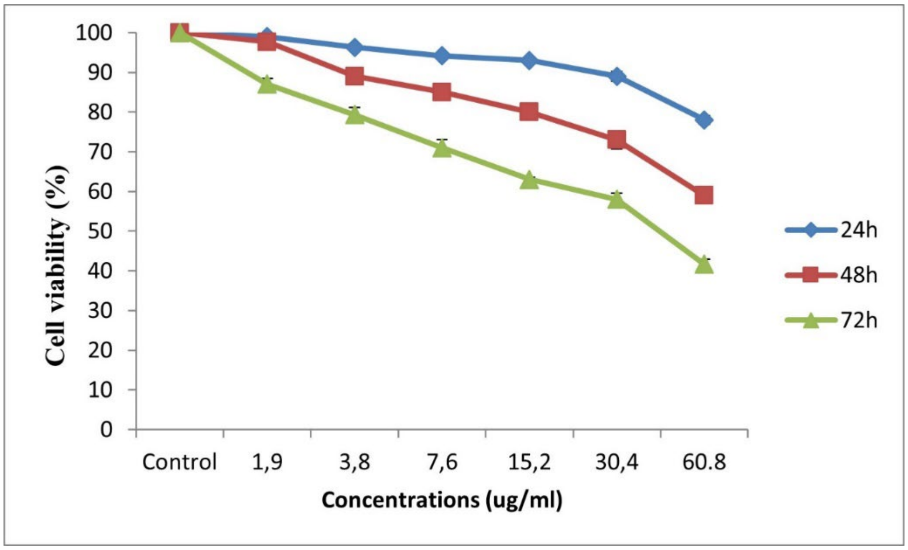

Figure 2. The effect of various concentrations of *Ceratophyllum demersum* L. ethanolic extract on the proliferation of *A. castellani* trophozoites at different hours

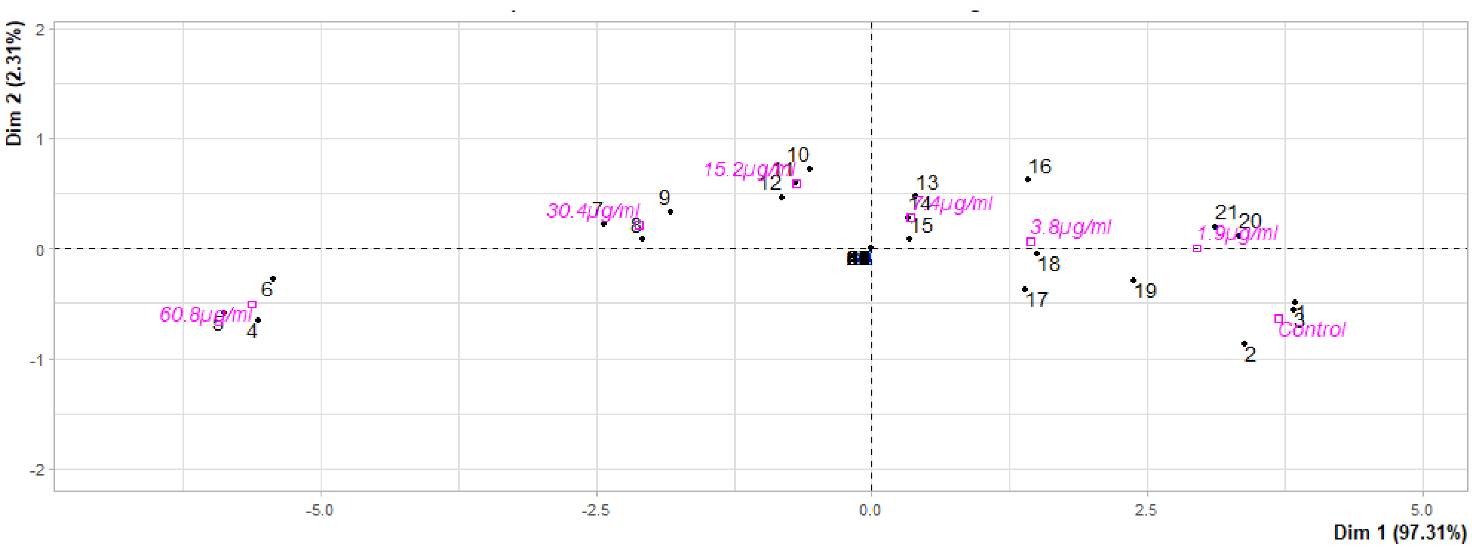

Figure 3. Representation of the individuals (and the categories)

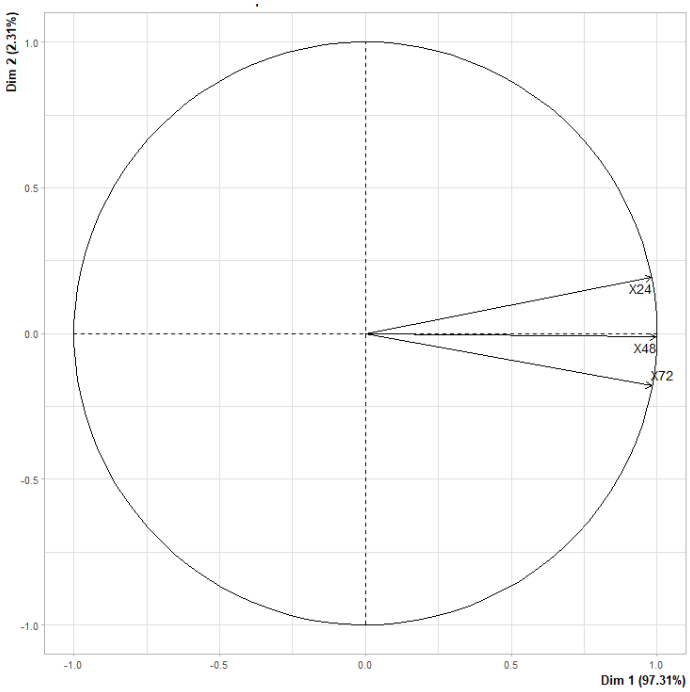

Figure 4. Representation of the active variables

Table 4. Eigenvalue and (Cumulative) percentage of variance

	Eigenvalue	% of the variance	Cumulative %
Dim. 1	2.9193	97.311	97.3
Dim. 2	0.0692	2.308	99.6

Awati et al. (2020) reported that the original phytoconstituents components of *C. demersum* are flavonoids, alkaloids, sterols, proteins, tannins, quercitrin, and volatile oil. Many studies have been reported on *C. demersum* L. because it is antidiarrhoeal and wound curative activity (Ashok et al., 2011; Brunton et al., 2006), antioxidant and anti-acetyl cholinesterase potential (Monferran et al., 2007; Lone et al., 2023), analgesic activity (Karale et al., 2013), anti-inflammatory and antineoplastic potential (Kurashov et al., 2016) anti-ulcer activity (Parmar et al.,1993; Niesink et al., 1996), antipyretic activity (Karale et al., 2013; Kurashov et al., 2016).

Extract from *C. demersum* has a high concentration of phytol, according to a study by Rashid et al. in 2023. Phytol has several beneficial properties, such as anti-inflammatory, antimicrobial, anticancer, and diuretic effects (Beulah et al., 2018; Ramya et al., 2021). Other studies have also discovered that phytol has anxiolytic, anticonvulsant, antinociceptive, cytotoxic, antioxidant, immune-modulating, and metabolism-modulating properties (Islam et al., 2018). Plant extracts have also been found to contain Vitamin E, as reported by Rashid et al. in 2024. Vitamin E has several health benefits, including antioxidant, antiaging, analgesic, antidiabetic, anti-inflammatory, antidermatitic, anticancer, antispasmodic, and hepatoprotective activities. Studies conducted by Nisha in 2018, Mujeeb et al. in 2014, and Ramya et al. in 2021 have confirmed these benefits.

According to our search, several in vitro studies have been performed on the effectiveness of *C. demersum L.* in treating anti-microbial activities. The antimicrobial effect of *C. demersum* extract using acetone, butanol, and methanol on *Staphylococcus aureus* and *Escherichia coli* and *Aspergillus niger* was investigated (Fareed et al., 2008; Malathy & Shaleesha, 2015). The antibacterial potential of different solvent extracts obtained from a free-floating aquatic plant, C. demersum, on fish bacterial pathogens was performed by Lone et al. (2023). One report was found on the effect of the methanol extract of *C. demersum* as an anti-leishmanial. The methanol extract of *C. demersum* in quantities of 25, 50, 100, 200, 400, and 800 µg/mL was investigated for its effect on an anti-leishmanial, anticancer, and antibacterial. The concentration of 800 µg/mL of *C. demersum* has been shown to have significant activity against Leishmania.

Although many studies in Türkiye show the amoebacidal effects of plant extracts on *A. castellanii* (Malatyali et al.,2012a; Malatyali et al.,2012b; Degerli et al., 2012a; Degerli et al., 2012b; Tepe et al., 2012; Kaynak et al., 2018; Kaynak et al., 2019; Koloren et al., 2019) have been reported, according to the internet search no report was found on the anti-*Acanthamoeba* effect of ethanol extract of *C. demersum*.

Despite the availability of a small number of chemotherapeutic agents for anti- *Acanthamoeba* therapy, the management of patients with AK, and GAE in particular, remains in great difficulty. Only in patients presenting early combined treatments may be promising. Furthermore, long-term use of the main drugs used in the treatment of AK brings other problems. Additionally, treatments can cause toxic keratopathy, encystation, and the development of resistant *Acanthamoeba* cysts. Recent studies suggest that microbial co-infections should be considered in cases of AK where *Acanthamoeba* therapy is ineffective. Co-infections make treatment difficult, and other supportive treatment methods are needed. This situation further complicates treatment regimens and requires additional therapeutic interventions (Elsheikha et al., 2020).

The study found that animals treated with aqueous and methanolic extract of *C. demersum* had faster wound contraction and rate of epithelialization in the excision wound model. This may be attributed to phytoconstituents like tannins and flavonoids, which promote wound healing due to their astringent and antimicrobial properties (Manjunath et al., 2005; 2007). Based on the above discussion, it can be concluded that both extracts of *C. demersum* have potent antidiarrheal and wound-healing activities. Furthermore, the study found that the extracts were equally effective as the standard drugs used for these purposes (Taranhalli et al., 2011). Various bioactive compounds in these plant extracts align with their traditional use for medicinal purposes in various cultures (Rashid et al., 2023).

This study aims to shed light on the effectiveness of this extract against *Acanthamoeba* trophozoites and to pave the way for potential applications as candidates for preventing infection with the parasite. The findings of this study are promising and highlight the potential of *C. demersum* as a natural medicine against acanthamoeba infections. Results indicated that ethanolic extract of *C. demersum* showed anti-*Acanthamoeba* activity against *A. castellanii* trophozoites, especially in higher concentrations. These results indicate that the aquatic macrophytes used for medical, pharmacological, biological, or environmental purposes also have an antiparasitic effect.

Conclusion

Aquatic plants have antimicrobial and antiparasitic effects and have been used for medical, pharmacological, biological, and environmental purposes. Although not pure antibiotic substances, plant extracts have significant effects (Ertürk et al., 2019). Recent studies have shown that the aquatic plant *C. demersum* contains bioactive compounds with great pharmacological potential and could contribute to the development of future drugs (Rashid et al., 2023). Hydrophytes are a natural resource that can be considered medicinal plants, and further research is required to isolate and characterize the phytochemical components of extracts obtained from these plants using different extraction methods. Developing plant-based therapeutics is a promising area that requires further research and development.

Compliance with Ethical Standards

Conflict of interest: The authors declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

Data availability: Data will be made available on request.

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Disclosure: -

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Spatial and temporal dynamics of fish-habitat interactions in Yuvarlakçay stream (Muğla, Türkiye)

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ABSTRACT

Human activities significantly impact freshwater ecosystems, and the dynamic nature of fluvial ecosystems makes resident species more vulnerable. The ecological importance of freshwater organisms is often overlooked in conservation concepts. The Yuvarlakçay Stream in Muğla, Türkiye, hosts endemic species but lacks conservation plans despite various threats. Freshwater habitat quality significantly affects fish survival, emphasising the need to establish fish-habitat relationships. This study aims to reveal habitat use and temporal/spatial interactions of fish in the ecosystem. In this context, Constrained Quadratic Ordination (CQO) and Point Abundance Sampling (PAS) with electrofishing are employed to understand fish-habitat relationships in the Yuvarlakçay Stream. The results revealed insignificant spatial variations over seasons within the fish populations. The endemic Aegean chub *Squalius fellowesii*, barbel *Barbus xanthos*, and spined loach *Cobitis fahireae* emerged as the most prevalent species in the study. Their presence was consistent across seasons, and their habitat relations exhibited overlaps. Water abstraction for agricultural and aquacultural purposes is defined as the major threat in the area, compounded by climate change-induced reductions in rainfall.

Keywords: *Anguilla anguilla, Rhodeus amarus*, Constrained Quadratic Ordination, *Knipowitshcia caunosi, Oncorhynchus mykiss*

Introduction

Globally, freshwater ecosystems encounter several threats and stressors, such as habitat degradation, pathway blockages, pollution, introduction of non-native species, overfishing and the impacts of climate change (Malmqvist & Rundle, 2002; Dudgeon et al., 2006). Paradoxically, despite the ecological significance of freshwater ecosystems (Carpenter et al., 1992), they often receive insufficient attention (Dudgeon et al., 2006; Butchart et al., 2010; Barbarossa et al., 2021). The dynamic nature of fluvial ecosystems further compounds the challenge, making resident species vulnerable in space and time (Angeler et al., 2014). The quality of freshwater habitats significantly influences fish survival within ecosystems (Carpenter et al., 2011). Changes in habitat availability can pose a threat to species persistence. In conservation, it is essential first to establish fish-habitat relationships and overall fish community structures (Yamazaki et al., 2006). For instance, significant gaps in knowledge and data availability among habitat requirements are discovered for many Italian species (Negro et al., 2021). Thus, species that depend on specific habitats can be at risk of extinction, and reduced water availability may lead to increased cohabitation and competition between species. Therefore, the habitat requirements that play a critical role for species should be determined within the ecosystems (Doll et al., 2021). Constrained Quadratic Ordination (CQO) is commonly employed to determine fish-habitat relations (Vilizzi et al., 2012). This technique focuses on species-specific habitat profiles and unveils how different species interact and coexist (Klaar et al., 2004; Vilizzi et al., 2012; Top et al., 2016, 2019). On the other hand, Point Abundance Sampling (PAS) using electrofishing (Persat & Copp, 1989) has proven to be a valuable approach for habitat utilisation studies, offering simplicity and a non-lethal method. With the help of these environment-friendly methods, we aimed in the present study to understand the overall structure of the fish community and significant threats in a small stream in Aegean Region-Türkiye. Environmental variables significantly influence fish habitat preferences and community structure, including substrate type, water velocity, and vegetation cover. (Vieira et al., 2020). Due to anthropogenic pressures and natural ecosystem dynamics, these habitat characteristics dictate endemic species' spatial and temporal distribution, affecting biodiversity and ecosystem resilience. Therefore, the particular focus of the study was to reveal (i) the habitat use of the resident species and (ii) temporal and spatial interactions of fish assemblages in the Yuvarlakçay stream, Muğla (SW, Türkiye).

Materials and Methods

Sampling Locations

The Yuvarlakçay stream is 15 km in length and feeds an important lake (Köyceğiz Lake) in south-west Türkiye (Figure 1). We determined five sampling sites based on elevation (Table 1, Figure 2) along the stream as representatives of ecologic zones (Simonović et al., 2017). The headwater section (S0, crenon) was not wadable or sampled due to possible insecure fieldwork. Therefore, the first locality was a wadable uppermost part of the stream (S1). The general habitat structure was mostly shallow (~0,5m), including high velocity along stones, and was poor in submerged vegetation. Sites 2, 3 and 4 were determined on the areas of the rhithron section of the stream (Figure 1, Table 1). Finally, the last sampling site of the study (S5) was the connection section to the lake of the stream along the potamon zone.

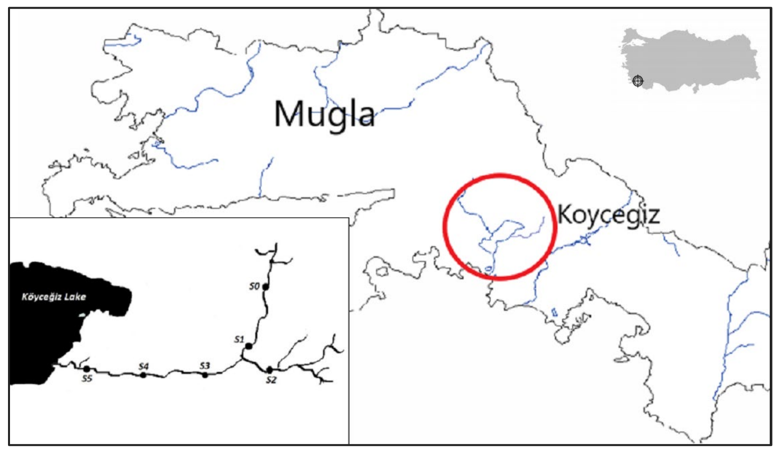

Figure 1. Map of the Yuvarlakçay stream and sampling locations

Point abundance sampling was applied via electrofishing from down to up direction following a zigzag manner (Tomanova et al., 2013). Fifty points were sampled on each site during a season. S4 was dried up in summer due to a common anthropogenic pressure (water abstraction), and no data was available. Therefore, 950 points were electrified along the stream (Table 1). On each randomly selected point, we recorded the physical variables, and these included depth, dominant substrate types, distance from the bank, submersed vegetation, submersed woody structure (roots, riparian), plant cover, velocity, light condition and turbidity (Vilizzi et al., 2012). These variables were measured as follows: Depth and Distance from the bank, with a one-meter-long pole labelled each 10 cm. Substrate types were classified based on particle size, measured in millimetres (mm), according to a modified Wentworth scale: mud (<0.062 mm), pebbles (4 mm to 64 mm), stones (64 mm to 256 mm), big stones (256 mm to 1024 mm), and rocks (>1024 mm). Submersed structures and plant cover were quantified as percentages within a 2m² surveyed area (Beyer et al., 2007). Water velocity was determined semi-quantitatively as described in Beyer et al. (2007) using the pole; upon immersion of the pole, (1) absent: no ripple effect around the pole was noted as zero water velocity; (2) weak: a gentle ripple effect (broken water) around the pole (>0 but <5cms-1); (3) moderate: an elevated ripple effect around the pole (5–10 cm s-1). The light condition was noted, including whether the discrete sampling point was in the sun or shaded. Turbidity: visually determined as clear, turbid and very turbid. For data analysis, nominal variables such as 'sunny'/'shady' were coded (e.g., sunny = 1, shady = 2). Water temperature was measured on each side with a temperature probe (YSI ProDSS) and represented in Table 2. The same person applied measurements on each season/site to sustain consistency.

Table 1. Geographical locations and total sampling points by each site and season

Sites	Elevation (m)	Lat-Lon	Winter	Spring	Summer	Autumn
S0	209	36.945-28.808	-	-	-	-
S1	108	36.918-28.796	50	50	50	50
S2	107	36.907-28.808	50	50	50	50
S3	58.4	36.906-28.771	50	50	50	50
S4	28.9	36.906-28.740	50	50	-	50
S5	6.9	36.907-28.711	50	50	50	50
Total			250	250	200	250

Figure 2. Habitat examples of Yuvarlakçay stream

Fish Metrics and Community Analysis

The fish survey was employed using binary data (presence/absence) on each described point by each pulse. A matrix bubble plot was used to illustrate the abundance of fish populations across sites and seasons. For this purpose, fish survey data were used to find the cumulative sum of each site species. Using the cumulative sum, the matrix bubble plot was generated using R-4.2.3 (R Core Team, 2022) with the library "tidyverse" to visually display each taxon amount.

The Shannon Diversity Index H determined the taxonomic diversity of fish at each sampling site (Welcomme, 1979). It considers both the richness (the number of different species present) and the evenness (EH, the relative abundance of those species). This index measures the uncertainty or entropy associated with species diversity in the ecosystem. A higher Shannon Diversity Index indicates greater diversity. It is calculated using the expression:

$H' = -\Sigma pi \times \ln(pi)$

$EH = H' / \ln(Si)$

In this formula, H': the Shannon Diversity Index, pi represents the proportion of individuals of the i-th species relative to the total number of individuals. Si is the number of species on site/season.

One-way ANOVA was used independently to analyse the effects of sites (spatial) and seasons (temporal) on fish species richness (Park et al., 2020).

CQO evaluated the degree of overlap and sharing of microhabitat preferences between species following the instructions of Vilizzi et al. (2012). With generalized linear models, this technique has been found suitable for shaping fish-environment relationships (Yee 2004, 2006). CQO is brief; a sample × species data matrix is related to a sample microhabitat variables data matrix, and the output is an ordination diagram. In this diagram, the x-axis represents the 'latent variable', which in the present case is a combination of physical descriptors (Table 3, note: Light and turbidity were not considered in the analysis due to the inconsistent records of light and indifference of turbidity among sites) whereas the y- axis plots the n scores (i.e. abundance or presence/absence). CQO estimates an optimal linear combination of the environmental variables and regresses the species data upon the latent variable using quadratic curves fitted across the species scores. Each response curve in an ordination diagram represents the distributional range of a certain fish species across the microhabitat gradient so that the relative position of the curve indicates the use/preference of a certain fish species for certain values (Table 4). Models were run in R-4.2.3 (R Core Team, 2022) using library VGAM v0.9-7 using following scripts: cqo (formula = cbind (Sp1, Sp2) ~ scale(habitat1) + scale(habitat2) +, family = binomialff (multiple.responses = TRUE), Rank = 1, df1.nl = 3 , Bestof = 100, Crow1positive = FALSE, I.tolerances = FALSE, data = Season).

Results and Discussion

Three hundred ninety-six specimens across 11 taxa were identified in the Yuvarlakçay Stream. The matrix bubble plots showed spatial variations over seasons within the fish population (see Figure 3). Notably, the statistical analysis revealed that there were no significant site-related impacts on overall diversity (F=1.287, $P > 0.05$), and the seasonal variations did not exert any influence on the community distribution ($F = 0.014$, $P > 0.05$) (Table 2). The uppermost sampling location -Site 1- has the lowest species number (Table 3, Figure 3). The potamon section of the Yuvarlakçay stream, i.e., Site 5, has the highest species diversity, hosting 10 species (Table 3). The Evenness Index (EH) exhibited a consistent pattern, with similar values observed across all sampling sites (Table 2).

The most prevalent fish species identified in the study included the endemic Aegean chub *Squalius fellowesii* (Günther, 1868), barbel *Barbus xanthos* (Güçlü et al., 2020) and the spined loach *Cobitis fahireae* (Linnaeus, 1758) (Table 3). In contrast, the European eel *Anguilla anguilla* (Linnaeus, 1758) and the endemic scraper *Capoeta aydinensis* (Turan et al., 2017) were found in smaller numbers. Mullet *Mugil cephalus* and the non-native rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), which had escaped from aquaculture facilities, were relatively rare, accounting for less than 2% of the total count in the study. Endemic Anatolian gizani *Ladigesocypris irideus* (Ladiges, 1960) and dwarf goby *Knipowitschia byblisia* (Balık et al., 2005; Ahnelt, 2011) were observed in limited numbers, while bitterling *Rhodeus amarus* (Bloch, 1782) was documented for the first time in the Yuvarlakçay Stream, with just one specimen recorded during the study.

Seasonal CQO results are presented in Figure 4. Table 4 displays the species scores for each component on the latent variable (Figure 4) and provides the scores for the descriptors. As shown in Figure 3, the response profiles or bell-shaped curves overlapped in most cases for Cyprinid species but exhibited variations in occurrence. These species were consistently discovered in areas with coarser substrates, near riparian trees with vegetation, and the stream bank. The spined loach, *Cobitis fahireae*, avoids deep waters and woody structures (such as those found along the stream bank). Instead, it primarily inhabits vegetated, finer substrates in shallow waters, particularly during summer. *Anguilla anguilla* was observed in significant numbers during the autumn season. Although they exhibited considerable overlap with other species in winter and spring, a shift in their distribution was noted during autumn and summer. For the ordination diagram, we included the most abundant fish species. The less abundant ones led to a confusing representation on the diagram, as they did not exhibit bell-shaped curves (Figure 4); however, these less abundant species were discussed separately.

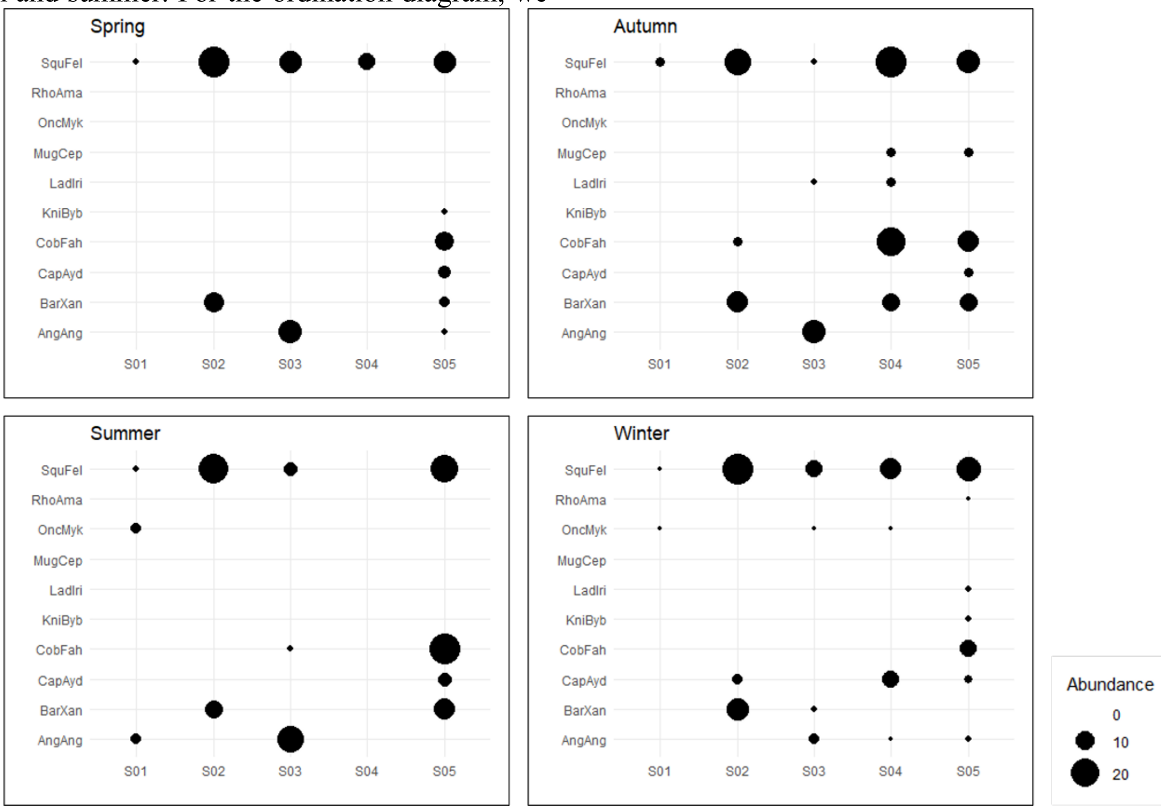

Figure 3. Species abundance bubble plot for the Yuvarlakçay Stream over sites and seasons. SquFel: *Squalius fellowesii*, RhoAma: *Rhodeus amarus*, OncMyk: *Oncorhynchus mykiss*, MugCep: *Mugil cephalus*, LadIri: *Ladigesocypris irideus*, KniByb: *Knipowitschia byblisia*, CobFah: *Cobitis fahireae*, CapAyd: *Capoeta aydinensis*, BarXan: *Barbus xanthos*, AngAng: *Anguilla anguilla*.

Table 2. Summary of data, sites, Shannon Diversity Index (H^{i}), Evenness (EH), Seasons, Sampling date and Temp (water temperature)

Sites	H^{i}	EH	Seasons	Sampling date	H^{i}	EH	Temp(°C)
S1	1.01	0.94	Winter	18.02.2022	1.60	0.77	6.6
S2	0.99	0.55	Spring	19.05.2022	1.66	0.75	17.8
S3	1.03	0.57	Summer	24.08.2022	1.57	0.81	24.8
S4	1.66	0.76	Autumn	29.10.2022	1.52	0.66	11.3
S5	1.80	0.72					

Table 3. Number of fish species sampled from each site during the study

Taxon/Sites	S1	S2	S3	S4	S5	Total (n)
Anguilla anguilla	2	-	33	1	3	39
Barbus xantos	-	36	2	6	15	59
Capoeta aydinensis	-	4	-	9	11	24
Cobitis fahireae	-	2	1	14	38	55
Knipowitschia byblisia	-	-	-	-	3	3
Ladigesocyrpris irideus	-	-	1	2	2	5
Mugil cephalus	-	-	-	2	2	4
Oncorhynchus mykiss	3	-	1	1	-	5
Rhodeus amarus	-	-	-	-	1	1
Squalius fellowesii	5	70	21	36	48	180
Larvae	-	2	-	14	5	21
Total number of species	3	5	6	9	10	11

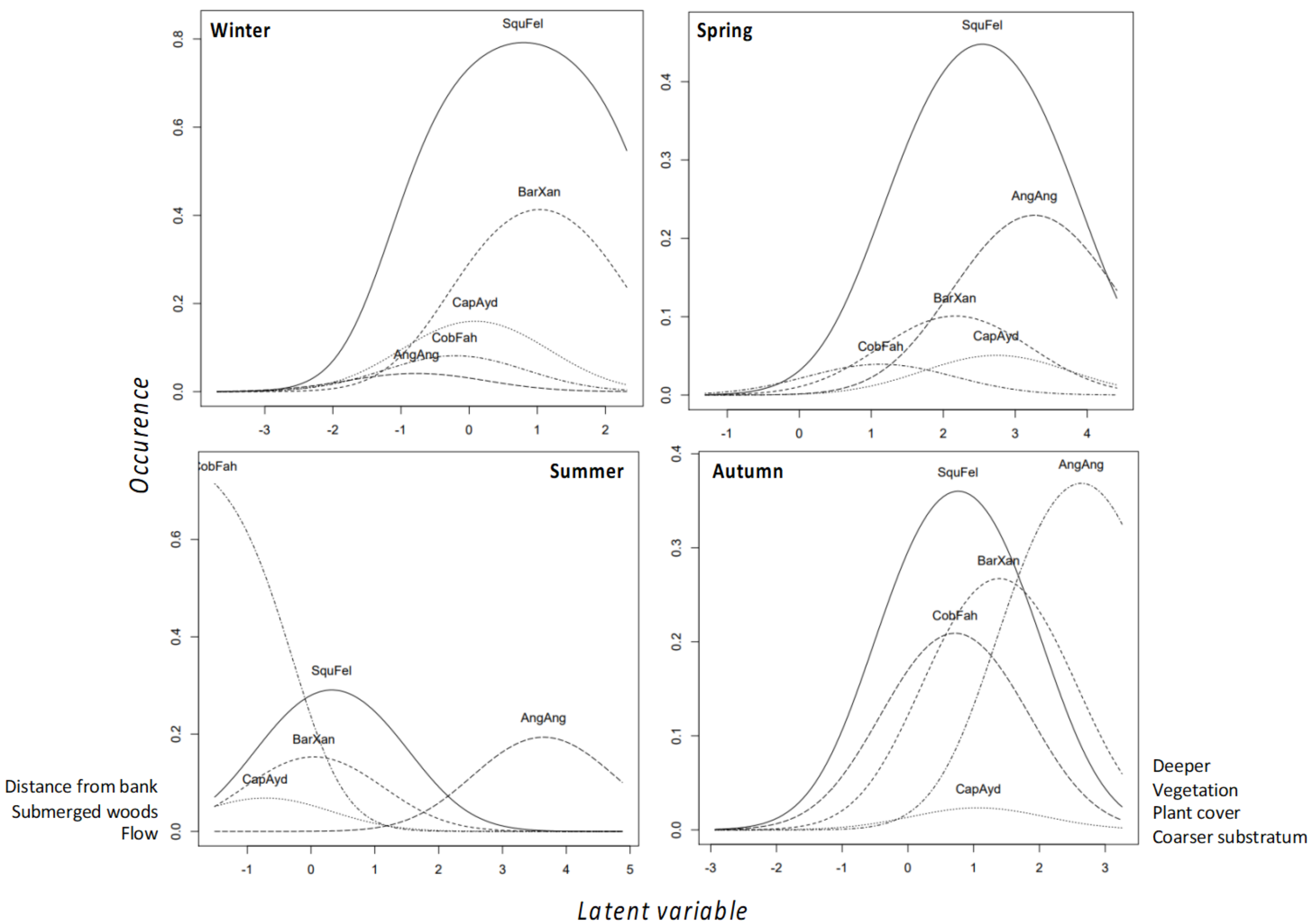

Figure 4. CQO ordination plots for species of the Yuvarlakçay Stream over seasons. A summary of the main microhabitat features (i.e., Table 4) is provided on each side of the latent variable. (SquFel: *Squalius fellowesii*, BarXan: *Barbus xanthos*, CapAyd: *Capoeta aydinensis*, AngAng: *Anguilla anguilla*, CobFah: *Cobitis fahireae*)

Species Diversity and Distribution

Identified species provide a foundational understanding of the fish community within the Yuvarlakçay Stream. Notably, the species diversity exhibited spatial variability (Table 3). While the uppermost sections of the stream (Site 1 & 2) displayed the lowest diversity, the potamon section of the stream (Site 5) hosted a remarkable total of 10 species. This section of the stream is a transition area between the river and the lake. This refers to the increased diversity and density of plant and animal species that typically occurs in these transition zones. This variation in species richness among sites highlights the importance of considering spatial factors in managing and conserving aquatic ecosystems.

Matrix bubble plots depicted the dynamic nature of the fish community (Figure 3), with changes occurring over the year. Despite these fluctuations, statistical analysis indicated that overall diversity remained stable across sites and seasons. The consistent pattern observed in the Evenness Index (EH) further emphasises the resilience and stability of the fish community in the face of environmental variations.

Species-Specific Insights

Insights into the habitat preferences of certain species provide valuable information for conservation efforts. *Squalius fellowesii, B. xanthos*, and *C. fahireae* emerged as the most prevalent species in the study. Their presence was consistent across seasons, and their distribution profiles exhibited overlaps. However, *A. anguilla* and *C. aydinensis* were encountered in smaller numbers, suggesting potential vulnerabilities or habitat preferences that warrant further investigation.

Anguilla anguilla was particularly abundant during the autumn season and mostly occurred on Site 3. They generally prefer being distant from the riverbank, found in a coarser substratum in the middle of the stream section with high flow. In autumn, they prefer hiding among riparian trees (i.e., woody structures). However, in spring, they avoid velocity, submersed woods and vegetation. The observation that *A. anguilla* was particularly abundant during different seasons could suggest that this altered presence is linked to their migratory behaviour or other ecological factors affecting their distribution. For instance, current velocities, inclination pitches, bottom materials and carrying capacities are potential factors in different habitat use and migration behaviours of eels (Glova et al., 1998). On the other hand, seasonal changes in food items/prey populations could also influence eel abundance, with eels possibly taking advantage of increased prey availability (Arai, 2022). The European eel has been listed as Critically Endangered in the latest International Union for Conservation of Nature (IUCN) Red List assessment (Pike et al., 2020). Thus, the data on local population structures have a critical role in the overall population trend (ICES, 2020; Ertürk et al., 2023), and their repartition in the Yuvarlakçay Stream during particular seasons emphasises the importance of considering temporal dynamics in ecological studies.

Capoeta aydinensis is notably abundant in a nearby stream, the Tersakan stream, as reported by Akbaş et al. (2019). These fish are predominantly found in muddy shelters along the stream bank in that stream. Interestingly, Akbaş et al. (2019) highlighted that they tend to inhabit turbid waters. However, it is worth noting that turbidity was not a significant factor in the present study. Consequently, the lower catches of *C. aydinensis* observed in our study may be attributed to the absence of turbidity, which could have acted as a stressor for this species.

Cobitis fahireae preferred highly vegetated flowing waters, particularly during the summer. As small-sized fish, they use small stones as shelters/shields and a large number of the specimens were caught from both submerged plants and filamentous algae. This behavioural adaptation aligns with its avoidance of deep waters and woody structures. These bottom-dwelling species are very sensitive to any disturbance of benthic structure, which is vulnerable to getting easily altered by human activities (Erkakan & Ekmekçi, 2000). The final photograph in Figure 2 is crucial as it clearly shows the stream structure characteristic of the Cobitis species' environment, and its destruction must be prohibited immediately.

The Yuvarlakçay Stream hosts several endemic species; however, the present study revealed that one of them, *Ladigesocypris irideus*, was rarely encountered, accounting for less than 2% of the total samples collected from the entire stream. Previous research by Top and Tarkan (2009) indicated that the Anatolian gizani prefers stagnant, deep, and ponded sections of streams, often among small pebbles. Despite its tolerance to high temperatures and low oxygen levels (Özdemir et al., 2015), the population trend of this small endemic species is declining (Yılmaz et al., 2015). Notably, *L. irideus* is classified as "Near Threatened" on the Red List (Yılmaz et al., 2015).

Knipowitschia spp. Inhabit various aquatic environments, including marine, freshwater, and brackish waters (IUCN, 2022). The present study discovered them at the nearest site to the lake, Site 5. This observation aligns with the expectation that they primarily inhabit Köyceğiz Lake (Balık et al., 2005; Ahnelt, 2011). Köyceğiz Lake is distinguished by its brackish water composition (Akın et al. (2005). This unique environmental condition would have provided a favourable habitat for a euryhaline population of *Knipowitschia byblisia* (Ahnelt, 2011).

The mullets, occasional residents in the area, were primarily captured at sites near the lake, specifically at Sites 4 and 5. Notably, mullets constitute the most numerous fish species within Köyceğiz Lake, demonstrating a remarkable tolerance to fluctuating environmental conditions (Akın et al., 2005).

Among the fish species observed in the entire stream, the aquaculture escapee *Oncorhynchus mykiss* stood out as the only non-native fish species, with only a few individuals recorded. The presence of escapees can potentially act as a stressor if they establish themselves in the ecosystem. However, it is important to note that the habitat suitability for rainbow trout in the Muğla region is limited, and there have been no officially confirmed established populations of this species (Yoğurtçuoğlu et al., 2021).

Conclusion

The comprehensive seasonal sampling effort conducted at various sites within the Yuvarlakçay stream revealed valuable insights into the fish populations and their ecological dynamics.

The results presented in this study provide knowledge of several key aspects of the stream's fish community and its response to seasonal and spatial variations.

The stability of overall diversity, species-specific patterns, and habitat preferences offer valuable insights for conserving and managing this unique aquatic ecosystem. Protecting the unique habitats in the Yuvarlakçay stream from environmental destructions such as water abstraction, pollution, artificial hydraulic barriers, trout farms, etc., is essential.

Further research, especially focusing on the less common species and their specific ecological requirements, will contribute to a more comprehensive understanding of the stream's biodiversity and its responses to environmental changes. As the vulnerability of endemic species continues to trend upward, this study becomes a crucial source of knowledge for prioritising protection plans.

Further, it is essential to recognise that water abstraction for agricultural and aquacultural purposes stands out as the major threat in the area, compounded by climate change-induced reductions in rainfall (Cai & Cowan, 2013), economic development, and the expanding human population (Squires, 2014). Additionally, the thriving tourism industry in the region places significant demands on freshwater resources, further increasing the strain on water sources, which are often polluted.

Compliance with Ethical Standards

Conflict of interest: The authors declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study was conducted by approval (2021/13-2) of Muğla Sıtkı Koçman University-Local Ethics Committee for Aquatic Animal Experimentation.

Data availability: Data will be made available on request.

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Research Article

Assessment of fishing gears, crafts, and socio-economic status of the Hilsa (*Tenualosa ilisha*) fishers' community near the Meghna River, Bangladesh

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ABSTRACT

An evaluation was conducted to assess the current state of fishing gears, crafts, and socio-economic aspects among the Hilsa (*Tenualosa ilisha* Hamilton, 1822) fishers communities residing along the Meghna River region in Gazaria and Munshiganj Sadar Upazila, located in the district of Munshiganj, Bangladesh. About 150 fishers were interviewed using pre-tested questionnaires from selected areas from July 2019 to June 2020, and the data that was obtained was analysed using appropriate statistical tools. The methods employed for Hilsa fishing included the use of drift gill nets (known as Ilish jal, Fash jal, and Poa jal) and seine nets (referred to as Jagat ber jal), while the crafts utilised were Chandi boat and Donga. Most fishers were 31-40 years old (53.33%) in Ismanirchar and (46.67%) in Kalirchar village. Many fishers were Hindus (80%) in Ismanirchar and Muslims (96%) in Kalirchar. Regarding educational level, 69.33% and 70.67% of fishers in Ismanirchar and Kalirchar can only sign, respectively. Semi-earthen toilets were the primary type of sanitary facilities found in the area. Approximately half of the fishers residing in the two villages owned land ranging from 0 to 15 decimals. A significant portion, around 80%, relied on loans from non-governmental organisations (NGOs), while the remaining fishers obtained financial assistance from their neighbours. Hilsa fishing was found to be the main occupation in the study area. The fishers' income increased from September to November and March to May. The primary issues identified included extortion from local criminal groups, insufficient access to credit, absence of proper storage facilities, ongoing conflicts between professional and non-professional fishers, as well as tensions between Muslim and Hindu fishers. To improve their socio-economic situation and ensure sustainable livelihoods, fishers require more institutional support, better organisation, enhanced technical assistance, and increased access to credit.

Keywords: Fishing gears, Fishing crafts, Livelihood, Hilsa fish, Fishers, Meghna River

Introduction

Bangladesh boasts abundant and varied inland waters, with a total inland area of 4.3 million hectares. Approximately 94% of this area is utilised for open-water capture fisheries, while the remaining 6% is designated for closed-water culture fisheries. The Meghna River, a significant water body in the region, is intricately linked with numerous channels, creating a vast expanse of water during the rainy season. Spanning approximately 1200 hectares in Gazaria Upazila, it supports around 1200 fishers relying on its resources for sustenance and protein intake. This fishery sustains about 40% of the local fisher's population, equivalent to 2% of the country's total population, providing crucial livelihood opportunities. The Hilsa fish (*Tenualosa Ilisha* Hamilton, 1822) holds significant importance as the primary aquatic resource and forms the cornerstone of the largest fishery industry in Bangladesh. Production of Hilsa fish was 566,593 tons (DoF, 2023) and a growth rate of 2.68 in the 2022-23 fiscal year (FRSS, 2022). The Hilsa fishery accounted for approximately 1% of the total gross domestic production (GDP) in Bangladesh's fisheries sector (DoF, 2023; Ahsan et al., 2014), contributing 12.22% to the nation's overall fish production (FRSS, 2022). The fisheries industry is paramount in Bangladesh's socio-economic advancement, serving as a primary source of animal protein, employment, food security, foreign revenue, and socio-economic progress (Ali et al., 2009; Siddique et al., 2013). The monofilament gill net is the primary gear utilised in capturing Hilsa fish, typically featuring stretched mesh sizes ranging from 50 to 140 mm. Siddique et al. (2013) noted the use of "chandi jal" in the Meghna River, with mesh sizes between 40 mm and 45 mm, net lengths of 650 m to 700 m, and widths of 10 m to 12 m. These nets are deployed from small motorised boats, the specifics of which vary based on fishing area and capacity. BCAS (1989) documented seven fishing crafts in the Meghna, Padma, and Jamuna rivers. In the Padma River region of Charghat and Bhaga Upazila, Ayenuddin et al. (2017) observed the use of drift gill nets alongside "chandi" boats and "dongas" for Hilsa fishing. Meanwhile, at the Meghna River estuary in Chandpur, Siddique et al. (2013) identified various fishing gears employed, including gill nets, seine nets, fixed purses, cast nets, drag nets, traps, hooks, lines, and wounding gears. Hilsa fishers constitute one of Bangladesh's most vulnerable communities, as Mia et al. (2015) highlighted. Their study revealed that approximately 75% of fishers reside in semi-earthen houses. In comparison, only 2.5% have access to half-built houses within the fishing community spanning a 4-kilometre area along the Meghna River in Ashuganj Upazila, Brahmanbaria district. Fishers faced dire sanitary conditions, with 50% using semi-earthen toilets. Family sizes ranged from 2-11 individuals, with smaller households (2-4 members) and larger ones (7+ members). The highest income of fishers (52.5%) from Bangladeshi taka (BDT) is 1,00,000-2,00,000/year, contrasting with the lowest income of fishers (5%) from BDT 25,000-50,000/year (Mia et al., 2015). Effective planning and development efforts for economically struggling sectors like fish farming require access to current socio-economic data. The absence of sufficient and reliable information on the socio-economic status of the target population hampers the successful execution of developmental programs (Ellis, 2000).

However, governmental and non-governmental organisations (NGOs) have yet to make minimal efforts to address and enhance the socio-economic conditions of Hilsa fishers in this region. There needs to be a clearer understanding of the impact of the banning period on the livelihoods of Hilsa fishers. To our knowledge, research has yet to be conducted on the effects of the banning season on the socio-economic conditions of Hilsa fishing communities near the Meghna River. With this in mind, this study aimed to assess the socio-economic and cultural profiles of fishers in the study area, predominantly inhabited by fishers who rely on fishing for their livelihoods. The objectives of this study were to examine the current status of Hilsa fishing gears and crafts in the study area and to evaluate the socio-economic status of Hilsa fishers residing near the Meghna River.

Materials and Methods

Study Area and Time Frame

The study areas were on the riverine fishers of Meghna River under Ismanirchar and Kalirchar village of Gazaria and Sadar Upazila of Munshiganj district (22°49'59.99" N to 90°49'59.99" E), respectively. Data were gathered from the selected survey areas from July 2019 to June 2020.

Sample Size and Sampling Procedure

About 150 fishers (75 from Ismanirchar village and 75 from Kalirchar village) were proportionately selected within 2 kilometres of the Meghna River. Data was collected using a thorough interview schedule consisting of a pre-tested structured questionnaire to accomplish the study's objectives.

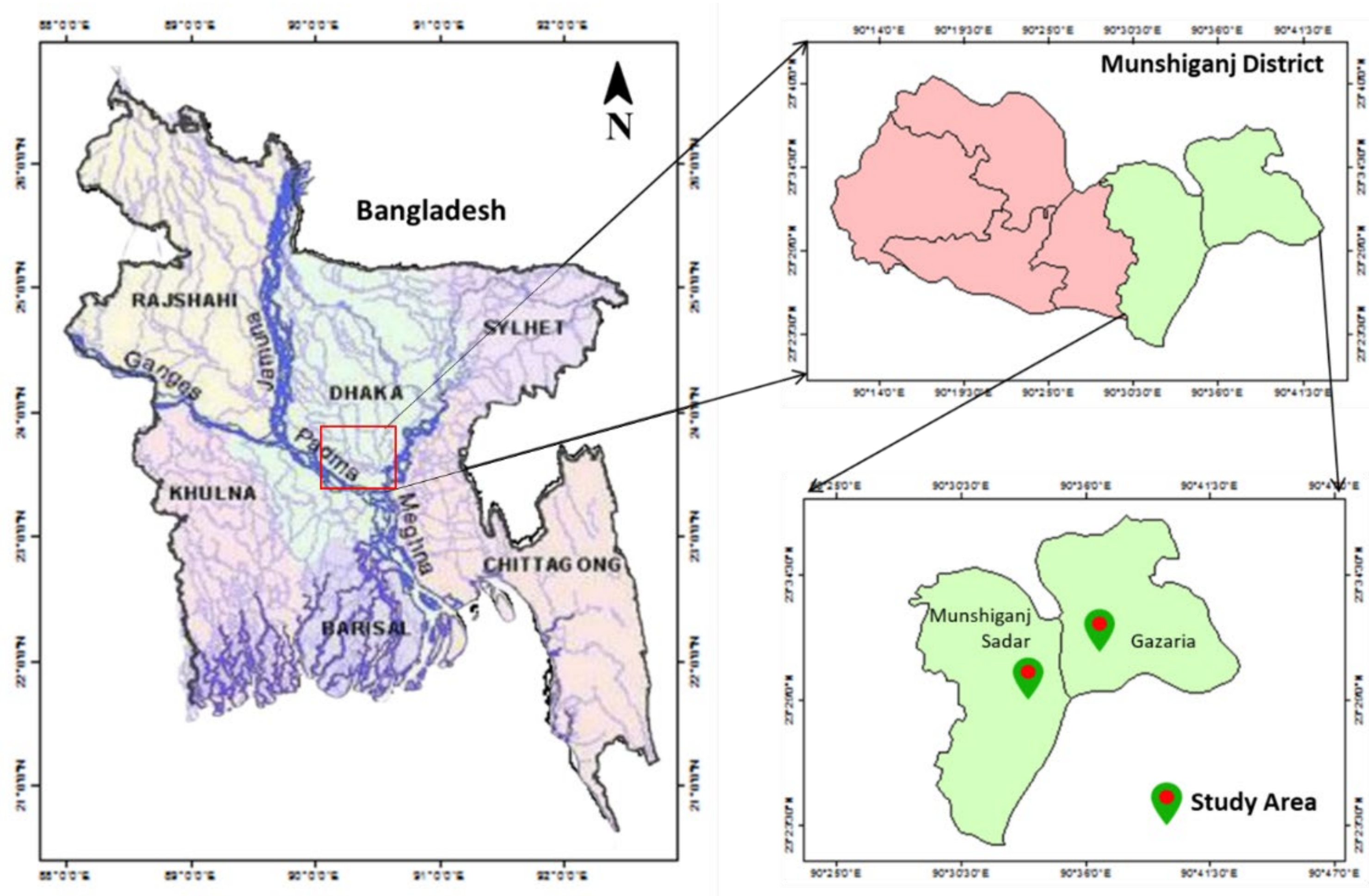

Figure 1: Study areas map under the Munshiganj district of Bangladesh.

Preparation of Questionnaire and Interviews

The questionnaire was carefully structured logically to facilitate fishers responding chronologically. Various parameters were employed to gather comprehensive data on the socio-economic status of fishers, such as categories of Hilsa fishers, fisher's types, age structure, family type, catch per unit effort (CPUE), etc. Catch per unit effort (CPUE) is an indirect measure of the abundance of a target species. Changes in the CPUE are inferred to signify changes to the target species' true abundance. A simple random sampling technique was utilised to select 150 fishers from the study area for questionnaire interviews. Interviews with fishers were conducted on-site at the river during their fishing activities. The collected data underwent validation through interviews with key individuals, including the Upazila Fisheries Officer (UFO), schoolteachers, local leaders, and NGO representatives. These interviews took place at the respondents' respective office locations.

Summarisation and Presentation of the Data

After collecting primary and secondary data from the field, the information was entered into master table sheets. Subsequently, all gathered data were carefully summarised, reviewed, and recorded. Finally, relevant tables and graphs were created using Microsoft Office 2019 to align with the study's objectives.

Results and Discussion

Fishing Gears Used in Catching Hilsa

Table 1 provides descriptions of the fishing gears utilised for catching Hilsa. Fisher's selection of nets for operation varies depending on factors such as current velocity, catch characteristics, and financial resources. Gill and seine nets were the primary types employed in the region, particularly for catching Hilsa (*Tenualosa ilisha*). During the Hilsa season, the majority of fishers were found to use chandi jal (drift gill net).

These nets typically ranged in length from 20 meters to 300 meters and in width from 1 meter to 5 meters, with stretched mesh sizes ranging from 35 to 150 millimetres. Using a gill net for Hilsa fishing commonly has a 50 to 140 mm mesh size (Halder et al., 2011). Sazzad (1993) observed that the gear used for Hilsa fishing were current jal, chandi jal, gulti jal, kona jal, jagotber jal, daba jal. It has been reported that chandi jal was mainly used in the Meghna River for Hilsa fishing with a mesh size of 40 mm to 45 mm and the length of the net was 650 m to 700 m, and the width was 10 m to 12 m (Siddique et al., 2013). Mondal et al. (2013) also found that chandi jal or Ilisha jal was very common in the Meghna River at Ramgati Upazila, Lakshmipur district and the species caught by this net was mainly Hilsa.

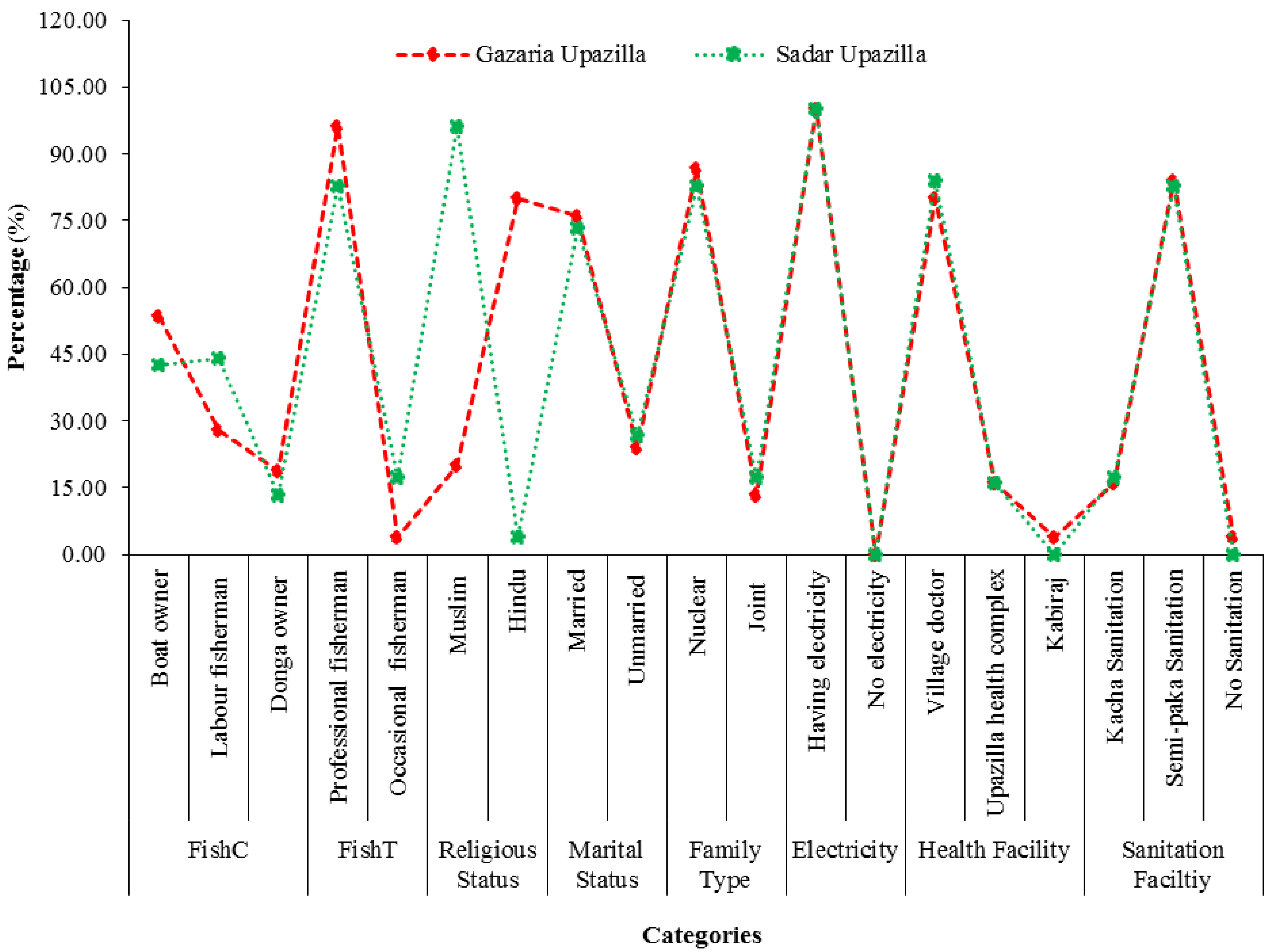

Figure 2. Fisher's categories (FishC), fisher's types (FishT), religious status, marital status, family type, electricity facility, health facility and sanitation facility of Hilsa fisher's communities.

Fishing Crafts Used in Catching Hilsa

Depending on the fishing craft, different gear sizes are used to catch fish for Hilsa fishing. The craft used in Bangladesh may have begun in ancient times when people started catching fish from rivers and other open water bodies. In the study area, small motorised boats such as Chandi nauka are predominantly utilised for Hilsa fishing (Table 1). Fishers also employed "Donga" to catch Hilsa fish. There was a total of 72 Chandi nauka and 24 Donga boats in the study area. Chandi nauka boats typically measure between 5 to 8 meters in length, 1.5 to 2 meters in width, and have a depth of 0.5 to 1 meter. These boats feature a flat bottom and pointed stern, requiring 2 to 6 persons for operation. In contrast, Donga boats have a length ranging from 3 to 4 meters, a width of 0.5 to 1 meter, and a depth between 0.4 to 0.7 meters. A slightly rounded bottom characterises them, is blunt and stern, and can be operated by only 1 to 3 persons. Bangladesh's fishermen commonly utilise various crafts with differing lengths, breadths, depths, shapes, and sizes.

Table 1: Description of fishing gear and crafts used in Hilsa fishing.

Fishing Gear

Type of gear	Name of gear	Habitat	Construction cost and life span	Number of crew	Length width and mesh size	CPUE (kg/gear/day)	Materials	Operation period	Major species caught
Drift gill net	Current jal or Net jal or Ilish jal or “angly” jal	River or Estuary	5000 to 30000/- & 3-4 yrs	2-6	100 -250 m; 2-3 m; 6-8 cm.	5-30	Synthetic monofilament fibre, rope, sinkers and floats	All the year-round	Hilsa, Poa
Drift gill net	Fash jal	River or Estuary	2000 to 10000/- & 1-3 yrs	1-2	20 -200 m; 1-1.5 m; 4-15 cm.	10-15	Synthetic monofilament fibre, rope, earthen sinkers and floats	All the year-round	Hilsa, Poa
Drift gill net	Poa jal	River or Estuary	2000 to 20000/- & 4-5 yrs	2-4	40 -100 m; 3-5 m; 3.5-4 cm.	5-10	Synthetic or cotton polyamide monofilament fibre, rope, sinkers and floats	All the year-round	Hilsa, Poa, Bata
Seine net	Jagot ber jal	River or Estuary	20000 to 50000/- & 5-7 yrs	4-8	100 -300 m; 5-7 m; 0.3-2.5 cm.	10-40	Nylon twine or double cotton twines or tier cord, rope, sinkers and floats	All the year round	Hilsa, Jatka, Pangus

Fishing Crafts

Name of the crafts	Size				Construction materials	Bottom	Stern or bow	Person per craft	Mechanised or manual	Durability (year)
	Length (m)	Width (m)	Depth (m)	Carrying capacity (kg)						
Chandi nauka	5-8	1.5-2	0.5-1	500-1000	Wood, Iron sheet, Motor	Flat	Pointed	2-6	Mechanised/ Motorized	3-8
Donga	3-4	0.5-1	0.4-0.7	200-500	Wood, Bamboo, Tin	Slightly rounded	Blunt	1-3	Manual	1-3

Rural carpenters typically construct crafts or boats based on their plans, using planks as the primary material. Traditionally, boat-making involves the use of wood and bamboo. In traditional construction, no approved design is followed; therefore, classification of the crafts is very difficult. In a study conducted by Sazzad (1993), it was discovered that the primary crafts utilised in the Meghna River consisted of small motorised boats as well as non-motorized boats, including chandi boats and dingi boats. Additionally, BCAS (1989) documented the operation of seven distinct types of fishing crafts in the Meghna, Padma, and Jamuna rivers. DoF (2019) stated that different types of 32 fishing boats were involved in Hilsa fishing from Bhola Sadar to Hatia Island. CIDA (1993) documented the use of various fishing crafts by fishers in the northeast region of Bangladesh, particularly in the greater Sylhet and Mymensingh districts. These included chandi boats, dinghi boats, khosa boats, bachari boats, and dongas.

Table 2: Age group, educational status, family size, earners-dependency ratio, fish carrying system and gender of Hilsa fishers' communities.

Categories		Study Area			
		Gazaria Upazila (Village Ismanirchar)		Sadar Upazila (Village Kalirchar)	
		Number	Percentage	Number	Percentage
Age (in 2022) group of fishers					
less than 20		8	10.67	12	16.00
21-30		15	20.00	18	24.00
31-40		40	53.33	35	46.67
41-60		10	13.33	10	13.33
Above 60		2	2.67	0	0
Total		75	100	75	100
Educational status of the Hilsa fishers in the study area					
Illiterate		8	10.67	5	6.67
Only can sign		52	69.33	53	70.67
Class 1-5		10	13.33	15	20.00
Class 6-10		3	4.00	1	1.33
Above SSC		2	2.67	1	1.33
Above HSC		0	0	0	0
Total		75	100	75	100
Family size of Hilsa fishers in the study area					
1-3		8	10.67	10	13.33
4-6		62	82.67	60	80.00
7-9		3	4.00	5	6.67
10 or above		2	2.66	0	0.00
Total		75	100	75	100
The percent distribution of earners and dependency ratio					
Total earners		115	31.08	109	31.59
Total dependents		285	77.02	236	68.41
Total		370	100	345	100
Earners: dependents		1:2.48		1: 2.17	
Status of school-going children of fishers.					
Non-school going		18	18.75	15	17.65
School going	Only boys	30	31.25	28	32.94
	Only girls	22	22.92	22	25.88
	Both boys girls	26	27.08	20	23.53
Total		96	100	85	100
Gender					
Male		75	100	75	100
Female		0	0	0	0
Total		75	100	75	100

Socio-Economic Status of the Fishers

Fisher's category

Different groups of people engaged in Hilsa fishing in the study area are given in Figure 2. The categories of fishers were done mainly as a boat owner (who has their own boat for Hilsa fishing), labour fishers (the fishers who have no boats and work in boat owner fishers' boats) and donga owners (who have their donga for catching Hilsa fish). In the study area, boat owner fishers were (53.33% in Ismanirchar and 42.67% in Kalirchar), labour fishers (28.00% in Ismanirchar and 44.00% in Kalirchar) and donga owners (18.67% in Ismanirchar and 13.33% in Kalirchar). Ayenuddin et al. (2017) classified Hilsa fishers into three categories: boat owners, labour fishers, and donga owners. They identified two types of Hilsa fishers: professional, accounting for 57% in Raowtha and 61.63% in Vanukor, respectively, and occasional, comprising 43% in Raowtha and 38.37% in Vanukor, respectively.

Fishers' type

In the study area, various fishers were identified, including professional fishers, who rely on fishing as their primary source of income throughout the year, and occasional fishers, who engage in fishing for part of the year (October to November) to earn income. These categories are depicted in Figure 2. Subsistence fishers, who catch fish solely for personal consumption, were not present in the area since the fishers primarily catch fish for commercial purposes. It was found that professional fishers comprised 96% of the population in the village of Ismanirchar of Gazaria Upazila, and occasional fishers comprised 4%. In contrast, professional and occasional fishers were 82.67% and 17.33% in the village of Kalirchar of Sadar Upazila. In their study, Faruque and Ahsan (2014) identified that 56.52% to 75.00% of fishers were classified as professional, 20.83% to 43.48% as occasional, and 0.00% to 7.69% as subsistence Hilsa fishers in the Padma River. Conversely, Mondal et al. (2013) reported that 82% of fishers were classified as professional, while 18% were categorised as seasonal in the Meghna River within Ramgati Upazila, under Lakshmipur district.

Religious status

The survey area's fishers belonged to two religions, Muslims and Hindus. About 80% Hindu and 20% Muslim fishers were found in the village of Ismanirchar of Gazaria Upazila (Figure 2). On the other hand, around 96% Muslim and only 4% Hindu fishers were found in the village of Kalirchar of Sadar Upazila. According to Faruque and Ahsan (2014), Muslim fishers predominantly engaged in Hilsa fishing in the Padma River, except in Horisonkorpur, where most were Hindus. Hossain et al. (2015) discovered that in the Punorvaba River under Sadar Upazila, Dinajpur, most fishers were Muslim, constituting 90% of the population. Similarly, Hassan and Mahmud (2002) observed that in the coastal fishing community at Kuakata, Patuakhali, 93.94% of fishers were Muslim. However, Islam et al. (2013) found that most fishers in the Jessore district were Hindu.

Marital status

About 76% of fishers in this study were married from Ismanirchar (Figure 2). Similarly, 73.33% of fishers were married, and 26.67% were unmarried in Kalirchar. Faruque and Ahsan (2014) found that 90-95% of the fishers were married and 5-10% unmarried in Padma River. Ahamed (1996) also reported that married fishers (94%) were dominant in Tangail.

Family size and type

The family size of fishers was categorised into two groups (Table 2). According to Kostori (2012), most families, comprising 4-6 members, accounted for 64% of the sample respondents. Similarly, Ali et al. (2009) found that 45% of fish farmers belonged to families with 4 to 5 members in the Mymensingh district. Abdullah-Bin-Farid et al. (2013) observed that a significant portion (48%) of fishers in the Baluhar Baor area of Jhenaidah, Bangladesh, had small families with fewer than 5 members, consistent with the present study's findings. Mahmud et al. (2015) noted a prevalence of medium-sized families (5-7 members) comprising 60% of fishers in the Paira River. These findings align closely with Kabir et al. (2012) and Hossain et al. (2009).

This study identified two types of family structures: nuclear families, consisting of married couples and their children, and joint families, which comprise a group of individuals connected by blood and legal ties (Figure 2). Around 86.67% nuclear and 13.33% joint families in Ismanirchar village and 82.67% nuclear and 17.33% joint families in Kalirchar village were found. According to Minar et al. (2012), most fishers, accounting for 86%, resided in joint family setups, while 16% lived in nuclear families along the Kirtonkhola River. In the Mymensingh district, Ali et al. (2009) reported that approximately 42.5% of fishers lived in nuclear families, with the remaining 57.5% residing in joint family arrangements. Hossain et al. (2015) found that 37% of fishers lived in joint families, while 63% lived in nuclear families along the Punorvaba River. Additionally, Bappa et al. (2014) noted that over 56% of fishers resided in nuclear families, while 44% lived in joint family settings.

Table 3. Recreational media, land holding capacity, living house conditions, source of drinking water and fish carrying system of Hilsa fisher's communities.

Categories		Study Area			
		Gazaria Upazila (Village Ismanirchar)		Sadar Upazila (Village Kalirchar)	
		Number	Percentage	Number	Percentage
Uses of recreational media by the Hilsa fishers					
Radio		0	0.00	3	4.00
Cassette player		12	16.00	8	16.67
Television		55	73.33	57	76.00
Mobile		62	82.67	66	88.00
Both Television & Mobile		48	64.00	52	69.33
None of them		15	20.00	12	16.00
Total		75	100	75	100.00
Distribution of total land holding by the Hilsa fishers					
Landless to 15 decimals		38	50.67	35	46.67
16 to 33 decimals		22	29.33	28	37.33
Above 33 decimals		15	20.00	12	16.00
Total		75	100	75	100
Condition of living house					
Kacha	Present	16	21.33	21	28.00
	Absent	0	0.00	0	0.00
Tin shed	Present	50	66.67	44	58.67
	Absent	0	0.00	0	0.00
Semi-pacca	Present	7	9.33	7	9.33
	Absent	0	0.00	0	0.00
Pacca	Present	2	2.67	3	4.00
	Absent	0	0.00	0	0.00
Total		75	100	75	100
Drinking water source					
Tube-wells		75	100.00	75	100.00
(a) Own		45	60.00	45	60.00
(b) Neighbor's		30	40.00	30	40.00
River water		0	0.00	0	0.00
Others		0	0.00	0	0.00
Total		75	100	75	100
Hilsa carrying system					
By foot		8	10.67	7	9.33
By van		3	4.00	2	2.67
By fishing craft		64	85.33	66	88.00
Total		75	100	75	100
Plastic tray		63	84.00	62	82.67
Cork sheet box		12	16.00	13	17.33
Wash fish		0	0.00	0	0.00
Use ice		0	0.00	0	0.00
Chemical use		0	0.00	0	0.00
Total		75	100	75	100

Table 4. Catch per unit effort (CPUE), fishing effort, occupation, training programs, organisations, funding sources, and various aids of Hilsa fishers' communities.

Categories		Study Area			
		Gazaria Upazila (Village Ismanirchar)		Sadar Upazila (Village Kalirchar)	
		Number	Percentage	Number	Percentage
Catch Per Unit Effort (CPUE) and Fishing effort					
CPUE (kg/gear/day)	Fishing effort (hour/day)				
1-5	1-2	12	16.00	7	9.33
6-10	2-3	58	77.33	60	80.00
11-20	3-4	5	6.67	8	10.67
Total		75	100	75	100
The fishers engaged in Hilsa fishing are occupied.					
Solely fishing		51	68.00	48	64.00
Fishing combined with agriculture		13	17.33	18	24.00
Fishing alongside day labour		8	10.67	5	6.67
Other related activities		3	4.00	4	5.33
Total		75	100	75	100
Training on fishing and fish quality taken by fishers					
Awareness of Jatka, Ma Ilish, Current net		75	100	75	100
Awareness of Special Combing Operation		75	100	75	100
Workshop on Fish Conservation Act 1950		75	100	75	100
Good Aquaculture Practice (GAP)		12	16.00	0	82.67
Others		0	0.00	0	0.00
Total		75	100	75	100
Name of organisation					
NGOs	Association for Social Advancement (ASSA)	25	33.33	24	32.00
	"Ekti Bari Ekti Khamer"	5	6.67	3	4.00
	Bangladesh Rural Advancement Committee (BRAC)	8	10.67	12	16.00
	Blue Bangla	22	29.33	20	26.67
Not required		15	20.00	16	21.33
Total		75	100	75	100
Source of funding					
None		7	9.33	6	8.00
Neighbour		5	6.67	5	6.67
Relatives		3	4.00	5	6.67
NGO		60	80.00	60	80.00
Total		75	100	75	100
Aids from GO and NGO for Hilsa fishers					
GO	VGF (Rice) during ban season	60	80.00	57	76.00
	AIG (Rickshaw, Van, Selai Machine)	24	32.00	18	24.00
	Others relief	42	56.00	33	44.00
NGO		0	0.00	0	0.00
Total		75	100	75	100

Table 5: General information about Hilsa size, price and consumer demand.

Size (kg)	Price (BDT)	Maximum caught size (kg)	Considering price maximum consumer demand size (kg)	Study area (in total)	
				Number	Percentage
0.4-0.5	300-400	0.4-0.6	0.6-0.8	150	100
0.6-0.8	500-700				
0.9-1.0	800-1000				
Above 1.0	1000-1500				
			Total	150	100

Electricity facility

In both Ismanirchar and Kalirchar villages, all fishers enjoyed full access to electricity (Figure 2). However, Hossain et al. (2015) discovered that approximately 36% of fishers residing near the Punorvaba River in Sadar Upazila, Dinajpur, lacked access to electricity. Similarly, Mia et al. (2015) noted that approximately 87.5% of respondents in the fisher community along the Meghna River in Ashuganj Upazila, Brahmanbaria District, had access to electricity, while 12.5% did not. Mahmud et al. (2015) found that a maximum of 82% of households had electricity connections, while 18% did not, among fishers residing along the Pira River.

Health facility

Regarding health facilities, approximately 80% of fishers in Ismanirchar village received healthcare services from village doctors, 16% from Upazila Health Complexes, and the remaining 4% sought assistance from quacks (Figure 2). In contrast, around 84% of fishers in Kalirchar village received healthcare services from village doctors, with the remaining 16% utilising services from Upazila Health Complexes. Faruque and Ahsan (2014) reported that approximately 68.14%, 24.05%, and 7.81% of fishers received healthcare from village doctors, Upazila Health Complexes, and quacks, respectively. Kabir et al. (2012) noted that 60% of fishers received healthcare from village doctors, 30% from Upazila Health Complexes, and 10% from private Bachelor of Medicine, Bachelor of Surgery (MBBS) doctors. Bappa et al. (2014) revealed that 64% of respondents sought treatment from quacks, while only 16% visited MBBS doctors for treatment. It is important to note that no facilities were provided by MBBS or trained registered doctors in the study area (Khan et al., 2013).

Sanitation facility

The hygiene standards among the fishers in the study area were notably low, as depicted in Figure 2. Faruque and Ahsan (2014) reported that approximately 88% of toilets were of "kacha" construction, with 10% being semi-pacca in the Charghat Upazila of Rajshahi, while 2% lacked any sanitary facilities, mirroring the current findings. Similarly, Kabir et al. (2012) noted that around 70% of fishers had access to sanitary facilities.

Age group

In assessing the potential productive human resources, understanding the age distribution of fishers is crucial. Various age groups of fishers were identified in the study area (Table 2). Kostori (2012) reported that individuals aged 20-30 comprised the primary workforce in the Chalan Beel community of Tarash Upazila, Sirajganj district, Bangladesh. Our findings were generally consistent with those of Faruque and Ahsan (2014) and Hossain et al. (2009). Minar et al. (2012) discovered that most fishers fell within the age brackets of 31 to 40 years (56%) in the Kirtonkhola River, Barisal. Similarly, Pravakar et al. (2013) observed that most fishers belonged to the age groups of 41-60 years (44%) and 20-30 years (20%) in Shahrasti Upazila, Chandpur district.

Educational status

This study categorised fishers into six groups based on their educational attainment (Table 2). Most fishers demonstrated the ability to sign their names, possibly indicating efforts by NGOs to promote educational development within the fishing community. Faruque and Ahsan (2014) discovered that 67.54% of Hilsa fishers in Charghat Upazila could only sign their names, while 16.62%, 14.05%, and 1.57% were classified as illiterate, primary-educated, and secondary-educated, respectively. Similarly, Minar et al. (2012) reported that 80% of riverine fishers in Barisal town were illiterate, with 12% able to sign their names and only 8% having completed primary education. Pravakar et al. (2013) found that in Shahrasti Upazila, Chandpur district, approximately 10% had no formal education, 16% had completed primary education, 48% had completed secondary education, 16% had attained higher secondary education, and 10% had obtained a bachelor's degree.

Table 6. Income of the Hilsa fishers in the study area.

Season	Highest catch	Categories of fishers	Income/day (in BDT)
During Hilsa fishing season (Sep-Nov and March-May)	Sep-Nov	Boat owner fishers	1500-2000
		Labor fishers	500-600
		Donga owner fishers	1000-1200
Other seasons of the year (Dec-Feb and July-Aug)	July-Aug	Boat owner fishers	500-1000
		Labor fishers	300-500
		Donga owner fishers	500-800

Percent distribution of earners and dependency ratio

Observations revealed that only males were earning money in the study area, while females typically assisted in net-making activities. However, in certain households, children under 18 were also found to contribute to the family's income (Table 2). About 31.08% were earners, and 77.02% were dependents in Ismanirchar village. In contrast, around 31.59% were earners, and 68.41% were dependents in Kalirchar village.

Status of school-going children of fishers

In the study area, it was observed that around 18.75% and 17.65% of the children of fishers were non-school-going in Ismanirchar and Kalirchar villages, respectively (Table 2). The remaining (63.60%) were school-going children, both boys and girls. Kostori (2012) noted that in the Chalan Beel area under Tarash Upazila of Sirajganj district in Bangladesh, 53% were boys, and 47% were school-going children.

Gender

No female fishers were found in this study area. All the fishers of the area (100%) were male. Society discourages hard work like Hilsa fishing and the involvement of females in fishing activities. These fishing activities of females were always rare, which corresponded with the findings of Faruque and Ahsan (2014) and Halder et al. (2011).

Recreational media

Most fishers used television and mobile phones as recreational media (64% in Ismanirchar village and 69.33% in Kalirchar village) (Table 3). According to Mahmud et al. (2015), approximately 34% of fishers utilised their radios, while 46% relied on television sets for recreational purposes and to stay informed about national news. Additionally, 20% of fishers spent leisure time conversing at tea stalls. Kostori (2012) discovered that around 36% of individuals in the Chalan Beel area of Sirajganj district used radio or television for recreation, while 64% did not possess any recreational devices.

Landholding

The individual with a large area of land leads the community, and his social status is comparatively higher than others (Table 3). Rahman et al. (2014) reported that the majority (52%) of the fishers living near Jamuna River under Shirajgonj District had land properties ranging from 1 to 20 decimal.

Living house condition

Four housing categories (Kacha, Tin shed, Semi-paka, and Paka) were identified (Table 3). Alam et al. (2009) reported that approximately 82.22% of household structures were categorised as kacha, with semi-paka and paka structures accounting for 11.11% and 6.66%, respectively, among Basantapur Beel fishers. Ahmed (2002) found that 62% of housing structures among fish farmers in the Mymensingh region were kacha. Similarly, Mia et al. (2015) observed that the majority (75-80%) of respondents had kacha houses, while 17.5% had tin sheds, and 2.5-7.5% had semi-paka or paka structures within the fisher's community of the Meghna River in Ashuganj Upazila, Brahmanbaria district, Bangladesh.

Drinking water facility

Most households of Hilsa fishers relied on tube-well water for drinking purposes, either utilising their tube-well or a neighbouring household (Table 3). This practice was widespread among fishers across various regions of Bangladesh, with similar findings reported by Alam et al. (2009), Kabir et al. (2012), Abdullah-Bin-Farid et al. (2013), and Hossain et al. (2015). In their study, Faruque and Ahsan (2014) also observed that 100% of Hilsa fisher households residing along the Padma River in Charghat Upazila utilised tube-well water. Additionally, Rahman et al. (2014) documented that approximately 83% of fishers in the Sirajganj District, situated along the Jamuna River, accessed water from their tube wells, while the remaining 17% relied on tube wells belonging to neighbours.

The transportation system of Hilsa

It was observed that Hilsa was transported by van and fishing craft using a plastic tray and cork sheet box. No one was found who washed fish or used ice and other chemicals throughout the study area (Table 3). According to Hossain (2018), Hilsa fish were typically packaged in plastic boxes, which were subsequently stored in large Styrofoam containers filled with ice before being sold to buyers, retailers, and local markets in Bangladesh. However, no literature was available regarding the pricing of Hilsa or the purchasing power of consumers in the Munshiganj district.

Catch per unit effort

About 16%, 77.33% and 6.67% fishers stated that catch per unit effort (CPUE) for 1-2, 2-3 and 3-4 fishing effort (hour/day) was 1-5, 6-10 and 11-20 kg/gear/day in Ismanirchar village, respectively (Table 4). On the other hand, 9.33%, 80.33% and 10.67% fishers stated that CPUE for 1-2, 2-3 and 3-4 fishing effort was 1-5, 6-10 and 11-20 kg/gear/day in Kalirchar village, respectively. Hossain et al. (2014) documented that most Hilsa-catching fishers were dominant and, on average, caught 35-40 kg of Hilsa fish daily. Siddique (2013) stated that the CPUE of ilish jal and fash jal were 120-150 kg and 30-50 kg, respectively.

Occupation

The occupation of the fishers was classified into distinct categories, including solely fishing, fishing combined with agriculture, fishing alongside day labour, and other related activities (Table 4). Previous research conducted by Faruque and Ahsan (2014) revealed that most fishers (ranging from 52.17% to 75.00%) were exclusively engaged in fishing activities in the Padma River. The findings of this study align closely with the conclusions drawn by Kabir et al. (2012) and Bappa et al. (2014).

Training on fishing and fish quality

About 100% of fishers were aware of brood Ilish (Gravid Hilsa), Jatka (Juvenile Hilsa), current net (drift gill net), special combing operation, and the Fish Conservation Act 1950 in both Ismanirchar and Kalirchar villages. Besides, around 16% of fishers received Good Aquaculture Practice (GAP) training in Ismanirchar village, whereas no one received GAP training in Kalirchar village (Table 4). Shuhaimi (2013) described that not much attention has been given to fishers in terms of fishing training.

Associated organisations

Most fishers (Ismanirchar and Kalirchar village) were engaged with different associated organisations such as ASSA, BRAC, and Blue Bangla, except a few (Table 4). Kostori (2012) showed that about 66% of the respondents were involved with a committee/association, and 34% were not involved with any association of the fishers in the Chalan Beel, Sirajganj district.

Source of funding

The maximum number of fishers depended on different NGOs to borrow money, followed by their relatives and neighbours in the study area (Table 4). The amount of loan for fishers varies from fisher to fisher, depending on processing costs, catch systems, boat and net size, and management. In contrast, very few fishers were not borrowed money from any sources. In their study, Mia et al. (2015) observed that 65.25% of fishers in the Meghna River area of Ashuganj Upazila, Brahmanbaria district, Bangladesh, received loans, while the remaining fishers did not have access to loans within the fisher community.

GO and NGO aids

Various GO and NGO aids provided relief facilities to the study area's fishers during different banning periods (Table 4). During the banning months, the Upazila Fisheries Officer (UFO) provided food assistance to 519 fisher families at Gazaria Upazila and 517 fisher families at Munshiganj Sadar Upazila at the rate of 40 kg/family/month (DoF, 2019).

Size and price of Hilsa

Hilsa size, 0.4-0.5 kg, 0.6-0.8 kg, 0.9-1.0 kg and above 1.0 kg Hilsa were the price of 300-400 BDT, 500-700 BDT, 800-1000 BDT and 1000-1500 BDT, respectively. During data collection, all fishers in the studied areas stated that a maximum of 0.4-0.6 kg sizes Hilsa was caught and 0.6-0.8 kg sizes Hilsa demanded by the maximum consumer (Table 5). Fishers of Munsiganj Sadar and Gazaria Upazila mentioned that Hilsa fishing, size and price depended on different seasons in the river Meghna (Sazzad, 1993). Farzana (2014) stated that the Hilsa species price remained more or less the same from July to August.

Income of fishers

Daily income and the highest fishing season for boat owner fishers, labour fishers and donga owner fishers were 1500-2000 BDT, 500-600 BDT and 1000-1200 BDT, respectively, during September to November, and were 500-1000 BDT, 300-500 BDT and 500-800 BDT, respectively during July to

August (Table 6). The income profile serves as a crucial economic gauge for measuring national development. Daily income stands out as a key indicator in comprehending the socio-economic status of fishers. Their income levels vary over time and are influenced by peak and lean seasons, although the demand for Hilsa remains relatively consistent year-round nationwide. During reduced catch rates, fishers often find themselves in the low-income bracket, necessitating borrowing to meet their daily needs. Consequently, they may experience poverty, ill health, and various diseases.

Conclusion

The socio-economic status of riverine fishers' communities was assessed based on family type, family size, and housing conditions. Fishers encountered various challenges, including limited education for their children, inadequate healthcare services, insufficient food supplies, and difficulties in saving during fishing bans. Nearly all fishers identified a lack of capital and viable alternatives during these bans as their primary concerns. Given fishers' crucial role in harvesting Hilsa fish under demanding conditions, the government must take proactive measures to improve their socio-economic well-being and nutritional status. Efforts should be made to raise awareness among fishers through governmental and non-governmental organisations (NGOs). A constructive approach is essential to establish a sustainable Hilsa fishery and integrate it into the broader framework of sustainable livelihoods at both community and national levels. Collaborative efforts between the government and various NGOs are necessary to implement effective management policies and provide additional support during fishing bans.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

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Determination of metals in different tissues of *Trachurus trachurus* from the Marmara Sea and evaluation of their health risks

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ABSTRACT

The present study aimed to determine the differences in metal levels of Aluminum (Al), Cadmium (Cd), Chromium (Cr), Copper (Cu), İron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb) and Zinc (Zn) between muscle and liver tissues of Trachurus trachurus, the correlation of metal accumulation in tissues with fish length and weight was examined. Health risks and nutritional adequacy were evaluated based on metal levels accumulated in fish muscle. The results demonstrated that fish liver accumulated higher metal levels than muscle. The investigation revealed that the majority of metals present in the tissues of the fish did not exhibit a significant correlation with their size ($p>0.05$). It can be concluded that fish are not an adequate source of these nutrients because their nutrient levels are below reference values. The fish were found to be nutrient deficient, as the levels of nutrients were below reference values. The estimated daily intakes of all metals accumulated in muscle were below reference doses. The target hazard coefficient and target hazard index values were less than 1. Lifetime carcinogenic risk values were below 10^{-4}, and total carcinogenic risk values were within the acceptable range. Pb and Cd concentrations in muscle exceeded the tolerable limit. The daily amount of fish that could be consumed without non-carcinogenic health risk was 181 grams for Cd and 295 grams for Pb. The daily amount of fish that could be consumed without carcinogenic health risk was 1.21 grams for Cd and 231 grams for Pb.

Keywords: *Trachurus trachurus*, Heavy metal, Risk assessment, Marmara Sea, Carcinogenic risk

Introduction

Many anthropogenic sources, including industrial, agricultural, domestic, and mineral wastes contaminate marine ecosystems. The presence of heavy metals in the sea can result in the exposure of humans and other organisms to these metals through a multitude of pathways. The primary pathway for human exposure to heavy metals found in the marine environment is consuming seafood. Since fish is the most consumed seafood product by humans, examining the accumulation of heavy metals in fish species is crucial.

Some heavy metals can exert a deleterious effect on organisms, while certain macroelements are essential for maintaining optimal health. Non-essential heavy metals, such as Al, Cd and Pb, can exhibit toxic effects even when consumed in low concentrations. (Cardoso et al., 2019). Heavy metals, including Cr, Cu, Fe, Mn, Ni and Zn, are essential elements. However, exposure to them in excessive concentrations can lead to a range of adverse health effects, including hepatic, renal, cardiovascular and neurological disorders. (Korkmaz et al., 2017).

Heavy metal pollution of the sea results in the accumulation of heavy metals in marine organisms. Living organisms cannot metabolise these pollutants, as they are stable, do not biodegrade, and therefore cannot accumulate. (Lozano-Bilbao et al., 2020). Organs such as the liver, gonads, kidneys, gills, and skin are target organs that accumulate metal in aquatic organisms. These toxic metals, which are absorbed into solids dissolved or undissolved in seawater, are absorbed by aquatic organisms through the gills and skin (Jabeen et al., 2018). Metal concentrations detected in the gills reflect the metal concentrations in the water, while concentrations in the liver indicate the accumulation of metals in vivo (Kalantzi et al., 2019; Tokatli, 2018; Yilmaz, 2003). It is well established that muscle tissue is not active in metal accumulation (Sunlu et al., 2001). Conversely, research has indicated that metal accumulation in skin tissues is more prevalent than in muscle tissues (Yazkan et al., 2002; Yilmaz, 2003).

Atlantic horse mackerel, Trachurus trachurus (Linnaeus, 1758), is a pelagic fish of significant economic importance (Bektas & Belduz, 2009; Turan et al., 2009). It is stated that this fish species is rich in minerals, vitamins and polyunsaturated fatty acids (Ozden, 2010). These fish species are common in the Aegean, Mediterranean and Black Sea, including mostly in the heavily polluted Marmara Sea (Erkan et al., 2020; Turan et al., 2009).

The Marmara Sea is influenced by many pressures, such as urban and industrial wastewater, maritime traffic, agricultural and settlement activities on land, and tourism. Recent studies have shown that the Marmara Sea remains polluted, particularly due to the increase in heavy metal, microplastic, and radionuclide concentrations (Almas et al., 2022; Baysal & Saygin, 2022; Gözel et al., 2022; Tan, 2021). Fishing activities continue to be conducted at a high intensity in this region. Consequently, it is necessary to monitor the metal content of seafood caught in this sea to know whether it suits human consumption. There are few studies on the content of metals and trace elements in *Trachurus trachurus* in the Marmara Sea region (Aydın & Tokalıoğlu, 2015; Mutlu et al., 2012; Yaman et al., 2013). Additionally, few risk assessments have been conducted for fish species cultivated in the Sea of Marmara. However, no carcinogenic or non-carcinogenic risk assessment exists for consuming fish from the Trachurus trachurus species. Humans may also consume liver, stomach, and muscle tissues when they consume fish. Fishermen can also recycle these tissues to feed the fish (Onsanit et al., 2010). For this purpose, the concentrations of Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined and their relationship with biometric data was analysed in the tissues (muscle and liver) of *Trachurus trachurus* species grown in the Marmara Sea. In addition, for people consuming this fish, the adequacy of nutrients was evaluated, and carcinogenic-noncarcinogenic risk assessment was carried out in muscle tissues.

Materials and Methods

Sample Collection and Preparation

Ten fish samples of Trachurus trachurus were randomly collected from local fishermen in Istanbul who fish in different parts of the Sea of Marmara. Sampling was conducted in December. No distinction was made between the sexes during sampling. The laboratory received the fish samples on the same day. The fish's length and weight were measured, and then samples were washed with distilled water for analysis. Each fish's muscle and liver tissue were dissected using a plastic knife. The tissues were homogenised into small pieces. Three fish muscle and liver tissue replicates were prepared and stored at -20°C until analysis.

Sample Digestion and Metal Analysis

The method employed by (Dirican et al., 2015) for the digestion of fish samples was utilised in this study. Weighed 1 g wet samples of muscle and liver tissues and placed in glass vials. 3 mL concentrated nitric acid was added to the samples and incubated at room temperature for 24 hours. Then, 1 mL of sulfuric acid and 1-2 drops of nitric acid were added and heated on a hot plate. Heating was continued until the dissolution of the samples was complete, and a transparent solution was obtained. The digested samples were diluted to 25 mL with distilled water. Sample solutions containing particles were filtered through Whatman No. 2 filter paper and ready for analysis. Flame Atomic Absorption Spectrometer - FAAS (Agilent 240 Duo) was calibrated with a standard solution of metals, and the method's accuracy was calculated using the standard addition method. The measurement parameters by FAAS are given in Table 1. The fish samples' Al, Cd, Cr, Cu, Fe, Ni, Mn, Pb and Zn metal concentrations were then measured three times with the spectrometer. The results were statistically evaluated, and carcinogenic and noncarcinogenic risk assessment was performed.

Risk Assessments

Estimated daily heavy metal intake of Trachurus trachurus

The estimated daily intake (EDI) is the amount of a substance that can be consumed daily based on body weight. The estimated daily heavy metal intake of *Trachurus trachurus* was calculated by using the equation below (Storelli et al., 2020; Uroko et al., 2020).

$$EDI = \frac{CM \times DIF}{BW} \quad (1)$$

Where;

CM = Concentration of metal (mg/kg). The mean metal concentration determined in the muscle tissue of fish was used.

DIF = Daily intake of fish (g/day). The amount of fish portion consumed daily in Turkiye (18.4 g/day) was used (GDFA, 2021).

BW = Body weight. The average body weight of fish consumers was 70 kg for an adult.

Target hazard quotient

The target hazard quotient (THQ) was calculated to assess the non-carcinogenic health risks of heavy metals. Trachurus trachurus was consumed. THQ values were calculated for each heavy metal using the following equation (Li et al., 2021).

$$THQ = \frac{EF \times ED \times DIF \times CM}{RfDo \times BW \times AT_{nc}} \times 10^{-3} \quad (2)$$

Where;

EF = exposure frequency (365 days/year) (USEPA, 1991).

ED = exposure duration (26 years) (USEPA, 2022a).

RfDo = chronic oral reference dose (mg/kg/day).

AT_{nc} = average exposure time to non-carcinogens (365 days/year × ED) (USEPA, 2022c).

If the THQ values are below 1, there is no negative effect on human health. If they are above 1, consumers may experience a negative health situation.

Target hazard index

The hazard index (THI) equation was used to determine the potential risk triggered by ingesting more than one heavy metal. The total risk calculation was made by taking the sum of the THQ values calculated for each metal. The hazard index percentage for each metal was also calculated. (USEPA, 1989).

$$HI = \sum_n^i THQ_{n\,Metal} \quad (3)$$

$$\%\, HI_n = \frac{THQ_n}{HI} \times 100 \quad (4)$$

Table 1. Measurement parameters for determination of the metals by FAAS.

	Al	Cd	Cr	Cu	Fe	Ni	Mn	Pb	Zn
Wavelength (nm)	309.3	326.1	357.9	324.8	372.0	352.5	279.5	283.3	213.9
Lamp Current (mA)	10.0	4.0	7.0	4.0	5.0	4.0	5.0	10.0	5.0
Slit Width (nm)	0.5	0.5	0.2	0.2	0.2	0.2	0.2	0.5	1.0
Air Flow (mL/min)	-	13.5	-	13.5	13.5	13.5	13.5	13.5	13.5
Acetylene Flow (mL/min)	6.8	2.0	6.8	2.0	2.0	2.0	2.0	2.0	2.0
N_2O Flow (mL/min)	10.0	-	10.0	-	-	-	-	-	-

Lifetime carcinogen risk

The lifetime carcinogen risk (LCR) refers to the increased likelihood of developing cancer during a person's lifetime due to exposure to a carcinogen. The lifetime cancer risk from consuming *Trachurus trachurus* fish was estimated using the following equation. (USEPA, 2022a; Varol et al., 2022).

$$LCR = \frac{EF \times ED \times DIF \times CM \times CSFo}{BW \times AT_{ca}} \times 10^{-3} \quad (5)$$

Where;

CSFo = oral cancer slope factor of the metals (mg/kg/day).

AT_{ca}= average exposure time to carcinogens (365 days/ year × LT) (USEPA, 2022c).

LT= Lifetime. Turkiye's average life expectancy was 78 years. (TUİK, 2018).

Total cancer risks

The total cancer risks (TCR) due to exposure to multiple carcinogenic heavy metals through the consumption of *Trachurus trachurus* fish was calculated as the sum of the cancer-producing risks of each heavy metal. (Liu et al., 2013).

$$TCR = \sum_k^i LCR_k \quad (6)$$

Safe consumption limits

The safe consumption limits (SCL) were calculated to determine the amount of fish that can be safely consumed for a given period with respect to non-carcinogenic and carcinogenic health effects. (USEPA, 2000; Varol et al., 2017).

$$SCL_{nc} = \frac{RfDo \times BW}{CM} \quad (7)$$

$$SCL_{ca} = \frac{ARL \times BW}{CM \times CSFo} \quad (8)$$

Where;

SCL_{nc} = non-carcinogenic safe consumption limit

SCL_{ca} = carcinogenic safe consumption limit

ARL = maximum acceptable individual lifetime risk level (10^{-5}) (USEPA, 2000).

Metal pollution index

The metal pollution index (MPI) was calculated to find and compare the total toxic element pollution in fish's liver and muscle tissues.

$$MPI=(CM_1 + CM_2 + \cdots \ldots \ldots + CM_n)^{1/n} \quad (9)$$

Statistical Analysis

All statistical analyses in this study were performed using the SPSS 25 program. Relationships between fish length-weight, length-metal concentration, and weight-metal concentration were evaluated by using the Pearson correlation test. Paired samples of the T-test and Wilcoxon test were used to compare metal concentrations in fish tissues.

Results and Discussion

Statistical Evaluations Between Heavy Metal Concentrations and Fish Size

The Pearson correlation test was employed to ascertain the relationships between heavy metal concentrations in fish tissues and fish sizes. While significant positive correlations were observed between Pb, Zn and Mn in liver and length-weight ($p<0.05$), no significant correlations were observed between Al, Cd, Cu and Ni and both length and weight ($p > 0.05$). A positive correlation was observed between Fe in the liver and length ($p < 0.05$), but no significant correlation with weight ($p>0.05$). However, no significant correlations were observed between all metals in muscles and length and weight ($p>0.05$). It was observed that there was a positive correlation between the length and weight of the fish ($p<0.01$).

The results demonstrated that only a few metals in the liver exhibited a statistically significant correlation with fish size. This suggests that the relationship between metals accumulated in tissues and fish size is insignificant and disconnected. A similar conclusion was reached in a previous study. (Varol & Kaçar, 2023). However, in contrast to our findings, some studies have observed a negative correlation between metal levels in tissues and fish size, and it has been postulated that metals accumulate at a higher rate in smaller fish. (Ge et al., 2020a; Merciai et al., 2014; Varol et al., 2022).

The Metal Concentration of Fish Tissues and Comparison with Food Safety Guidelines

The accuracy of the proposed method was checked by determining the metal ions using the standard addition method due to the lack of certified reference materials. 0.05 mg/kg and 0.1 mg/kg of standard solutions were added to muscle and liver samples. Validation parameters such as linearity, LOD, LOQ, RSD%, etc., are given in Table 2.

Table 2. The analytical values in FAAS

	Linear regression (y=ax+b)	Correlation coefficients (R^2)	LOD (mg/L)	LOQ (mg/L)	RSD (%)	Recovery (%)
Al	0.0013x – 0.0003	0.9996	0.23	0.77	1.7-7.5	99.6
Cd	0.03x+ 0.0039	0.9995	0.0018	0.006	0.4-4.8	99.9
Cr	0.2125x–0.0038	0.9987	0.015	0.05	0.4-3.6	100.0
Cu	0.2261x+0.0009	0.9997	0.007	0.023	0.2-2.5	97.7
Fe	0.0062x – 0.0005	0.9996	0.24	0.81	0.7-4.8	99.8
Mn	0.0757x+0.0055	0.9984	0.0039	0.013	0.3-4.3	100.0
Ni	0.1137x+0.0005	0.9997	0.001	0.01	0.5-6.2	98.9
Pb	0.0737x–0.0015	0.9992	0.002	0.007	0.3-2.4	102.0
Zn	0.1724x+0.0188	0.9995	0.069	0.23	0.2-1.6	102.2

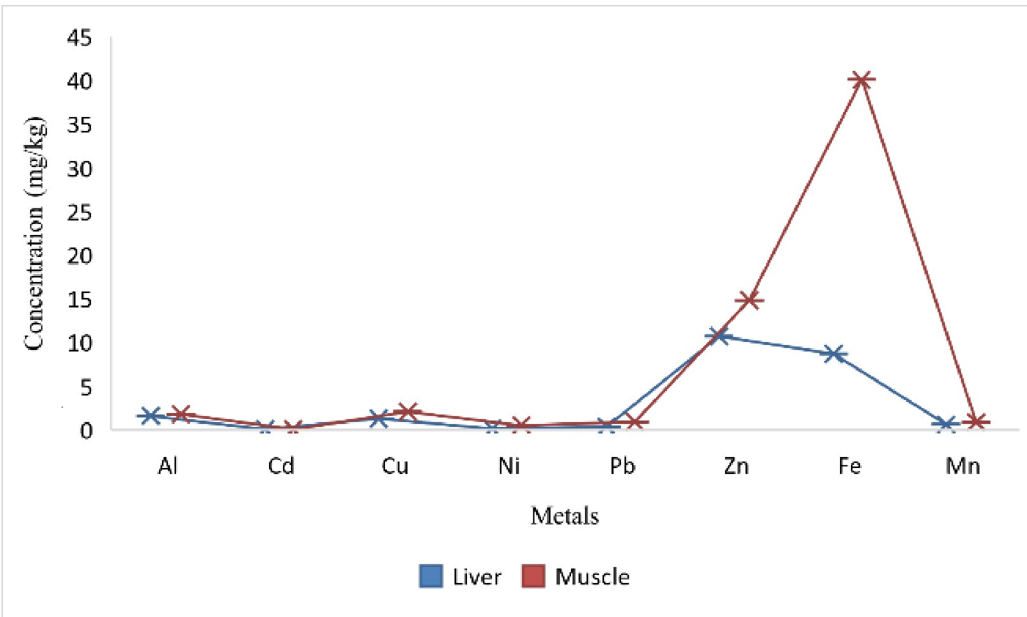

Figure 1. Comparison of metal levels detected in liver and muscle tissues.

Chromium was found below the detection limit in muscle tissues. Among the metals determined in muscle tissue, the highest concentrations are zinc (10.72 $mgkg^{-1}$) and iron (8.69 $mgkg^{-1}$), and the lowest concentrations are cadmium (0.039 $mgkg^{-1}$) and nickel (0.12 $mgkg^{-1}$). Metal concentrations in muscle tissue are in the order Zn>Fe>Al>Cu> Mn>Pb>Ni>Cd.

The chromium concentration in the liver tissues was below the detection limit. Among the metals determined in liver tissue, the highest concentration was observed in iron (40.05 $mgkg^{-1}$), and the lowest was detected in cadmium (0.048 $mgkg^{-1}$). The order of metal concentration determined in liver tissue is as follows: Fe>Zn>Cu>Al>Pb>Mn>Ni>Cd.

When comparing the metal concentrations in the liver and muscles, it was found that all metal concentrations were higher in the liver than in the muscle (Figure 1). Al, Cd and Zn accumulated similarly in both tissues. Cu and Mn accumulated about twice as much in the liver as in muscle. Fe and Ni were significantly higher in the liver than in the muscle. The concentration of Pb in the liver was 2.5 times higher than in the muscle. The liver is metabolically active tissue such as gills, accumulating metals in higher concentrations than other tissues. The liver reflects the fish's past exposure to metals (Ge et al., 2020b; Vetsis et al., 2021; Yilmaz, 2003). On the other hand, muscle tissue has lower metabolic activity than the liver and a longer metal deposition time (Varol et al., 2020; Varol et al., 2022; Vetsis et al., 2021). For this reason, a large amount of metal accumulation is not observed in muscle. Our study confirms that fish tend to accumulate higher levels of metals in their liver. In this respect, our study is sensitive to other studies that compare metal concentrations in various tissues of other fish species (Dirican et al., 2015; Pan et al., 2022; Varol et al., 2022; Vetsis et al., 2021; Yazkan et al., 2002). The average total length-weight and the heavy metal contents detected in the *Trachurus trachurus* fishes are summarised in Table 3.

The contribution of essential elements necessary for human health to the nutritional adequacy of the fish was evaluated by comparing them with the nutrient reference values (NRVs) given in Table 4. The evaluation was limited to muscle tissue samples as humans consume fish muscle tissue. NRVs of Cr, Cu, Fe, Mn, and Zn were 0.04, 1.0, 14.0, 2.0 and 10.0 mg/100g fish, respectively. Since Cr could not be detected in fish muscles, it did not contribute to its nutritional value. The Cu, Fe, Mn, and Zn concentrations in 100 g fish were 0.128, 0.869, 0.059 and 1.072 mg/100 g, respectively. The concentrations of all elements were found to be well below the NRVs. This indicates that the NRV for these elements cannot be achieved even if one portion (200 g) of fish is consumed. This fish species appears to not meet the dietary requirements for the intake of the mentioned elements.

Al, Cd and Pb are non-essential elements. Al is the third most abundant element in the Earth's crust and has no means of biodegradability. Therefore, it readily bioaccumulates in tissues. (Exley & Mold, 2015). This can make Al toxic to aquatic organisms and consumers (Igbokwe et al., 2019). Cd and Pb are known as toxic elements and have no biological function (Genchi et al., 2020). Cd has been proven to be a human carcinogen and has been shown to cause neurodegenerative diseases such as Parkinson's disease (Tamás et al., 2018; Tinkov et al., 2018). It is known that exposure to Pb causes mental disorders and irreversible errors in the mental development of children (O'Connor et al., 2020; Stanaway et al., 2018). The non-essential element concentrations detected in fish muscles were evaluated by comparing them with the maximum permissible limits (ML) values in Table 4. Al (1.59 mg/kg) and Cd (0.039 mg/kg) concentrations were below the reference ML. Pb (0.36 mg/kg) and Mn (0.59 mg/kg) slightly exceeded the reference ML values. The majority of studies conducted with different fish species at different times have shown that consuming fish grown in the Marmara Sea is not risky for health (Cucu et al., 2019; Dökmeci, 2021; Güngör & Kara, 2018). Furthermore, some studies corroborate our findings and suggest that certain metals exceed acceptable limits and should be consumed with care (Köker et al., 2021)

Risk Assessment

Estimated daily intake (EDI)

The EDI represents the estimated amount of metals found in fish that can be consumed daily over a lifetime without posing a health risk. EDI values of all metals were compared with the chronic oral reference doses (RfDo), as shown in Table 4. Fe and Zn stand out in fish muscle tissues with the highest EDI values, whereas Cd, Ni, and Pb have the lowest EDI values (Table 5). EDI values of all metals analysed were much lower than RfDo. These results were correlated with the daily fish consumption in Turkey (18.4 g/day). Consequently, ingesting 18.4 g of these fish daily does not present a health risk associated with the metals. There is one study in the literature that reports results contrary to our study. In this study examining different Trachurus species (*Trachurus mediterraneus*) growing in Marmara, the estimated weekly intake was found to be above the tolerable weekly intake in some regions (Köker et al., 2021).

Target hazard quotient (THQ)

The objective of THQ is to ascertain the non-carcinogenic health risks associated with fish analysed. THQ values of metals determined in fish muscle were evaluated according to whether they were below "1". THQ values of all metals analysed in fish muscle were found below "1". (Table 5.) For this reason, there is no noncarcinogenic health risk for metals in the case of feeding with these fish species grown in the Marmara Sea. Similarly, in a study conducted in 2019, It has been observed that there is no risk in terms of Cd and other metals (except As) in different fish species obtained from the Marmara Sea (Dökmeci et al., 2019).

Target hazard index (THI)

The THI value is employed to ascertain the cumulative non-carcinogenic health risk. The THI value and the percentage of each metal to the THI value (% THI) are presented in Table 5. A THI value exceeding 1 signifies that the ingestion of fish may potentially result in non-carcinogenic health hazards in individuals. The THI value (1.88×10^{-1}) in fish was below 1, indicating that a non-carcinogenic health risk does not arise if this fish species from the Sea of Marmara is consumed. Upon examination of the contribution of each metal to the THI value, it becomes evident that the metal with the largest share is Cd (54%), followed by Pb (33.2%). Consequently, it can be posited that Pb and Cd metals represent potential non-carcinogenic risk sources in these fish. Nevertheless, as long as the limits for fish consumption are not exceeded, there is no non-carcinogenic health risk from Pb and Cd metals.

Table 3. Metal concentrations and biological parameters in *Trachurus trachurus*.

	Concentration (mg/kg)			
	Muscle		Liver	
	Min-Max	Mean±SD	Min-Max	Mean±SD
Al	0.73-2.15	1.59±0.38	1.25-2.51	1.76±0.43
Cd	0.022-0.047	0.039±0.0079	0.030-0.064	0.048±0.018
Cr	ND		ND	
Cu	0.89-1.74	1.28±0.29	1.14-3.88	2.05±0.83
Fe	5.61-12.17	8.69±1.76	12.52-105.80	40.05±33.07
Mn	0.39-0.71	0.59±0.094	0.46-1.31	0.86±0.26
Ni	0.14-0.092	0.12±0.018	0.10-0.92	0.51±0.37
Pb	ND±3.56	0.36±1.13	ND±4.92	0.90±1.91
Zn	8.69-13.32	10.72±1.61	10.10-19.11	14.80±3.02
	Properties			
Weight (g)	65.0-170.9	108.0±35.6		
Length (cm)	20.4-27.7	23.3±2.5		

ND: Not detected

Table 4. Maximum permissible limits (ML), nutrient reference values (NRV), chronic oral reference doses (RfDo) and cancer slope factors (CSFo) used for health risk assessment

	ML (mg/kg)	NRV[3] (mg/100g)	RfDo (mg/kg-day)	CSFo[6] $(mg/kg/day)^{-1}$
Al	5.0[1]		1.0E+00[4]	
Cd	0.05[2]		1.0E-04[4]	1.5E+01
Cr	1.0[1]	0.04	3.0E-03[4]	4.2E-01
Cu	4.0[1]	1.0	4.0E-02[4]	
Fe	40.0[1]	14.0	7.0E-01[4]	
Mn	0.55[1]	2.0	1.4E-01[4]	
Ni	0.14[1]		2.0E-02[4]	9.1E-01
Pb	0.3[2]		4.0E-03[5]	8.5E-03
Zn	5.0[1]	10.0	3.0E-01[4]	

[1] (EDQM, 2022); [2] (EU, 2006); [3] (EU, 2011); [4] (USEPA, 2023a); [5] (Nag & Cummins, 2022); [6] (OEHHA, 2023)

Table 5. The results of the calculation of the health risks associated with fish consumption, based on the elemental content of the *Trachurus trachurus*

	Al	Cd	Cu	Fe	Mn	Ni	Pb	Zn
EDI (mg/kg bw/day)	4.17E-04	1.02E-05	3.36E-04	2.28E-03	1.54E-04	3.14E-05	9.36E-05	2.82E-03
THQ	4.17E-04	1.02E-01	8.40E-03	3.26E-03	1.10E-03	1.57E-03	6.24E-02	9.39E-03
THI (%)	0.222	54	4.46	1.73	0.584	0.834	33.2	4.99
LCR		5.08E-05				9.52E-06	2.65E-07	
SCL_{nc} (kg fish/day)	4.41E+01	1.81E-01	2.19E+00	5.64E+00	1.67E+01	1.17E+01	2.95E-01	1.96E+00
SCL_{ca} (kg fish/day)		1.21E-03				6.44E-03	2.31E-01	
THI	1.88E-01							
TCR	6.06E-05							
MPI_{Muscle} (mg/kg fish)	1.42							
MPI_{Liver} (mg/kg fish)	1.58							
MPI_{Total} (mg/kg fish)	3.0							

Lifetime carcinogen risk (LCR) and total cancer risks (TCR)

LCRs were calculated for the metals Cd, Ni and Pb using CSFo values determined by OEHHA. The following criteria were used to conduct the evaluations: if the calculated LCRs fall between 10^{-4} and 10^{-6}, the carcinogenic risk is deemed acceptable. There is no carcinogenic risk if the LCRs are less than 10^{-6}. Conversely, if the LCRs exceed 10^{-4}, the risk is deemed unacceptable (USEPA, 2023). The LCR values calculated for fish are presented in Table 5. The data indicate that the lifetime cancer risk for Cd ($5.08x10^{-5}$) metal is within acceptable limits when consumed in the indicated quantities. No lifetime cancer risk exists for Ni ($9.52x10^{-6}$) and Pb ($2.65x10^{-7}$) metals.

The TCR was calculated to estimate the lifetime probability of developing cancer in individuals exposed to a potential carcinogen through fish consumption. It was evaluated in a manner analogous to that employed for the LCR. The data indicate that the calculated TCR ($6,06x10^{-5}$) is within the minimum acceptable range (10^{-6}-10^{-4}) and does not carry a total cancer risk. Consequently, the evidence indicates that fish do not pose a lifelong cancer risk due to heavy metals.

Safe consumption limits (SCL)

The SCLnc values were calculated separately for each metal to determine the daily quantity of fish a 70-kilogram individual can consume without incurring a non-carcinogenic health risk. Among the metals, Cd ($1.81x10^{-1}$ kg fish/day) and Pb ($2.95x10^{-1}$ kg fish/day) had the lowest SCLnc values, while Al ($4.41x10^{1}$ kg fish/day) had the highest SCLnc value (Table 5). Accordingly, it can be concluded that no health risk is associated with the metals analysed, except for Cd and Pb. However, Cd may pose a non-carcinogenic health risk if more than 181 g of this fish is consumed daily. Similarly, this fish's regular consumption of more than 295 g per day may pose a non-carcinogenic health risk for Pb metal. As the determined consumption values were above the average daily fish consumption in Turkey (18.4 g/day), it was concluded that consuming these fish was not a significant risk.

The SCLca values of the metals Cd, Ni and Pb were calculated to determine the daily amount of fish that a person weighing 70 kg can consume without the risk of developing cancer. The mean SCLca values for Cd, Ni and Pb were $1.21x10^{-3}$ kg fish/day, $6.44x10^{-3}$ kg fish/day and $2.31x10^{-1}$ kg fish/day, respectively (Table 5). For the metals Cd and Ni, the amount of fish that can be consumed without a carcinogenic risk is lower than the average reference consumption of fish (18.4 g/day). Therefore, regular daily consumption of this fish species may be unfavourable regarding Cd and Ni metals. For Pb, the safe consumption limit is higher than the average fish consumption in Turkey. Therefore, carcinogenic health effects are expected if the reference consumption levels are exceeded for Cd and Ni but not for Pb.

Metal pollution index (MPI)

MPI is calculated to assess the environmental pollution level (Bilgin et al., 2023; Fahmy et al., 2023). The degree of contamination can be classified as low, initial, or increasing, depending on the value of MPI. The contamination level is considered low if the MPI is less than one. If MPI equals one, the contamination level is at its initial stage. Finally, if the MPI is greater than one, the contamination level increases (Fahmy et al., 2023). In this study, MPI values in liver and muscle were 1.58 mg/kg fish and 1.42 mg/kg fish, respectively. The concentration of metals in muscle and liver tissues is just above 1. This may indicate that the pollution level of the Marmara Sea is increasing. Furthermore, it has been reported that excessive metal accumulation in the liver compared to muscle indicates water pollution (George, Biju, Martin, & Gerson, 2022). In this study, the quantity of metals accumulated in the liver is less than in the muscle, but they are close to each other. A 2017 study concluded that the water quality of the Marmara Sea does not present a threat to human health or aquatic life (Bozkurt Kopuz & Kara, 2020). A 2020 study proposed that the elevated metal concentrations observed in biota were likely the result of the presence of chemical and environmental wastes in the Marmara Sea (Karabayir, Taskin, Simsek, Aksu, & Caglar, 2020). All these data may indicate that the Marmara Sea is under threat of pollution.

Conclusion

Among the metals examined, Pb and Cd exceeded the tolerable limit, with Pb exceeding the maximum permissible limit. Concentrations of metals were higher in the liver compared to muscle. There was no correlation between metal levels in muscle and fish size, but there was a positive correlation between some metal levels in liver and fish size. The health risk assessment calculations showed that there is no non-carcinogenic health risk associated with the consumption of these fish. The carcinogenic health risk calculations indicated that if the consumption limits were exceeded, there was a Cd and

Ni risk but no lifetime cancer risk. The environmental pollution level of the fish was determined by calculating the metal pollution index, which suggests that the pollution level of the Marmara Sea may be at the initial stage. Consequently, it is recommended that further detailed studies be conducted to control pollution and monitor fish and seafood.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

Data availability: Data will be made available on request.

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Geographic expansion of the blackbellied angler (*Lophius budegassa*) towards the east along the Turkish coast of the Black Sea

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ABSTRACT

The natural range of *Lophius budegassa*, originating from the Atlanto-Mediterranean region, extends from the Mediterranean Sea basin to the Sea of Marmara. However, its occurrence along the Turkish coast of the Black Sea has previously been limited to the Sinop coast in the Western Black Sea. This study provides the first scientific evidence of *L. budegassa* extending to the easternmost part of the Black Sea, based on capturing two specimens under different circumstances. This observation underscores the dynamic nature of marine ecosystems and the interplay of ecological and anthropogenic factors. The congruence between species temperature preferences and actual water temperatures in the Black Sea suggests suitable environmental conditions for *L. budegassa*. However, the absence of an established stock and the discovery of only two large-sized adult individuals suggest a potential isolated event rather than a significant range expansion. Factors such as navigation errors, food availability, or reproductive behaviour may contribute to the presence of *L. budegassa* in this region.

Keywords: Anglerfish, Lophiidae, First record, Range expansion, Türkiye

Introduction

The Lophiidae family encompasses around 30 species spread across four genera globally (Fricke et al., 2024). The family is represented by two prominent species in Turkish territorial waters (Bilecenoğlu et al., 2014): blackbellied angler (*Lophius budegassa* Spinola, 1807) and angler (*L. piscatorius* Linnaeus, 1758). *L. budegassa* is distributed primarily in the eastern central Atlantic region, spanning from the British Isles to Senegal (La Mesa & De Rossi, 2008), and also extends its presence from the Mediterranean Sea to the Black Sea (Sümer et al., 2016). They are commonly found between depths of 100 and 500 meters, with occurrences documented at depths reaching 1000 meters (Ungaro et al., 2002). *L. budegassa*, being a benthic species, thrives across various habitats, including muddy, sandy, and rocky environments. It has the potential to reach a maximum age of 25 years and a standard length of up to 100 cm (Vakily et al., 2002). Predominantly feeding on teleost fish and decapod crustaceans, *L. budegassa* demonstrates a diverse dietary spectrum (Şenbahar & Özaydın, 2021), establishing its role as an opportunistic apex predator (Ainouche & Nouar, 2018). *L. budegassa*, being a commercially significant species, is actively targeted by trawl and gill net fishing fleets (ICES, 2013). However, anglerfishes are also frequently caught incidentally as a non-target species in beam trawl fisheries (Karadurmuş, 2022). Anglerfishes, including *L. budegassa*, are increasingly valued in the fishing industry, leading to a rise in demand within Turkish fish markets (Şenbahar & Özaydın, 2019). According to the latest stock assessments reported from FAO catch statistics, *L. budegassa* is generally considered overfished in the Mediterranean Sea basin. This species is listed among the rare species in the Black Sea Red Data Book and classified as a vulnerable species in Türkiye due to the high risk of endangerment in the wild.

The distribution of the *L. budegassa* of Atlanto-Mediterranean origin in the Mediterranean Sea basin extends to the Ionian Sea (Barcala et al., 2019), the Aegean Sea (Yığın et al., 2015), and even the Sea of Marmara (Daban et al., 2021). The first scientifically supported evidence of the existence of *L. budegassa* in the Black Sea was reported by Sümer et al. (2016) with a single individual caught off Sinop province. After this record, to date, there is no evidence in scientific or media databases for the existence of species on the Turkish coast of the Black Sea. This paper documents that the geographical range of the *L. budegassa* extends to the easternmost part of the Black Sea.

Materials and Methods

During the study, two anglerfish individuals were captured (Figure 1), each under different circumstances. The General Fisheries Commission covers both sampling points for the Mediterranean Geographical Sub-Area 29, Division 37.4.2. Detailed information regarding the captures is as follows:

The first specimen (Sp1) was captured on July 20, 2018, using a trammel net at a depth of 35 meters off the coast of Hopa, Artvin (41°25'31"N - 41°21'24"E), situated in the easternmost part of the Black Sea.

The second specimen (Sp2) was caught on January 4, 2024, by a trammel net at a depth of 18 meters off the coast of Perşembe, Ordu (41°04'42"N - 37°48'36"E).

Taxonomic identification of both specimens was conducted according to the fish identification key (Fischer et al., 1987). Furthermore, the scientific name verification was checked from FishBase (Froese & Pauly, 2024). Both species, *L. budegassa* and its congener *L. piscatorius*, belonging to the *Lophius* genus, are very similar, with *L. budegassa* being characterised by 9-10 soft rays on the second dorsal fin, 8-9 rays on the anal fin, and dark peritoneum (Fischer et al., 1987). Total length (TL) was measured using an ichthyometer with a precision of 0.1 cm, while body weight (W) was recorded using an electronic scale accurate to 0.01 g. Sex identification was performed based on gonad colour and shape (Gunderson, 1994).

Results and Discussion

This study documented the sex, size, and weight information for two *L. budegassa* specimens captured during the long-term period. Sp1 was identified as a female, measuring a TL of 39.05 cm and weighing 930.56 g. Sp2 was classified as a male, exhibiting a TL of 40.50 cm and a W of 993.27 g. These size ranges are smaller than previously reported size (with 51.8 cm in TL) from the Sinop coast in the Black Sea (Sümer et al., 2016).

The recent discovery of new L. budegassa individuals in the easternmost part of the Black Sea raise questions about the potential spread and adaptation of the species to this region. This expansion is a compelling phenomenon that reflects the dynamic nature of marine ecosystems and the intricate interplay of various ecological and anthropogenic factors over time. Shifts in water temperature, salinity levels, and nutrient availability, possibly influenced by climate change and anthropogenic activities in the Black Sea (Oral et al., 2013;

Zengin, 2019), may have created favorable conditions for the *L. budegassa* to establish populations in new habitats. This phenomenon, defined as the Mediterraneanizing of the Black Sea (Oral et al., 2013), has resulted in the entrance and adaptation of many new fish species of Atlanto-Mediterranean origin to the Black Sea (Yankova et al., 2013; Aydın & Karadurmuş, 2023). The annual average surface water temperature of the Black Sea, approximately 16°C (Bengil & Mavruk, 2019), aligns closely with the preferred habitat temperature range of *L. budegassa*, which falls between 16.5°C to 17.5°C (Barcala et al., 2019). This indicates that the temperature conditions in the Black Sea are generally conducive to the species' existence in the region. The close match between the preferred temperature range of the anglerfish and the actual water temperatures suggests that the Black Sea provides suitable environmental conditions to support the presence and potential proliferation of *L. budegassa*.

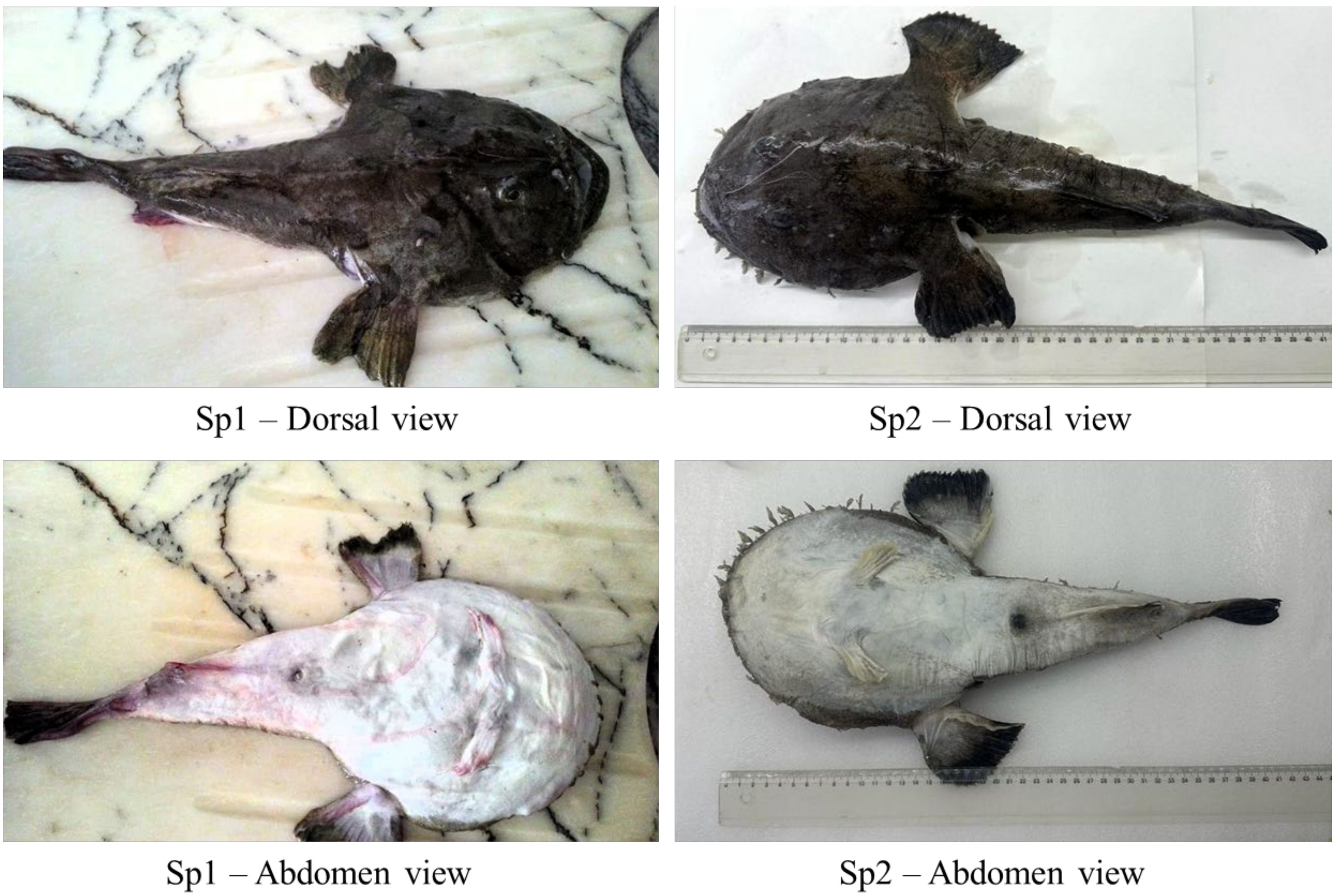

Figure 1. The female specimen of *Lophius budegassa* with 39.05 cm total length was captured from the Hopa, and the male specimen of *Lophius budegassa* with 40.50 cm total length was captured from the Perşembe

The absence of natural predators and competitors in the Black Sea ecosystem may have further facilitated the spread of *L. budegassa* populations. The main diet of the species consists of more than 80% of local fish species, such as whiting and mackerel (Negzaoui-Garali et al., 2008; Şenbahar & Özaydın, 2021). The abundance of target species in the Black Sea could likely support the development and distribution of the *L. budegassa* by providing a readily available food source. A consistent food supply allows individuals to meet their nutritional needs and allocate resources towards growth, reproduction, and survival. It is plausible that the captured specimens may be immature, considering the variability in maturity thresholds reported across different regions (Colmenero et al., 2013; Yığın et al., 2015). The relatively high reproductive capacity of the species, as indicated by the average potential fertility of around 80,000 oocytes per kilogram of mature female (Colmenero et al., 2013), suggests that individuals with immature reproductive organs may still possess the potential for significant reproductive output once they reach maturity. The long lifespan, late maturation, and slow growth rate of *L. budegassa* (Knudsen, 2015) render it vulnerable to overfishing. These traits increase their vulnerability to exploitation and make it challenging for the population to replenish itself under intensive fishing pressure.

Conclusion

This study sheds light on the geographical expansion of *L. budegassa* to the easternmost region of the Black Sea. The absence of an established stock and the discovery of only two large-sized adult individuals suggest that *L. budegassa* in the easternmost part of the Black Sea may be an isolated event rather than a significant range expansion. Specimens may have strayed from their typical habitat due to various factors such as navigation errors, food availability, or reproductive behaviour. Further research, including monitoring the area and population assessments, would be necessary to determine the long-term implications of this discovery and whether the species has spread and adapted to the region.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

Data availability: Data will be made available on request.

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4. **Research Funding and Conflicts of Interest:**

   Research funding sources and conflicts of interest should be clearly stated. It is important to disclose and not conceal conflicts of interest.

5. **Language:**

   Articles should be written to a scientific journal standard, and care should be taken regarding grammar and spelling errors.

Editors' Responsibilities

1. **Maintaining High Scientific Standards:**

   To ensure that the articles published in the journal comply with high scientific standards.

   To ensure full compliance with ethical rules and journal policies.

2. **Managing the Article Evaluation Process:**

   To effectively manage the article evaluation process and support a rapid publication process.

   To adopt the principles of double-blind arbitration and maintain the principles of expertise and impartiality in selecting arbitrators.

3. **Making Editorial Decisions:**

   Consider referee evaluations to make decisions about accepting or rejecting articles for publication.

   Maintaining transparency and openness in the editorial process.

4. **Contact with Authors:**

   Maintaining effective and constructive communication with authors.

   They provide authors with regular updates on the status of their articles, correction requests, and publication dates.

5. **Managing Journal Policies:**

   Keep the journal's policies and guidelines updated and revise them as needed.

   To provide a reliable platform between readers and writers.

Responsibilities of Referees

1. **Objectivity and Expertise:**

   To comply with the principles of double-blind refereeing and to evaluate articles impartially.

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   Informing editors promptly in case of delays.

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   Provide clear and constructive feedback to authors and suggest improving the article when necessary.

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Preparation of the Manuscript

Manuscripts prepared in Microsoft Word must be converted into a single file before submission. Please start with the title page and insert your graphics (schemes, figures, *etc.*) and tables in the one main text (Word Office file).

Title (should be clear, descriptive, and not too long)

Full Name(s) and Surname (s) of author(s)

ORCID ID for all author (s) (http://orcid.org/)

Authors complete correspondence Address (es) of affiliations and e-mail (s)

Abstract

Keywords (indexing terms), usually 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standards

- **Conflict of Interest:** When you (or your employer or sponsor) have a financial, commercial, legal, or professional relationship with other organisations 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 ethics committee's approval, and the authors may argue that they do not need support for their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit, and Service Evaluations" document, evaluate the study with the editorial board, and decide whether or not it needs approval.
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  One of the following should be selected and stated in the submitted article;

  1. No data was used for the research described in the article.
  2. The data that has been used is confidential.
  3. The authors do not have permission to share the data.
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References

Tables (all tables given in the main text)

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Manuscript Types

Original Articles: This is the most essential type of article since it provides new information based on original research. The main text should contain **"Title", "Abstract", "Introduction", "Materials and Methods", "Results and Discussion", "Conclusion", "Compliance with Ethical Standards"**, and **"References" sections.**

Statistical analysis to support conclusions is usually necessary. International statistical reporting standards must conduct statistical analyses. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section, and the statistical software used during the process must be specified.

Units should be prepared by the International System of Units (SI).

Review Articles: Reviews prepared by authors with extensive knowledge of a particular field

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and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. The journal may even invite these authors. Reviews should describe, discuss, and evaluate the current knowledge level of a research topic and should guide future studies. The main text should start with the Introduction and end with the Conclusion sections. Authors may choose to use any subheadings in between those sections.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking features of a previously published article. Articles on subjects within the journal's scope that might attract the readers' attention, particularly educative cases, may also be submitted as a "Short Communication". Readers can also comment on the published manuscripts as a "Short Communication". The main text should contain **"Title", "Abstract", "Introduction", "Materials and Methods", "Results and Discussion", "Conclusion", "Compliance with Ethical Standards**", and **"References"** sections.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤30	200	40
Review Article	no limits	200	60
Short Communication	≤5	200	20

Tables

Tables should be included in the main document and 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 in the tables should be defined below them 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 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 support the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted through the submission system in the main document's Word files (in JPEG or PNG format). 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 (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the primary 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 the USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)."

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Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

The citation style and methods that comply with the scientific standards that should be used in the "Aquatic Research" journal for the sources used by the authors in their works are given below.

Reference System is APA 6th Edition (with minor changes)

The APA style calls for three kinds of information to be included in in-text citations. The author's last name and the work's publication date must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Bhujel, 2014).

....(Mol & Erkan, 2009).

....(Alofa et al., 2023).

....(Mol & Erkan, 2009; Bhujel, 2014; Alofa et al., 2023).

Citations for a Reference Section:

An article

Alofa, C.S., Olodo, I.Y., Chabi Kpéra Orou Nari, M., Abou, Y. (2023). Effects of the fresh and dried housefly (*Musca domestica*) larvae in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758): growth, feed utilisation efficiency, body composition, and biological indices. *Aquatic Research*, 6(1), 1-10. https://doi.org/10.3153/AR23001
(if a DOI number is available)

A book in print

Bhujel, R.C. (2014). A manual for tilapia business. CABI Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 199 p. ISBN 978-1-78064-136-2. https://doi.org/10.1079/9781780641362.0000
(if a DOI number is available)

A book chapter

Craddock, N. (1997). Practical management in the food industry A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpage

CDC (2020). Rift Valley Fever | CDC. https://www.cdc.gov/vhf/rvf/index.html (accessed 20.08.2020).

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 15 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 cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-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 two days of their receipt of the proof.