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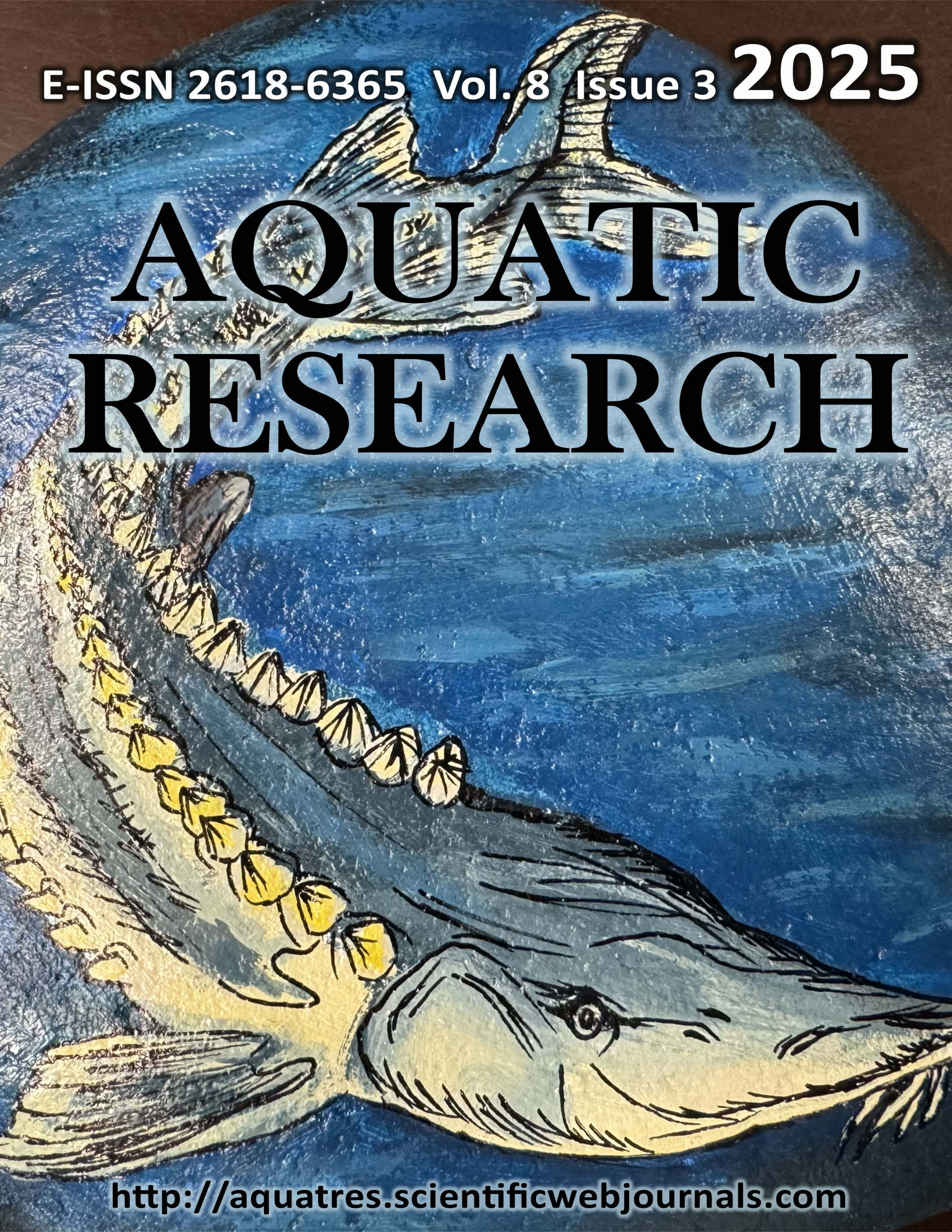

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Aquatic Research 8(3), 148-155 (2025) • https://doi.org/10.3153/AR25015 *Research Article*

Aquatic macroinvertebrate diversity and water quality assessment in Vembannur Wetland, Tamil Nadu, India

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ABSTRACT

Wetlands are vital components of the ecosystem, offering both ecological and economic benefits. This study was conducted in the Vambannur wetland, located in Rajakkamangalam block, Kanyakumari, Tamil Nadu, to assess the diversity of aquatic macroinvertebrates and water quality across three sites in September 2024. A total of 237 individuals, 15 genera, 11 families and 8 orders were identified, with Gastropods being the most dominant order. Key biodiversity indices, including Shannon, Simpson, Menhinick, Margalef and Berger-Parker, were calculated, revealing diversity in sites 2 and 3 compared to site 1. The results highlight the significance of macroinvertebrate diversity in determining wetland quality. Principal Component Analysis (PCA) is used to explore the relationship between physicochemical parameters and macroinvertebrate communities, providing insights into wetland health.

Keywords: Aquatic macroinvertebrates, Water quality, Diversity indices, Biodiversity, Vembannur wetland

Introduction

Wetlands are among the most vital ecosystems on Earth, acting as transitional zones between terrestrial and aquatic habitats (Xu et al., 2019). These ecosystems are crucial for preserving biodiversity, regulating water cycles, and providing numerous other ecological services, including water purification, flood regulation, and carbon storage (Singh et al., 2006). The significance of wetlands in maintaining ecological balance is further underscored by their role in supporting a diverse range of species, enhancing local economies through fishing and water resource management, and mitigating environmental damage caused by human activities (McLaughlin & Cohen, 2013). In India, the country's varied topography and climatic conditions have fostered the development of a diverse range of wetland ecosystems. These wetlands are home to approximately 20% of the nation's biodiversity, playing an indispensable role in maintaining ecological stability (Deepa & Ramachandra, 1999).

The global importance of wetlands has become increasingly apparent as human activities continue to threaten their existence (Junk et al., 2013). Over the past century, wetlands have faced widespread degradation, with 64% of the world's wetland areas impacted by urbanisation, agricultural expansion, and industrial development (Ballut-Dajud et al., 2022). This degradation often results in the contamination of wetland water, loss of biodiversity, and a decrease in the vital services they provide to both nature and society. Despite these threats, awareness of the ecological and economic values of wetlands has grown, with many nations, including India, taking steps toward their protection and conservation. India currently recognises the importance of wetlands through the establishment of 85 Ramsar sites, which highlights the country's commitment to conserving these critical ecosystems for biodiversity preservation (Prasad et al., 2002). In the context of this growing concern, aquatic macroinvertebrates have emerged as effective bioindicators of wetland health. These organisms are susceptible to environmental changes, making them invaluable for monitoring water quality and ecosystem stability. Previous studies have demonstrated that macroinvertebrates respond rapidly to both chemical pollutants and habitat alterations, with their diversity and abundance providing valuable insights into the ecological condition of a wetland (Dodson et al., 2001; Sharma et al., 2009). By examining these organisms, researchers can assess the degree of environmental stress and identify potential areas for conservation efforts. The presence of diverse macroinvertebrate communities, for instance, often correlates with higher water quality and a more balanced ecosystem. In contrast, a decline in their diversity may signal pollution or habitat degradation.

The purpose of the current study is to investigate the diversity of macroinvertebrates and evaluate the water quality in the Vembannur wetland, a crucial wetland located in southern India. This wetland, like many others, faces the dual pressures of natural and anthropogenic changes, including urban encroachment and agricultural runoff. The central question driving this study is: What is the current status of macroinvertebrate diversity and water quality in the Vembannur wetland, and how do these factors reflect the overall ecological health of the ecosystem? By addressing this question, the study aims to provide a comprehensive assessment of the wetland's ecological condition, offering valuable data to inform future conservation strategies. The rationale for this research stems from the need to enhance our understanding of wetland ecosystems, particularly in regions like India, where rapid development poses significant threats to ecological integrity. Although considerable research has been conducted on the general importance of wetlands and macroinvertebrates as bioindicators, studies focusing on specific wetlands in India, such as Vembannur, remain limited. The findings of this study will not only contribute to the body of knowledge about the ecological health of this particular wetland. However, they will also offer insights into the broader issues of wetland conservation in the region. Through a detailed analysis of macroinvertebrate diversity and water quality, this research will help identify potential environmental stressors and provide a baseline for future monitoring efforts.

Materials and Methods

Study Area

The Vembannur Wetland is a freshwater urban pond located in Rajakkamangalam Block of Kanyakumari District, Tamil Nadu, India. This artificial wetland spans approximately 19.75 hectares, with an average depth of 7 feet, and was developed through human intervention. The surrounding area includes residential settlements, railway quarters, and roadways, with some sewage inflow impacting the water quality. Vembannur Wetland is recognized for its biodiversity, particularly in bird and fauna species. Since April 8, 2022, it has been listed by UNESCO for its ecological importance.

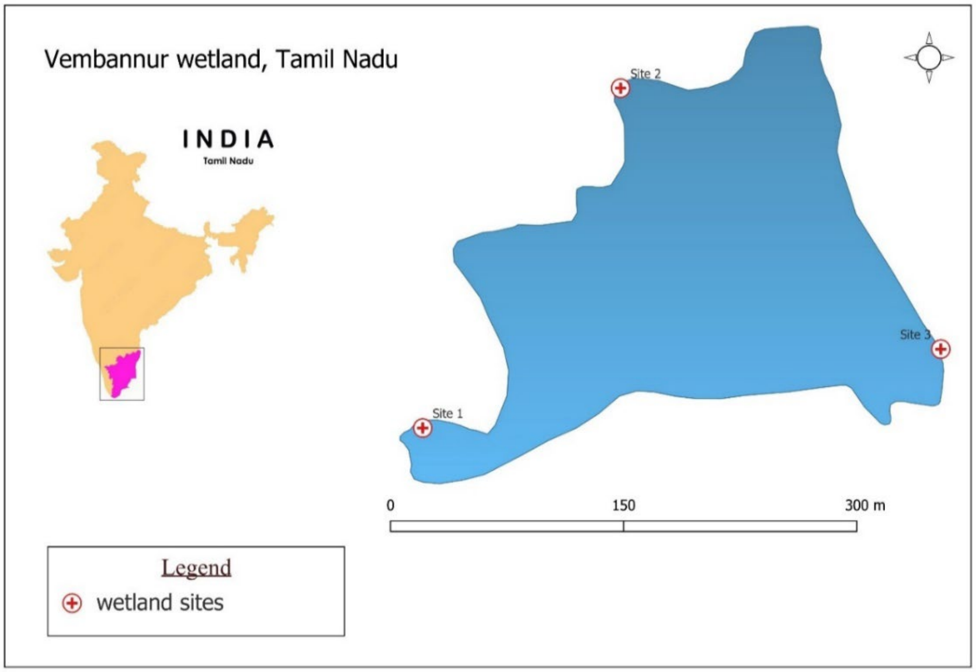

Figure 1. Geographical locations of sampling sites for macroinvertebrate and physicochemical water quality assessments

A field survey was conducted in September 2024, focusing on macroinvertebrate sampling and physicochemical analyses across three sites selected based on bird activity, accessibility, and environmental conditions. The latitude and longitude of each sampling site are detailed in Figure 1. The general land use patterns in the basin range from large-scale agriculture near the wetland to small-scale farming on elevated land. These patterns reflect the district's topography and highlight the importance of agriculture for local livelihoods.

Sampling Design

During the autumn season, macroinvertebrate samples and physicochemical parameters were collected from three distinct sites within the Vembannur Wetland to assess water quality and biodiversity. Site 1 is located near a roadside, Site 2 is adjacent to cultivated farmland, and Site 3 is situated in an area with moderate human influence. These sites were selected to represent different levels of anthropogenic impact on the wetland ecosystem. The latitude and longitude coordinates for the sites are as follows: Site 1 is at 8°10'47.8"N, 77°22'23.7"E, Site 2 is at 8°10'48.6"N, 77°22'42.9"E, and Site 3 is at 8°11'02.5"N, 77°22'39.8"E.

Water samples (1 litre each) were collected in pre-cleaned plastic containers for the analysis of environmental factors. On-site measurements were taken for parameters such as water temperature, surface temperature, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), and pH using a multi-parameter digital meter. The samples were then transported to the laboratory, where they underwent further analysis for chlorides, phosphates, water hardness, total alkalinity, ammonia, calcium, nitrate, nitrite, and magnesium, following the methods outlined by the American Public Health Association (APHA, 2005).

Macroinvertebrate Sampling

Aquatic macroinvertebrate samples were collected using a multi-habitat sampling technique as recommended by the United States Environmental Protection Agency (US-EPA) (Rico-Sánchez et al., 2021). Sampling was carried out for 5 minutes at each site using a 500 µm mesh kick net. Specimens were preserved in 70% ethanol for laboratory identification, following the methods described by Andrew et al. (2008) and Thorp et al. (2009).

Diversity Indices

The diversity and community composition of macroinvertebrates were assessed using diversity indices, including Taxa

Richness, Shannon Diversity Index, Simpson Diversity Index, Evenness Index, Menhinick's Richness Index, Margalef Diversity Index, and Berger-Parker Dominance Index.

Statistical Analysis

Physicochemical parameters and taxonomic richness data were analysed to assess site-wise relationships. Cluster analysis was employed to assess the similarity among sites based on environmental factors. At the same time, Principal Component Analysis (PCA) was applied to reduce the dataset's dimensionality, facilitating a more straightforward interpretation of macroinvertebrate diversity and environmental parameters. Multivariate relationship analysis and correlation analysis were performed using PAST software (Shrestha & Kazama, 2007).

Results and Discussion

Physicochemical Parameters of the Water

The physicochemical parameters of the water from Vembannur Wetland were measured across three sampling sites. Water temperature at site 1 is 26.40 °C, at site 2, 270 °C, and at site 3, 26.70 °C. The pH values remained consistent across all sites, with slight variations ranging from 7.1 to 7.2. Electrical conductivity exhibited minor fluctuations at sites 290µS/cm, 274µS/cm and 278 µS/cm, respectively. Total dissolved solids (TDS) were highest at Site 1 (174 mg/L) and recorded the same values at Sites 2 and 3 (137 mg/L). The chi-square test revealed no significant differences in the physicochemical parameters in sites ($p>0.05$).

Total hardness showed a minor difference between sites, with Sites 1 and 2 measuring 75 mg/L and Site 3 showing a slightly higher value at 100 mg/L. Other parameters, such as depth, Total alkalinity, calcium, magnesium, chloride, phosphate, nitrate, nitrite, and ammonia, were consistent across all three sites, showing no notable spatial variation. Depth was constant at 7 meters across all sites, while total alkalinity, calcium, and magnesium were recorded at 100 mg/L, 50 mg/L, and 75 mg/L, respectively. Chloride levels were uniform at 35.46 mg/L, phosphate at 0.612 mg /L, nitrate at 0.2744 mg /L, nitrite at 25 mg /L, and Ammonia at 2.44 mg /L.

Principal Component Analysis

Principal Component Analysis (PCA) revealed a clear spatial separation of sites based on factors of electrical conductivity, depth, total dissolved solids, total hardness, total alkalinity, calcium, magnesium, chloride, phosphate, nitrate, nitrite and ammonia (Figure 2). The total variance explained by PCA analysis is 80%. The analysis also revealed a significant relationship ($p < 0.05$) in the physicochemical parameters across the sites, highlighting the influence of these factors on the environmental dynamics of the Vembannur wetland.

Phylogenetic Tree

The phylogenetic tree (dendrogram) represents the hierarchical clustering of the study sites (site 1, site 2, and site 3) based on their physicochemical parameters using Ward's linkage method, which minimises variance within clusters (Figure 3). The tree reveals that sites 2 and 3 are closely clustered, indicating a high degree of similarity in their physicochemical characteristics, while site 1 stands apart, reflecting distinct environmental conditions. Parameters such as aerial temperature, total dissolved solids, and electrical conductivity contribute significantly to this differentiation, with total hardness playing a key role in the clustering of Site 3. This clustering highlights spatial variations in water quality across the Vembannur Wetland, with site 1 potentially being influenced by unique natural or anthropogenic factors. Such analyses are essential for identifying patterns in water quality and prioritising targeted conservation and management strategies for these wetland ecosystems.

Taxonomic Composition of Macroinvertebrates

The taxonomic composition of aquatic macroinvertebrates across the three sample sites in Vembannur Wetland is presented in Table 2. A total of 237 individuals were recorded, representing 3 classes, 8 orders, 11 families, and 15 taxa. Among these, gastropods were dominant, particularly within the family Viviparidae, which were abundant in all environmental conditions. These organisms thrive on organic debris, contributing to the wetland's ecosystem function (Madomguia et al., 2016). Site 1, which features sparse trees and minimal vegetation, had a relatively lower abundance, while site 2, with more intact vegetation, exhibited greater biodiversity and better water quality. Site 3, which had minimal vegetation and a small stream, also showed moderate biodiversity but indicated fair environmental conditions. Several key macroinvertebrate groups were recorded, including gastropods, malacostraca, and insecta. Mollusca showed notable diversity with four taxa present, and decapods were also observed in the wetland. Orders such as Ephemeroptera, Hemiptera, Odonata, and Diptera were well-represented. Meanwhile, orders such as Plecoptera, Tricoptera, and Oligochaeta were less dominant but are crucial for understanding macroinvertebrate diversity in the wetland (Fındık & Aras, 2024).

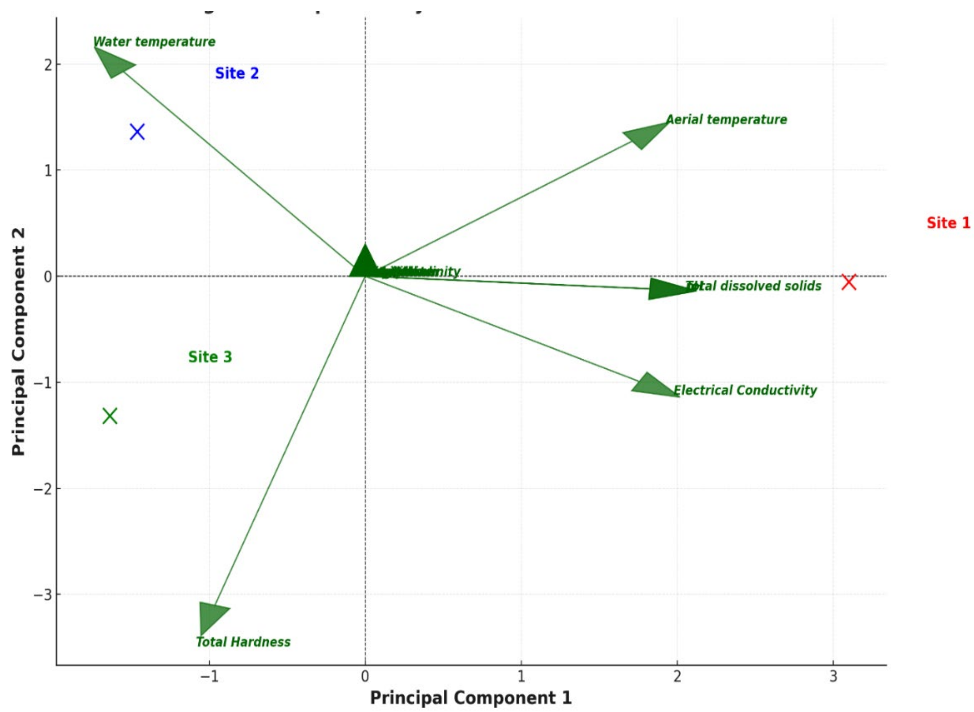

Figure 2. Principal Component Analysis showing the differentiation among sampling sites and their relationships based on physicochemical data

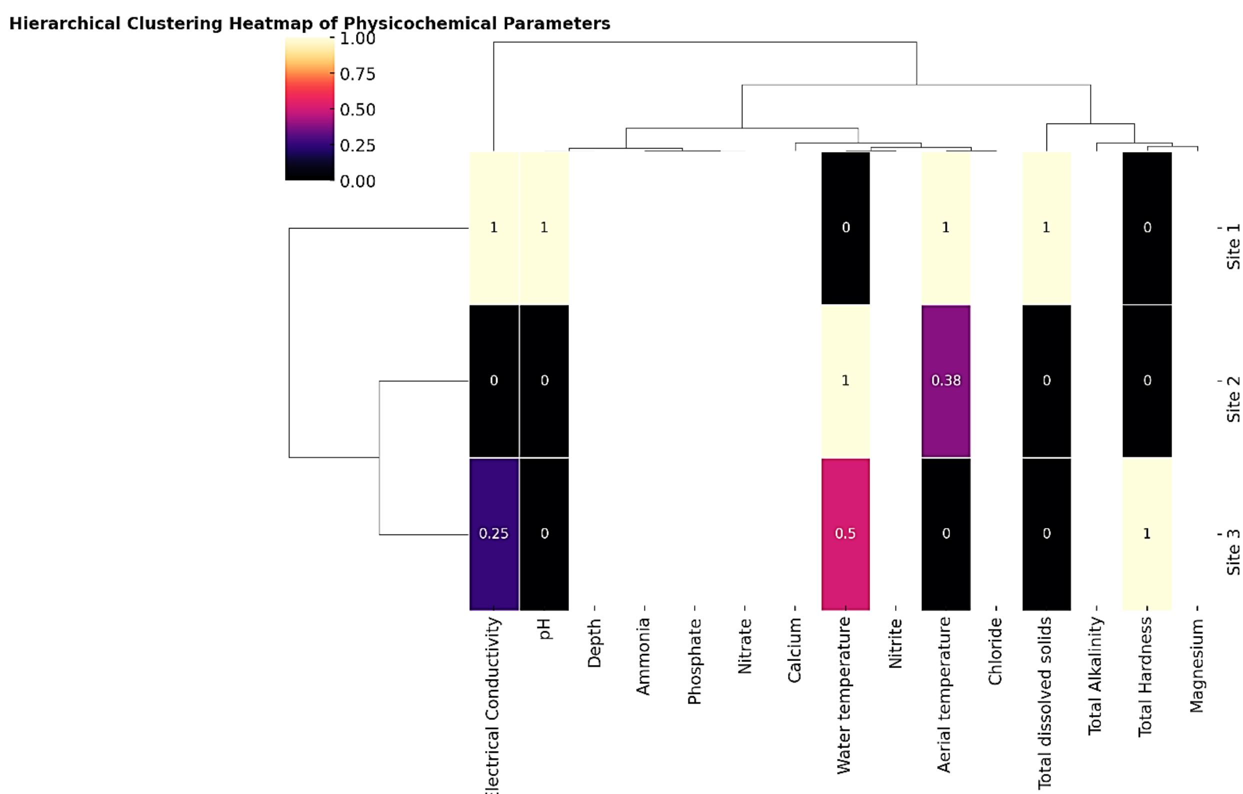

Figure 3. Phylogenetic tree constructed using physicochemical data, illustrating the similarity among the sampling sites

Diversity Indices

The diversity indices calculated for the macroinvertebrate communities show the diversity in comparing two site 2, with a Shannon-Wiener index (H) of 2.069 and Simpson's dominance index (1-D) of 0.8567, is showing balance diversity evenness was also moderate at this site (e^H/S = 0.7438), indicating a more even distribution of species. In contrast, Site 1 showed the lowest status, with an H value of 1.806 and a dominance index of 0.7917. Site 3 had the lowest status diversity (H = 1.905; D-1 = 0.8172), with the evenness index suggesting better species distribution (Table 3).

All three sites show a decreasing trend in diversity as the correlation of macroinvertebrates increases. Site 1 exhibits the steepest decline, suggesting a more sensitive decrease in diversity with increased correlation. Site 2 follows, with a more gradual decline, while Site 3 experiences the least significant drop in diversity. This suggests that as the correlation between macroinvertebrates strengthens, the overall diversity tends to decrease, but the rate of change varies across the sites (Figure 4). The data indicate that site 2 is unpolluted, while sites 1 and 3 exhibit moderate pollution. The proximity of these latter sites to agricultural land suggests that runoff during rain events may introduce pollutants, affecting water quality and biodiversity.

This study examined Physical-chemical characteristics such as temperature, DO, and pH, which varied significantly across the three sites. Site 2 exhibited a neutral pH (7.1), which contributed to greater macroinvertebrate diversity. In contrast, sites with higher TDS levels (Site 1 at 174 mg/L and Sites 2 and 3 at 134 mg/L) supported more diverse communities good TDS level in acceptable in drinking and insect survival in TDS level is 300 above this danger for insect survival, and this good condition as well as sites biological normal condition (Jiang et al., 2010; Zeybek et al., 2012). Higher temperatures (26.4–27°C) also correlated positively with macroinvertebrate abundance, suggesting that these conditions are conducive to their survival (Hauer & Hill, 2007). Identifying 237 individuals across 15 taxa of macroinvertebrates, indicating moderate biodiversity in Vembannur wetland (Andem et al., 2013). The presence of various taxa suggests that human activity, birds, and aquatic insects have a significant impact on diversity and abundance. Although the wetlands are moderately polluted, the community structure and functional diversity of the macroinvertebrates remain relatively intact. Hydrological factors such as water depth and habitat structure play a central role in influencing the distribution of insects. Chironomidae, for example, were found to be indicators of organic pollution, corroborating findings from previous studies (Basu et al., 2013; Koumba et al., 2017). The important elements of a biological community are measured by the community structure index, richness, and evenness, according to Barbour & Michael (1999), as well as the availability of equal to habit to diversity indices. A diversity index site 2 and 3 are good, and site one is polluted in water, as described in the diversity index. A comparison of diversity between site 1, site 3, and site 2 reveals moderate pollution in the water sources. The presence of pollution-sensitive taxa, such as Ephemeroptera, at specific sites further supports this conclusion. The population structure is primarily influenced by water depth, habitat type, and wetland features, as well as comparisons of water quality and aquatic macroinvertebrates, indicating good and moderate richness and abundance.

Table 2. Taxonomic composition of aquatic macroinvertebrates at the sampling sites

Classes	Orders	Families	Taxons	S1	S2	S3
Gastropoda	Architaenioglossa	Viviparidae	*Idiopoma* sp.	-	+	++
			Viviparus sp.	++	+	-
	Basommatophora	Planorbidae	*Gryalus* sp.	-	+	+
			Indoplanorbis sp.	+	+	++
Malacostraca	Decapoda	Palaemonidae	*Palaemonetes* sp.	-	++	-
		Penaeidae	*Litopenaeus* sp.	-	+	-
Insecta	Ephemeroptera	Tricorythidae	*Madecassorythus* sp.	+	-	++
	Hemiptera	Belostomatidae	*Diplonychus* sp.	+	-	++
	Odonata	Gomphidae	*Gomphidia* sp.	-	+	-
		Libellulidae	*Brachymesia* sp.	+	+	-
		Coenagrionidae	*Ischura* sp.	-	++	-
			Argia sp.	+	++	+
	Diptera	Chironomidae	*Chironomus* sp.	+	-	-
			Parochlus sp.	-	-	+
		Culicidae	*Culex* sp.	+	++	+

Table 3. Diversity index of aquatic macroinvertebrates in Vembannur Wetland

Index	S1	S2	S3
Taxa	8	10	8
Shannon_ H	0.753	0.875	0.811
Simpson_ D	0.208	0.143	0.183
Evenness e^H/S	0.7114	0.7438	0.8397
Menhinick's	1.099	1.054	0.8251
Margalef's	1.763	2	1.541
Berger-Parker	0.3774	0.2333	0.3511

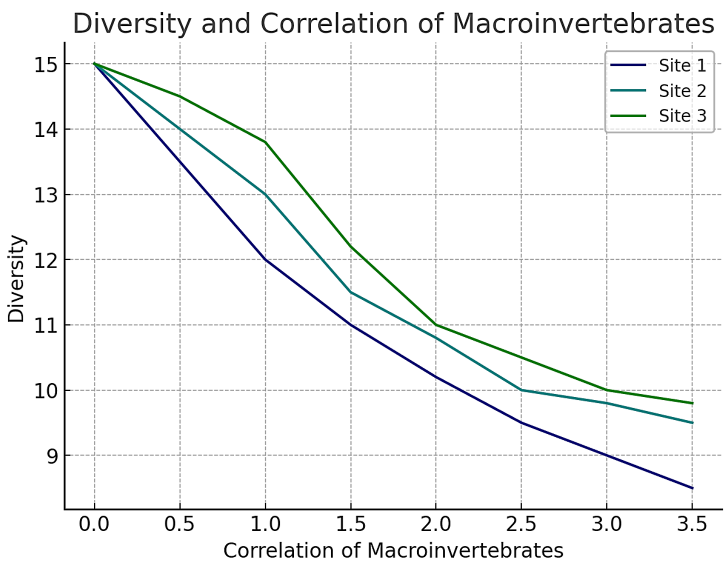

Figure 4. Correlation between macroinvertebrate diversity in Vembannur Wetland

Conclusion

This study highlights the significance of macroinvertebrates as bioindicators for assessing the health of wetlands and water quality. By documenting diversity indices and conducting water quality assessments, it becomes evident that sites 2 and 3 are relatively unpolluted, while Site 1 exhibits moderate pollution due to agricultural runoff. Macroinvertebrate assemblages offer valuable insights into the ecological condition of wetlands, and future research should explore their potential for long-term ecosystem monitoring.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: Data will be made available on request from the author.

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Investigation on marine *Staphylococcus* spp. isolated from the Sinop coastal areas, the Black Sea in Türkiye serves as a reservoir for antibiotic resistance genes

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ABSTRACT

Epidemiological surveillance of the *Staphylococci* genus, which harbours strains with high morbidity and mortality rates, is a crucial step in detecting and preventing diseases and disease agents. This study aimed to isolate, characterise, and screen some antibiotic resistance genes of possible *Staphylococcus* spp. strains from seawater samples taken from three points in Sinop, which is suitable for swimming from almost every point. Classical microbiological techniques were used for the isolation and possible identification of the strains. A fragment of the 16S rRNA gene region (216 bp) was amplified and analysed by the SSCP technique to determine their diversity among themselves. For antibiotic resistance genes, both classical PCR and multiplex PCR techniques were used. As a result, 29 probable *Staphylococcus* spp. strains were isolated, and according to SSCP analysis, it was determined that the strains had a similarity rate of 50% or more among themselves and within the scope of different stations. In addition, *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM*, and *blaZ* resistance genes of the strains were observed as 8 (27.5%), 3 (10.3%), 2 (6.8%), 2 (6.8%), 14 (48.2%), 27 (93.1%) and 29 (100%), respectively. Furthermore, *mecA* was positively correlated with *ermB* and *ermB* was positively correlated with *ermC* at the $p < 0.05$ significance level. In comparison, *ermB* was negatively correlated with t*etM* at the $p < 0.05$ significance level. In conclusion, the presence of *Staphylococcus* spp. strains, which are reservoirs of antibiotic resistance genes and have the potential to transfer these genes to other bacteria through gene transfer, have been shown in this study to be prevalent in marine environments, where they can be easily transmitted. The importance of taking precautions has been emphasised.

Keywords: *Staphylococcus* spp., SSCP, *mecA*, Seawater, Antibiotic resistance genes

Introduction

Due to the mortality and financial losses caused by infections, antimicrobial resistance (AMR) is becoming a significant issue in communities. An analysis conducted in 2019 estimated that antibiotic resistance resulted in the deaths of 1.27 million individuals in Europe alone (Brauge et al., 2024). The prevalence of such infections is a significant concern associated with this condition. Marine water has been reported to be one of the most harmful settings for them. Millions of cutaneous, acute respiratory, and gastrointestinal disorders are expected to occur yearly as a result of microbial pollution in marine water habitats (Goodwin et al., 2012). Due to their high mortality rates and multidrug resistance (MDR), the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) have been identified as the most significant risk factors for these illnesses. The fact that the ESKAPE group demonstrates diverse antibiotic resistance and transfers its resistance genes to other bacteria through horizontal gene transfer is one of the most critical considerations (Ackers-Johnson et al., 2024).

A range of Staphylococcus species currently produces serious infections. Humans are hosts to 17 of the 47 species and 24 subspecies that make up the genus *Staphylococcus*. The three that are most hazardous to humans are *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Thirty percent of nosocomial infections and 1-5% of community infections have been ascribed to *S. aureus* (Alabbosh et al., 2023).

The emergence of antibiotic-resistant strains of *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), is a global concern and cause for concern when this ESKAPE category is evaluated. The World Health Organisation's (WHO) categorisation of MRSA as a high-priority disease draws increased attention due to its resistance to numerous medications (Cui et al., 2024). According to a past study on *S. aureus* in coastal locations, swimmers can contract the bacterium from saltwater by shedding it through their skin, nose, and respiratory system. *S. aureus* has occasionally been identified in high quantities in both the water and the sand on recreational beaches; this can be directly linked to the number of swimmers and the quantity of people utilising the beach (Akanbi et al., 2017).

Research has been used to track and document the emergence of antibiotic resistance in *S. aureus* strains throughout time. These comprise multidrug-resistant *S. aureus* (resistant to penicillin-G, chloramphenicol, tetracycline, and erythromycin) in the late 1950s and penicillin-G-resistant *S. aureus* that developed penicillinase (penicillin-hydrolysis enzyme) in the mid-1940s. Methicillin, a β-lactam antibiotic that works against penicillin-resistant *S. aureus*, was initially sold in the 1960s, but strains of MRSA were found a year later. All β-lactams were unsuccessful against this MRSA (Contractor et al., 2024).

Methicillin resistance is imparted by the production of penicillin-binding protein 2a (PBP2a), which is carried by *mecA* on staphylococcal cassette chromosome *mec* (SCC*mec*). This mobile genetic element carries cassette chromosome recombinase genes. Even though SCC*mec* transfer is unusual in *S. aureus*, methicillin-sensitive *S. aureus* (MSSA) strains convert into MRSA after getting *mecA* embedded in SCC*mec*; all MRSA clones seem to include specific kinds of SCC*mec* (Takahashi et al., 2024; Vittorakis et al., 2024; Wan et al., 2025). In addition to *mecA*, resistance genes against tetracyclines and aminoglycosides can also be transferred in the SCC*mec* cassette, one of the 14 different cassettes found in staphylococci. Tetracycline decreases the creation of proteins by interacting with 16S rRNA genes. The *tetK* and *tetL* genes encode efflux pumps that actively remove tetracyclines from the cells, which is the primary mechanism of bacterial resistance to these antibiotics. The alternate technique utilizes ribosome protection proteins, which are generated by the *tetO* and *tetM* genes. These proteins either break down tetracyclines or inhibit them from binding by modifying the ribosome's structure (Szemraj et al., 2025). MRSA strains can demonstrate multidrug resistance, or resistance to antibiotics from various classes, including aminoglycosides, macrolides, tetracyclines, and fluoroquinolones, in addition to their innate resistance to β-lactam antibiotics (Michalik et al., 2025).

S. aureus has acquired diverse resistance mechanisms to β-lactam antibiotics, primarily associated with the *blaZ* and *mecA* genes. *mecA* is not effectively inhibited by β-lactams and permits bacterial cell wall cross-linking even in the presence of antibiotics. The *blaZ* gene produces the β-lactamase enzyme, which breaks down susceptible β-lactam antibiotics (Hnini et al., 2024). *S. aureus* can also have resistance genes such as the erythromycin resistance genes *erm*(B) and *erm*(C), the chloramphenicol resistance gene *cat*, and the lincomycin resistance gene *lnu*(A), in addition to the ones mentioned above (Cui et al., 2024).

This work aimed to characterise *Staphylococcus* spp. strains isolated from seawater samples in Sinop, Turkey, and to screen them for the antibiotic resistance genes *mecA*, *blaZ*, *tetK*, *tetM*, *ermA*, *ermB*, and *ermC*.

Materials and Methods

Collection of Samples

Water samples were obtained under aseptic conditions on June 4, June 10, June 26, July 9, and July 16, 2024, from Karakum (coordinates: 42°00'56"N 35°11'07"E), Taşocağı (coordinates: 42°01'03"N 35°10'10" E), and Kumkapı (coordinates: 42°01'33"N 35°08'21" E) in the center of Sinop, Turkey (Figure 1). Seawater samples were obtained in 500 mL sterile bottles from a few millimetres beneath the surface, ensuring no exposure to air, and transported to the laboratory in a cold chain bag within two hours for immediate examination.

Isolation of Stapylococcus spp. *Strains*

Fifteen different water samples were inoculated on Mannitol Salt Agar (MSA, Merck, Germany) and incubated at 30°C for 24 hours immediately after being brought to the laboratory to isolate possible *Staphylococcus* strains. At the end of the period, yellow-colored colonies were selected and grown in Nutrient Broth (Merck), and pure cultures were prepared (Faria et al., 2009). Isolates selected as single colonies were stored at -80°C in 20% glycerol stock. Gram staining, catalase, and oxidase tests were performed for the identification of probable *Staphylococcus* strains and further tests were performed with 29 strains selected according to these tests.

Genomic DNA isolation and 16S rRNA SSCP analysis

Genomic DNA isolation of the 29 strains was performed according to Sambrook et al. (1989) and stored at -20°C until analysis.

For single-stranded conformation polymorphism (SSCP) analysis performed with primers P11P and P13P (Table 1) selected to amplify the 216 bp fragment expressed as V6 in the 16S rRNA gene region, the method was as described in a previous study conducted in the same laboratory (Avsar et al., 2017). Briefly, the amplification conditions for SSCP included an initial denaturation at 95°C for 4 minutes. This was followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes. Two microliters of PCR product were added to 10 µL of denaturation solution (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanole and 10 mM NaOH). The mixture was heated to 95°C for 5 minutes and then immediately cooled on ice. The mixture was analysed by non-denaturing polyacrylamide gel electrophoresis (39:1 acrylamide: bisacrylamide) in 0.5 x TBE (16 x 18 cm) for 27 h at 18°C at a constant power of 5 mA on a Hoefer electrophoresis system (Hoefer Inc., Holliston, MA, USA). The gel was silver nitrate-stained and photographed. After that, a dendrogram based on UPGMA was produced using GelJ (Heras et al., 2015).

Figure 1. Map of the stations where samples were taken (Google map 2025). Karakum, Taşocağı, and Kumkapı stations are the places where people swim during the sampling period

Table 1. All primer sequences and sizes used in the study

Gene		The primer sequences (5'- 3')	Size of amplified product (bp)
P11P	Forward	**GAGGAAGGTGGGGATGACGT**	**216**
P13P	Revers	**AGGCCCGGGAACGTATTCAC**	
mecA	Forward	**AAAATCGATGGTAAAGGTTGGC**	**533**
	Revers	**AGTTCTGCAGTACCGGATTTGC**	
ermA	Forward	**AAGCGGTAAACCCCTCTGA**	**190**
	Revers	**TTCGCAAATCCCTTCTCAAC**	
ermB	Forward	**CTATCTGATTGTTGAAGAAGGATT**	**142**
	Revers	**GTTTACTCTTGGTTTAGGATGAAA**	
ermC	Forward	**AATCGTCAATTCCTGCATGT**	**299**
	Revers	**TAATCGTGGAATACGGGTTTG**	
tetK	Forward	**GTAGCGACAATAGGTAATAGT**	**360**
	Revers	**GTAGTGACAATAAACCTCCTA**	
tetM	Forward	**AGTGGAGCGATTACAGAA**	**158**
	Revers	**CATATGTCCTGGCGTGTCTA**	
blaZ	Forward	**ACTTCAACACCTGCTGCTTTC**	**173**
	Revers	**TGACCACTTTTATCAGCAACC**	

Detection of Antibiotic Resistance Genes

The primer base sequences of *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ*, preferred to detect antibiotic resistance genes, are given in Table 1.

Amplification of the *mecA* gene was performed using *mecA*-F and *mecA*-R primers, and a PCR product of 533 bp was obtained. PCR was performed in a volume of 25 µL with PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 µL 10 µM dNTPs, 0.4 µL Taq polymerase (DreamTaq) and 0.125 µM concentration of each primer. For amplification, initial denaturation was performed using 40 cycles of amplification at 94°C for 3 min, 94°C for 30 s, 55°C for 30 s and 72°C for 30 s; this reaction was followed by an additional extension of 5 min at 72°C (Lee, 2003). Sequences of other primers preferred for antibiotic resistance were taken from Duran et al. (2012). In addition, multiplex PCR was performed using tet and erm (annealing was performed at 55 °C for the multiplex PCR) primer sets, except for blaZ (annealing was performed at 54°C for the PCR) in the screening of these gene regions (Duran et al., 2012).

Statistical Analyses

The Pearson correlation was utilised to examine the association between antibiotic resistance genes using Past 4.03 statistical software; *p*-values lower than 0.05 were considered statistically significant.

Results and Discussion

According to Gram staining and catalase and oxidase tests, a total of 29 putative *Staphylococcus* strains were identified. Cocci morphology (more like grape clusters) is known to be specific for these bacteria, which makes their differentiation by classical microbiological tests easier. Out of the 29 strains among these strains, 11 were isolated from Karakum, 10 from Taşocağı, and 8 from Kumkapı (Table 2).

A 216 bp fragment of 16S rRNA was analysed using SSCP to assess the similarity among 29 isolated *Staphylococcus* spp. Bacteria, resulting in the construction of a UPGMA dendrogram (Figure 2). Consequently, it was established that the strains exhibited 50% or greater similarity to one another. Upon evaluation of the strains isolated from three distinct stations, it was ascertained that they exhibited diversity and were categorised into various clusters. Although techniques such as SSCP are still employed for the identification and diversity of potentially harmful bacterial groups such as *Staphylococcus*, faster methods have been studied in recent years. Zhao et al. (2024) indicated that the HiFi-loop-mediated isothermal amplification (LAMP) method created for expedited identification of MRSA and MSSA strains was exceptionally effective. In another study, Zhang et al. (2025) achieved an ultra-simple self-assembly of gold nanoparticles (AuNPs) using Nisin. They successfully applied lateral flow immunoassay

(LFIA) by utilising Nisin as a recognition element in combination with a smartphone to construct a dual-read detection sensor for the rapid detection of MRSA. In another work, Yang et al. (2025) developed an AI-driven colour-coded multiplex hydrogel LAMP approach that has promising possibilities for the digital quantification of antibiotic-resistant bacteria (such as *Escherichia coli* and MRSA) in the food market. In another investigation, Li et al. (2025) used the SELEX (the Systematic Evolution of Ligands by Exponential Enrichment) approach for optical detection of *S. aureus* in fewer than 9 hours. Here, they completed a specific identification based on a magnetic bead target enrichment and rolling circle amplification approach to enhance sensitivity, utilising a dual aptamer combined recognition target strategy. These approaches highlight only a few of them, clearly indicating that the timely diagnosis of *Staphylococcus* species, especially in food and environmental samples, is vital for preventing possible infections.

The antibiotic resistance genes of the 29 tested *Staphylococcus* spp. strains were screened and displayed in Table 2 and Figure 3A-B. Accordingly, *mecA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ* resistance genes were found as 8 (27.5%), 3 (10.3%), 2 (6.8%), 2 (6.8%), 14 (48.2%), 27 (93.1%) and 29 (100%), respectively. It is notable that *mecA*, an indicator of methicillin-resistant *Staphylococcus* strains, was found in 8 strains from three distinct stations. In addition, it is interesting that all strains possessed the *blaZ* resistance gene and the *tetM* resistance gene was identified in all but two of them.

In the context of this work, although we have focused on separating Staphylococcus strains from seawater, we can observe that this group of bacteria is widespread and strongly represented. The importance of this group becomes clear when considering the studies completed in the last few years alone. Some of these studies are as follows: Oshamika et al. (2024) examined antibiograms and harmful genes of *S. aureus* in asthmatic children. Ackers-Johnson et al. (2024) isolated *Staphylococcus* spp. from door knobs and push panels from a hospital in Liverpool and looked at their antibiotic resistance. Agredo-Campos et al. (2025) isolated *S. aureus* from milk tanks and studied their characterisation, antibiograms, pathogenicity and antibiotic resistance. Desire et al. (2024) isolated MRSA from smoked fish and analysed the behavioural and genotypic characterisation of the *mecA* genes. Dewi et al. (2024) studied the antibiograms, *mecA* and PVL gene presence of multidrug-resistant *Staphylococcus* isolated from an international airport. Olivo et al. (2024) isolated methicillin-resistant *Staphylococcus* spp. from individuals in contact with sick horses at a veterinary hospital and investigated their characteristics and the presence of the *mecA* gene. These research studies, conducted in various fields over the last one to two years, highlight the importance and prevalence of the genus *Staphylococcus*.

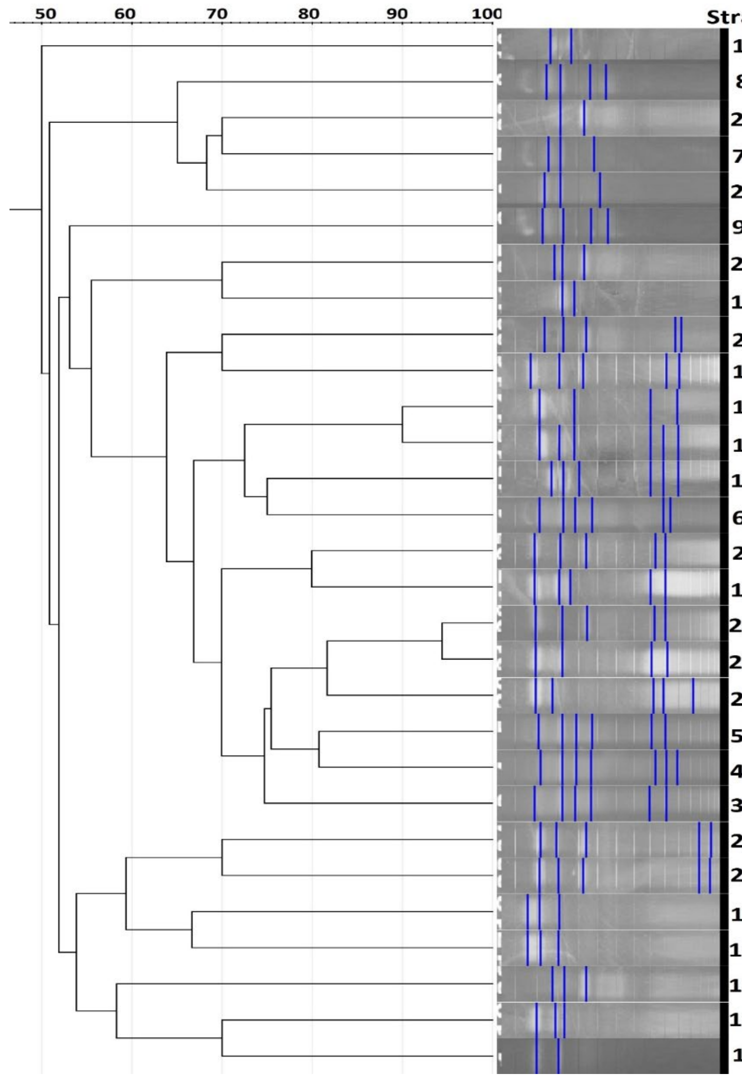

Figure 2. Phylogenetic tree constructed based on UPGMA analysis

In addition to these studies, there have been many studies in which *Staphylococcus* species have been isolated from seawater samples, characterised, and their resistance to antibiotics and resistance genes, including *mecA*, have been investigated (Soge et al., 2006; Harekeh et al., 2006; Goodwin et al., 2012; Akanbi et al., 2017; Gerken et al., 2021; Brauge et al., 2024). The fact that our work is in many ways comparable to previous studies on staphylococci isolated from seawater demonstrates the inevitability of protection against possibly pathogenic *Staphylococcus* for seawater.

In addition, the Pearson's correlation between different antibiotic resistance genes was studied and displayed in Figure 4.

Accordingly, the *mecA* had a significant impact on the *erm* resistance genes, especially with the *ermB* at the $p < 0.05$ level. On the other hand, the *mecA* demonstrated a very modest level of negative connection with both the *tet* resistance genes. In addition, the *ermB* was favorably linked with the *ermC* (+ 0.46) and negatively connected with the *tetM* (- 0.46) at a $p < 0.05$ significance level. Alkuraythi et al. (2024) evaluated the correlation coefficient between *mecA*, *tet* and *ermC* resistance genes in *S. aureus* strains and found that their results were parallel to our findings. In another work, Fontana et al. (2021) examined the correlation between *ermA*, *B*, *C* and *tetK* resistance genes in coagulase-negative *Staphylococcus* isolates obtained by them. While the connection between *ermA*, *ermB*, and *tetK* was the same as our findings, a difference was observed in the correlation between *ermC* and *tetK*, albeit extremely modest.

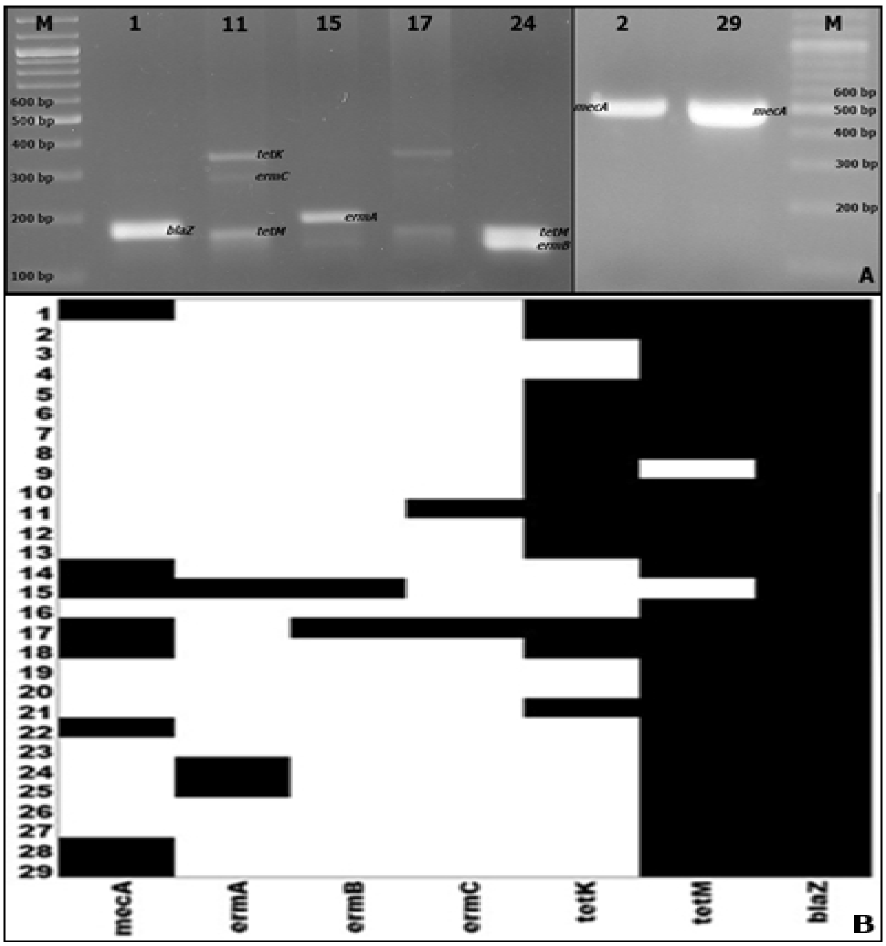

Figure 3. Antibiotic resistance genes screening results; (A) Agarose gel images of tested antibiotic resistance genes. M (Marker - 1000 bp GeneRuler); *blaZ* (173 bp) for strain 1; *tetK* (360 bp), *ermC* (299 bp) and *tetM* (158 bp) for strain 11; *ermA* (190 bp) for strain 15; *blaZ* and *tetM* for strain 17; *tetM* and *ermB* (142 bp) for strain 24; *mecA* (533 bp) for strains 2 and 29. (B) Results for all the strains show that the black part opposite the gene is positive for the strain, while the white part is negative. This type of graph was generated from the Past 4.03 program

Table 2. Stations where strains were isolated and the antibiotic resistance gene screening results

Strain	Location	*mecA*	*ermA*	*ermB*	*ermC*	*tetK*	*tetM*	*blaZ*
1	Karakum	+	-	-	-	+	+	+
2	Karakum	-	-	-	-	+	+	+
3	Karakum	-	-	-	-	-	+	+
4	Karakum	-	-	-	-	-	+	+
5	Karakum	-	-	-	-	+	+	+
6	Karakum	-	-	-	-	+	+	+
7	Karakum	-	-	-	-	+	+	+
8	Karakum	-	-	-	-	+	+	+
9	Karakum	-	-	-	-	+	-	+
10	Karakum	-	-	-	-	+	+	+
11	Karakum	-	-	-	+	+	+	+
12	Taşocağı	-	-	-	-	+	+	+
13	Taşocağı	-	-	-	-	+	+	+
14	Taşocağı	+	-	-	-	-	+	+
15	Taşocağı	+	+	+	-	-	-	+
16	Taşocağı	-	-	-	-	-	+	+
17	Taşocağı	+	-	+	+	+	+	+
18	Taşocağı	+	-	-	-	+	+	+
19	Taşocağı	-	-	-	-	-	+	+
20	Taşocağı	-	-	-	-	-	+	+
21	Taşocağı	-	-	-	-	+	+	+
22	Kumkapı	+	-	-	-	-	+	+
23	Kumkapı	-	-	-	-	-	+	+
24	Kumkapı	-	+	-	-	-	+	+
25	Kumkapı	-	+	-	-	-	+	+
26	Kumkapı	-	-	-	-	-	+	+
27	Kumkapı	-	-	-	-	-	+	+
28	Kumkapı	+	-	-	-	-	+	+
29	Kumkapı	+	-	-	-	-	+	+

(+) positive result; (−) negative result

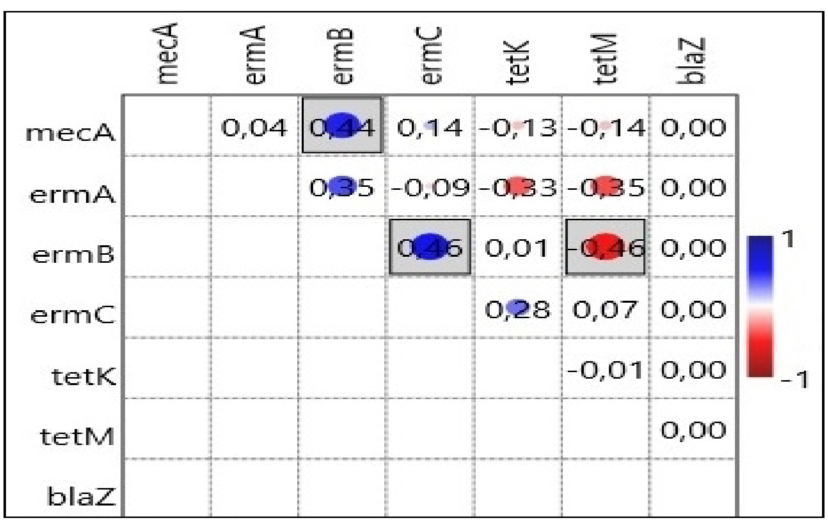

Figure 4. Pearson correlation results between antibiotic resistance genes. Boxes indicate $p < 0.05$ significance value.

Conclusion

Within the framework of this investigation, *Staphylococcus* spp. strains were isolated from samples collected at three different swimming locations in Sinop Province during the period when people typically swim in the sea, and their diversity was examined. In addition, specific antibiotic resistance genes were screened, and some strains were identified as harbouring various antibiotic resistance genes, primarily *mecA* (27.5%). It is significant because they are both reserved for resistance genes and are likely to convey these genes, possibly by horizontal gene transfer. The presence of such bacterial groups in the seas, which are employed primarily for leisure purposes and most significantly for fishing, should lead to severe measures. Otherwise, the impact of the transmission of the genus *Staphylococcus*, which has a high morbidity and mortality rate among certain of its species, which are frequently recorded in the literature, through seawater may be catastrophic.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: Data will be made available on request from the author.

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Disclosure: -

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Evaluation of the anti-*Acanthamoeba* potential of *Myriophyllum spicatum* on *Acanthamoeba castellanii* trophozoites

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ABSTRACT

Myriophyllum spicatum is a submerged aquatic macrophyte known for its allelopathic and antimicrobial properties. This study investigated the amoebicidal activity of ethanolic *M. spicatum* leaf extract against *Acanthamoeba castellanii* trophozoites. Plant samples were collected from the Terme River (Samsun), extracted with ethanol, and tested at concentrations ranging from 1.5 to 48 µg/mL over 24, 48, and 72 hours. Trophozoite viability was assessed using trypan blue staining, and statistical analysis was performed with SPSS and Jamovi software. Results showed significant dose- and time-dependent reductions in trophozoite viability, with an IC50 value of 26.5 µg/mL at 72 hours. The highest inhibition was observed at 48 µg/mL, resulting in a 33% reduction in viability. Principal Component Analysis revealed distinct clustering of higher concentrations from lower doses and control. The study highlights *M. spicatum*'s potential as a natural anti-amoebic agent. Given the parasite's resistance to conventional drugs and the increasing threat of *Acanthamoeba* infections, particularly in developing regions, plant-based compounds offer promising alternatives. Further research is recommended to evaluate the extract's effects on the cyst stage and explore its therapeutic applications. This study is the first to report the anti-Acanthamoeba effect of *M. spicatum*, contributing novel insight into aquatic plant-based antiparasitic agents.

Keywords: Eurasian water milfoil, *Myriophyllum spicatum, Acanthamoeba castellanii, Amoebicidal activity*

Introduction

Myriophyllum spicatum (Haloragaceae) is a cosmopolitan aquatic plant and one of the most species-rich genera among aquatic angiosperms. Approximately 68 species of this genus have been documented worldwide. In Türkiye, two species of this genus are known: *Myriophyllum spicatum* and *M. verticillatum*. Some species are considered invasive macrophytes because of their rapid vegetative growth and strong competitive ability (Taş et al., 2018; Liao et al., 2020). *M. spicatum* is the most common and heavily managed invasive aquatic plant due to concerns about its impact on native plant communities and human recreation (Hoff & Thum, 2022). In Türkiye, *M. spicatum* (Eurasian watermilfoil) is widely found in lentic and lotic shallow water ecosystems (rivers, lakes, wetlands, irrigation systems), and it is a cosmopolitan submerged macrophyte. Macrophytes play important roles in aquatic ecosystems, including absorbing nutrients and heavy metals, reducing suspended solid loads, providing clean and clear water environments, improving water quality, and creating habitats for aquatic animals (Taş & Topaldemir, 2021; Ustaoğlu et al., 2022). Additionally, macrophytes are also studied in ethnobotany (Topaldemir & Taş, 2024).

Submerged macrophytes not only have effective nutrient uptake capabilities but also possess unique allelopathic properties. Some *Myriophyllum* species (*M. aquaticum* (Vell.) Verdc., *M. spicatum*, *M. verticillatum*) release allelochemicals (Gross & Garric, 2019). Allelochemicals such as tellimagrandin II and gallic acid, isolated from *Myriophyllum* extracts, have been demonstrated to affect phytoplankton growth negatively (Leu et al., 2002), and *M. spicatum* is known to have a strong ability to suppress the growth of the globally known cyanobacterium *Microcystis aeruginosa*, which forms harmful blooms (He et al., 2016; Jeong et al., 2021; Jeong et al., 2024). Allelochemicals, which contain phenolic compounds, leach from plants into the water column, causing either synergistic or antagonistic effects on other organisms (Gross & Garric, 2019; Wang & Liu, 2023). However, only a small portion of the metabolites present in the plants are released into the environment (Gross & Bakker, 2012).

It has been reported that *M. spicatum* can significantly impact the formation and distribution of mysids and fish larvae in coastal ecosystems, resulting in substantial mortality among mysids (Lindén & Lehtiniemi, 2005). *M. aquaticum* also produces chemicals with allelopathic effects, which impact zooplankton. For example, depending on the source of the total phenols used, significant differences in survival and reproduction were observed in cultures of the rotifer Brachionus havanaensis Rousselet. Rotifers exposed to total phenols extracted from the plant at concentrations of 12.2 μg/g did not survive for more than a week (Viveros-Legorreta et al., 2022).

The amount of phenolic compounds in aquatic plants varies depending on the region and season in which the plants are collected. A study examining the in vitro antimicrobial effects of *M. spicatum* collected from different aquatic systems in Türkiye (Miliç River, Samsun, and Ulugöl Lake, Ordu) reported that the ethanolic extracts of the plant exhibited effects on test organisms. The antimicrobial effect of samples from the Miliç River was found to be more effective than that of samples from Ulugöl Lake (Ertürk et al., 2020). The literature review revealed no studies investigating the anti-*Acanthamoeba* properties of *M. spicatum*.

A. castellanii, a free-living amoeba species commonly found in water and soil, can cause severe human infections and exists in both trophozoite and cyst forms. Belonging to the Acanthamoebidae family, this species proliferates through mitosis, allowing it to spread widely (De Lacerda & Lira, 2021).

A. castellanii is a resilient organism that thrives in various environments. It can be found in swimming pools, drinking and utility water systems, sewage, hot springs, aquariums, ventilation systems, healthcare facilities, dental treatment areas, dialysis units, contact lens storage cases, disinfectants, cell cultures, particular plant species, and among vertebrates. Humans can become infected through several routes, including the nasal passages, throat, infected brain or lung tissues, skin wounds, and corneal tissue, particularly in patients with keratitis (Aykur & Dagci, 2023; Kaynak et al., 2024).

A. castellanii can enter the human body through the respiratory system, eyes, and skin, leading to a variety of severe conditions. One of the most common is *Acanthamoeba* keratitis (AK), which is frequently seen in contact lens users and can cause painful eye infections, potentially leading to blindness if left untreated. Less common but potentially fatal diseases caused by this amoeba include Granulomatous Amoebic Encephalitis (GAE) and Cutaneous Acanthamoebiasis (Ceniklioglu & Duzlu, 2022).

The drugs used to treat *Acanthamoeba* infections are generally ineffective, and long-term treatments are often poorly tolerated by patients. Additionally, the drugs have serious side effects, and the parasite's resistance to the medications complicates the treatment process. Furthermore, the cyst form of the parasite is resistant to both drugs and disinfectants, and its eukaryotic structure makes treatment more challenging. For these reasons, ongoing research aims to develop effective, well-tolerated, and non-cytotoxic drugs for both the trophozoite and cyst forms of *Acanthamoeba* (Fiori et al., 2006; Kaynak et al., 2019).

In this study, the effects of the ethanolic extract of *M. spicatum* collected from the Terme River (Samsun) on the cell viability of *Acanthamoeba castellanii* trophozoites were investigated. The effects of different concentrations of *M. spicatum* extract at various time intervals were determined, and the amoebicidal activity of the submerged macrophyte was evaluated.

Materials and Methods

Collection and Preparation of the Macrophytes Extract

Myriophyllum spicatum was collected from Terme Creek in Terme, Samsun, and brought to the laboratory in cold plastic containers. The macrophyte was weighed using a precision scale (100 g) and then ground with 250 mL of ethanol. The mixture was placed in a shaker and incubated at 100 rpm for 48 hours. After the incubation, the mixture was filtered through filter paper, and the ethanol was removed using an evaporator at 35°C. The resulting concentration of the extract was determined to be 48 µg/mL. Various concentrations of *M. spicatum* extract (1.5, 3, 6, 12, 24, and 48 µg/mL) were prepared by serial dilution with distilled water modified by Kaynak et al. (2019).

Culture of A. castellanii

A 1 mL suspension of *E. coli* (ATCC 25922) was prepared and added to the Ringer agar medium. Subsequently, 300 µL of *A. castellanii* (ATCC 30010) strain was added to the same medium and inoculated using a sterile pipette tip. After incubating the mixture at 26°C for 96 hours, the trophozoites were washed with Ringer Broth medium, collected, and assessed for both their count per mL and viability using a 0.4% trypan blue stain on a Thoma slide. The experiment began with an initial count of 2×10^6/mL trophozoites and 100% viability (Kaynak et al., 2019).

Determination of the Amoebicidal Activity Assays

Different concentrations of *M. spicatum* extracts (1.5, 3, 6, 12, 24, and 48 µg/mL) were placed in Eppendorf tubes, with 200 µL of each extract added to each tube. Following this, 200 µL of *A. castellanii* trophozoites was added to the corresponding Eppendorf tubes. A negative control (trophozoites + sterile distilled water) and a positive control (trophozoites + 0.05% Chlorhexidine gluconate) were also prepared. These tubes were incubated at 26°C for 24, 48, and 72 hours. At the end of each incubation period, 25 µL of 0.4% trypan blue was mixed with 25 µL of the parasite and *M. spicatum* extract solution and then incubated at room temperature for 5 minutes. Live and dead trophozoites were counted separately using a Thoma slide. The counts were performed three times (Kaynak et al., 2019).

Statistical Analysis

The data for changes observed at 24, 48, and 72 hours in test groups containing *M. spicatum* extracts were recorded using Microsoft Excel and IBM SPSS Statistics 27.0.1. The results are presented as a mean (Mean) ± standard deviation (SD). A 1% error margin was accepted in the comparison of "p" values (probability), and confidence intervals were set at 99% probability. The effectiveness of the extracts in terms of amoebicidal activity was assessed using post hoc multiple comparison analysis and Tukey pairwise comparisons in IBM SPSS Statistics 27.0.1. Logarithmic regression analysis was employed to determine the 50% inhibitory concentration (IC50) values for various *M. spicatum* extract concentrations that were lethal to trophozoites. These values were derived from logarithmic regression graphs. The percentage of cell death resulting from the amoebicidal effects of the *M. spicatum* extract concentrations at different time points was calculated in comparison to the control cells. Inhibitor concentrations producing 50% cell death were tested in three replicate experiments. Additionally, Principal Component Analysis (PCA) in Jamovi 2.4.11 was used to visualise explanatory variables and summarise the outcomes across all extract concentrations.

Results and Discussion

The viability of *Acanthamoeba* trophozoites was assessed during the experiment at 24, 48, and 72 hours at 26°C. Trophozoite growth was halted in *M. spicatum* leaf extract with an IC50/72h of 26.5 µg/mL (Table 1). More substantial inhibitory effects were observed at concentrations of 12, 24, and 48 µg/mL after 72 hours of exposure to the extract, showing significant activity against *Acanthamoeba* trophozoites. The *M. spicatum* extract exhibited lethal effects on most trophozoites at 48 and 24 µg/mL by the end of the 72 hours (Figure 1). The results were expressed as percentage inhibition with control cells (Figure 1). Among the different ethanolic *M. spicatum* extracts tested, the 1.5 µg/mL concentration exhibited the lowest anti-amoebic activity with a 72-hour IC50 value.

The results are presented as mean standard errors, as shown in Table 1.

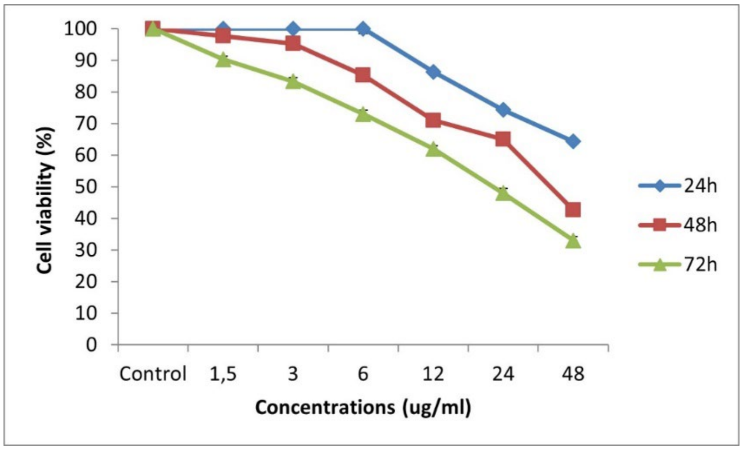

Figure 1. Amoebicidal effect of *M. spicatum* extract on *A. castellanii* trophozoites at different doses and time points

Tables 2 and 3 indicate which means are similar or different. Data are presented as mean ± SD. Statistically significant differences were observed between values marked with different letters, such as a and b. The statistical analysis revealed that the application time of the doses had a significant effect on viability, with $p<0.001$. Upon examining the impact of application times on viability, it was found that viability decreased as the exposure time increased. The highest viability was recorded after 24 hours, while the lowest viability was observed after 72 hours.

Furthermore, when the 48 µg/mL extract was compared at different time intervals, significant statistical differences in cell viability were observed between the 48-hour and 72-hour periods. Similarly, when the 24 µg/mL extract was compared across the three time points, significant differences in cell viability were observed between the 24-hour, 48-hour, and 72-hour measurements. For the 12 µg/mL concentration, comparisons across the three time intervals revealed statistically significant changes in cell viability at 24, 48, and 72 hours for all concentrations (Table 3).

To summarise the % viability data for all extract concentrations on A. castellanii trophozoites, Principal Component Analysis (PCA) was used to visualise the explanatory variables. The results revealed that the doses of 48, 24, and 12 µg/mL were negatively correlated with all other doses (1.5, 3, and 6 µg/mL), as well as the control group, with a clear separation on either side of the axis (Figure 2).

Aquatic macrophytes living underwater in natural rivers and stagnant waters are exposed to various abiotic stress factors, including solar radiation, temperature fluctuations, and mechanical stress caused by water flow. *M. spicatum* is an underwater plant that lives completely submerged, showing rapid growth and strong environmental adaptability. Due to its allelopathic effects, *M. spicatum* is a competitive species that exhibits rapid spreading strategies. It grows on all continents except Antarctica and is commonly found in colonies in eutrophic waters (Liu et al., 2019). Its strategy to thrive in eutrophic environments involves producing allelopathic chemical compounds. Several scientific studies have reported that *M. spicatum* produces chemical compounds that can inhibit the growth of cyanobacteria, showing cyanobacterialidal effects (Liu et al., 2007; Shao et al., 2009; Nakai et al., 2012; Jeong et al., 2021; Zuo et al., 2023).

The chemical composition of *M. spicatum* extract has been extensively studied, revealing a rich variety of bioactive compounds. In addition to polyphenols and fatty acids, the plant also contains alkaloids, terpenoids, and carotenoids, which have been linked to its allelopathic and antimicrobial properties. Specifically, *M. spicatum* has been reported to produce alkaloids such as *myriophylline*, which have shown potential as natural herbicides, aiding in the suppression of aquatic weed growth (Chen et al., 2017). Furthermore, carotenoids, including lutein and β-carotene, are present in the plant, contributing to its antioxidant activities and potential role in mitigating oxidative stress in aquatic environments (Li et al., 2020). The synergistic effects of these compounds may enhance the plant's ability to combat harmful algal blooms, particularly those caused by cyanobacteria, by interfering with their growth and photosynthetic activity (Liao et al., 2020). Studies have also highlighted the presence of saponins in *M. spicatum*, which are known for their antifungal and antimicrobial properties, further adding to its potential as a bioactive agent in aquatic ecosystem management (Zhao et al., 2018). The diversity of these compounds underscores the importance of *M. spicatum* as a natural source of biocides, offering promise for both environmental and pharmaceutical applications.

Table 1. The percentages of cell viability in *Acanthamoeba* trophozoites after being exposed to Various concentrations of *Myriophyllum spicatum* leaf extracts for 72 hours

The life cycle phase of *A. castellanii*	The different doses of *M. spicatum* leaf extracts	Cell viability percentage ± standard error (SE)
Trophozoites	48 µg/mL	33±1.20
	24 µg/mL	48±1.38
	12 µg/mL	62±0.92

Table 2. Mean ± SD values representing the percentages of cell viability of *Acanthamoeba castellanii* trophozoites following exposure to different concentrations of *Myriophyllum spicatum* ethanolic extract at different time intervals

Time (Hours)	Dose (µg/mL)	Mean±SD
24	Control	$100.00{\pm}0.00^{a}$
	1.5	$100.00{\pm}0.00^{a}$
	3	$100.00{\pm}0.00^{a}$
	6	$100.00{\pm}0.00^{a}$
	12	$86.33{\pm}1.52^{d}$
	24	$74.33{\pm}0.57^{e}$
	48	$64.33{\pm}1.52^{g}$
48	Control	$100.00{\pm}0.00^{a}$
	1.5	$97.66{\pm}0.57^{ab}$
	3	$95.33{\pm}1.15^{b}$
	6	$85.33{\pm}1.52^{d}$
	12	$71.00{\pm}1.73^{f}$
	24	$65.33{\pm}1.15^{g}$
	48	$42.66{\pm}2.51^{i}$
72	Control	$100.00{\pm}0.00^{a}$
	1.5	$90.33{\pm}1.52^{c}$
	3	$83.33{\pm}2.08^{d}$
	6	$73.00{\pm}2.64^{ef}$
	12	$62.00{\pm}1.52^{g}$
	24	$48.00{\pm}2.00^{h}$
	48	$33.00{\pm}2.00^{j}$
p	<0.01	

The data are presented as mean ± SD. Statistical significance between values denoted by different letters, such as a and b, is considered meaningful

Table 3. Overview of the impact of *Myriophyllum spicatum* L. ethanol extract on the cell viability of *Acanthamoeba castellanii* trophozoites at different concentrations and exposure times (Mean ± SD)

Dose/Time	24 h	48 h	72 h	∑
Control	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
1.5 µg/mL	100.00±0.00[a]	97.66±0.57[ab]	90.33±1.52[c]	96.00±9.16[b]
3 µg/mL	100.00±0.00[a]	95.33±1.15[b]	83.33±2.08[d]	92.88±7.54[c]
6 µg/mL	100.00±0.00[a]	85.33±1.52[d]	73.00±2.64[ef]	86.11±11.80[d]
12 µg/mL	86.33±1.52[d]	71.00±1.73[f]	62.00±1.52[g]	74.55±9.16[e]
24 µg/mL	74.33±0.57[e]	65.33±1.15[g]	48.00±2.00[h]	63.88±9.80[f]
48 µg/mL	64.33±1.52[g]	42.66±2.51[i]	33.00±2.00[j]	48.33±12.29[g]
∑	89.28±14.05[a]	79.61±20.02[b]	100.00±0.00[a]	

Statistical differences were found between the values indicated as superscripts and those represented by different letters (a,b,…j = $p < 0.01$)

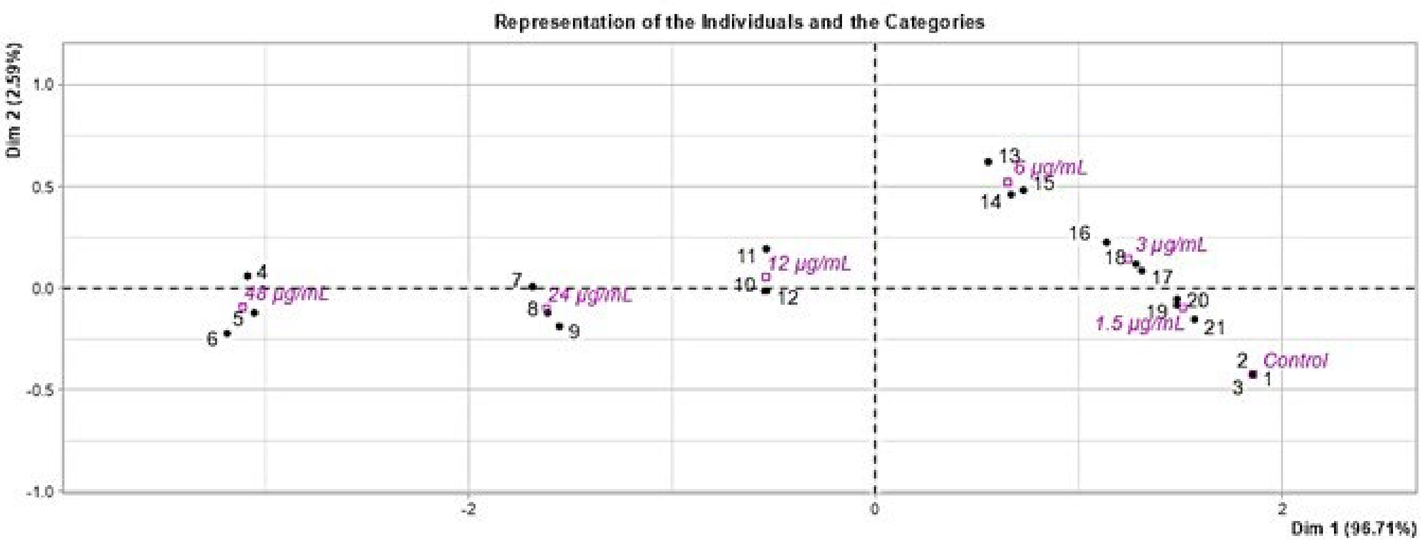

Figure 2. The PCA plot of *Myriophyllum spicatum* extract at various concentrations

M. spicatum produces significant amounts of fatty acids and polyphenols to suppress the cyanobacterium *Microcystis aeruginosa* (Nakai et al., 2012; Maredová et al., 2021). This helps reduce water turbidity by preventing excessive phytoplankton growth in eutrophic waters, facilitating the restoration, recolonisation, and regeneration of submerged macrophytes with proper light penetration (Švanys et al., 2014). Therefore, *M. spicatum* represents a promising natural source for preventing cyanobacterial harmful algal blooms (CyanoHABs) and can be used for the restoration of eutrophic water bodies by creating a cleaner aquatic environment. As a natural product, it could also be an important source for innovative drug development.

Allelochemicals released by aquatic plants containing secondary metabolites are distinguished by excellent biocompatibility, biological degradability, net algal inhibition, and minimal ecological harm (Zuo et al., 2023). However, different phytoplankton species show varying sensitivities to allelochemicals. For example, cyanobacteria and diatoms are more sensitive than green algae, while epiphytic species are less sensitive than phytoplankton. Additionally, environmental factors (such as light, temperature, and nutrients) can significantly affect the allelopathic impact of submerged macrophytes (Xi et al., 2009).

Acanthamoeba is an opportunistic protozoan commonly found in nature (mainly in water and soil). *Acanthamoeba* usually exists in two forms: trophozoites and cysts. Pathogenic species can lead to profound blindness due to *Acanthamoeba* keratitis (AK) and the rare granulomatous amoebic encephalitis (GAE), as well as skin and lung infections. In recent years, there has been an increase in *Acanthamoeba* infections, particularly AK. As a result, there has been growing scientific interest in the diagnosis, treatment, and prevention of *Acanthamoeba* infections (Wang et al., 2023).

To combat infections caused by *Acanthamoeba*, plant-based compounds may be utilised due to their bioactive molecular activities. By focusing on plants with antiparasitic properties, new drugs could be developed to treat *Acanthamoeba* infections (Mitsuwan et al., 2020).

With global warming, global water scarcity, and the reliance on household water tanks in developing countries, infections caused by free-living amoebas, such as Acanthamoeba, are expected to rise. Therefore, the development of new disinfectants that can effectively target pathogenic, free-living amoebas is a crucial issue (Siddiqui et al., 2022). Secondary metabolites obtained from natural sources are often considered to have more "drug-likeness and biological friendliness" compared to fully synthetic compounds, making them promising candidates for disinfectants, contact lens disinfectants, and drug development (Chin et al., 2006).

Aquatic plants (macrophytes) with anti-*Acanthamoeba* activity have rarely been studied. The ethanolic extract of the submerged aquatic plant *Ceratophyllum demersum* (coontail) was found to exhibit amoebicidal activity against *Acanthamoeba castellanii* trophozoites in an in vitro environment. After 72 hours of incubation, it was observed that the viability of trophozoites treated with *C. demersum* extract at concentrations of 30.4 and 60.8 µg/mL decreased to 42% and 58.33%, respectively (Taş et al., 2024).

No specific information is found in the current scientific literature regarding the amoebicidal (anti-amoebic) effect of *M. spicatum*. However, research on the antimicrobial and antioxidant properties of aquatic plants is increasing. It is known that aquatic plants, such as Ranunculus sphaerospermus, exhibit antibacterial and antioxidant properties (Ertürk et al., 2019). *M. spicatum* has antimicrobial effects (Ertürk et al., 2020), and *C. demersum* has anti-amoebic effects (Taş et al., 2024). Therefore, further research is needed to evaluate the potential anti-amoebic effects of *M. spicatum*. A study examining the in vitro antimicrobial effects of ethanol extracts from *M. spicatum* collected from two different locations on test organisms (four Gram-positive bacteria, four Gram-negative bacteria, and two fungi) found that the highest activity was observed against the Gram-positive bacterium *Bacillus subtilis* (inhibition zone diameter 24.50 ±6.24 mm/25 µL). The plant extract was more effective against Gram-positive bacteria compared to Gram-negative bacteria. Furthermore, the *M. spicatum* extract from the Miliç River (Samsun) was found to have a higher antimicrobial effect than the extract from Ulugöl Lake (Ordu) (Ertürk et al., 2020). The antimicrobial activity of *M. spicatum* suggests that it may also have potential anti-amoebic effects. In traditional medicine, *M. spicatum* is used externally as a wound-healing agent (Bolotova, 2015).

Although various phenolics, terpenes, and other phytochemicals have been isolated from *M. spicatum*, there is no literature on its pharmacological uses. At least 18 different phenolic compounds have been reported in *M. spicatum*, with ellagic acid, gallic acid, and various tannic acids being the most abundant compounds (Lambrechts et al., 2020).

Given the richness of these bioactive compounds, the plant presents potential for therapeutic applications, including antimicrobial and antiparasitic activities. One such area of interest may be the treatment of *Acanthamoeba* infections. That is why we examined the effect of *M. spicatum* on the trophozoite form of *Acanthamoeba. Acanthamoeba* infections are particularly challenging due to the parasite's ability to form cysts, which play a crucial role in its persistence and resistance to treatment. However, as noted, our study was specifically designed to evaluate the effects of *M. spicatum* on the trophozoite form of *Acanthamoeba*. Further studies are needed to assess the effects of the tested agents on various stages of the cyst life cycle.

Conclusion

The results indicated the amoebicidal activity of the ethanolic leaf extract of *M. spicatum* against *A. castellanii* trophozoites. It was observed that the parasite's survival rate decreased with increasing extract concentration. Additionally, when the impact of different application times on viability was analysed, it was found that the parasite's survival rate declined as the exposure time increased. Based on the findings, it can be inferred that the extract of *M. spicatum* could serve as a potential alternative or complementary treatment for Acanthamoeba infections under controlled conditions.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Determining the population dynamics of *Istiophorus albicans* (Latreille, 1804) in coastal waters of the Gulf of Guinea using length frequency data

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ABSTRACT

Atlantic sailfish catches in Ghana have declined to levels below historical levels, despite their economic and nutritional significance, raising concerns about a potential collapse of the fishery. This preliminary study aimed to assess the exploitation status of Atlantic sailfish (*Istiophorus albicans*) from the coast of Ghana, utilising FiSAT II software. Of the length distribution of 713 specimens sampled from Dixcove and Tema fishing communities, asymptotic length ($L\infty$), growth rate (K), and age at length zero (t0) were 325.5 cm, 0.59 yr^{-1}, and - 0.13, respectively. Total mortality rate (Z), natural mortality rate (M), and fishing mortality rate (F) were 3.05 yr^{-1}, 0.44 yr^{-1}, and 2.61 $yr^{-1,}$ respectively. The length at capture (Lc_{50} = 186.15 cm) exceeded the length at maturity (Lm_{50} = 150.6 cm). The current exploitation rate (E = 0.86) exceeded the maximum sustainable exploitation rate (E < Emax), indicating a potential collapse of the fishery without effective management measures. Based on the findings, there is an urgent need to implement effective management strategies to sustain the Atlantic sailfish population along Ghana's coast.

Keywords: Growth, Mortality, Exploitation, Ghana, Stock assessment, Atlantic sailfish

Introduction

The Atlantic sailfish (*Istiophorus albicans*, Latreille, 1804) belongs to the family Istiophoridae within the order Carangiformes (ICCAT, 2010). This opportunistic carnivorous species is recognised for its distinctive sail-like dorsal fin, featuring an elongated, pointed rostrum and sharp, small teeth adapted for grasping prey. It can grow up to 315 cm (ICCAT, 2010; Hugi, 2022). The Atlantic sailfish is a pelagic species that inhabits the tropical and temperate waters of the Atlantic Ocean, preferring depths of up to 200 meters (Nakamura, 1985; Hugi, 2022).

In Ghana, Atlantic sailfish are common in fishing communities such as Dixcove, Tema, Sekondi, among others (Kwei & Ofori-Adu, 2005). They are primarily caught by artisanal fishermen using drift gillnets, longlines, and hooks. Individuals of Atlantic sailfish are harvested for fresh, smoked, and frozen consumption, contributing to local nutrition both directly and indirectly (Kwei & Ofori-Adu, 2005). Economically, the Atlantic sailfish is vital to the livelihoods of coastal fishing communities in Ghana. However, the stock has exhibited a sharp decline in recent years, with recorded landings from 693 metric tons in 1993 to 63 metric tons in 2021 (ICCAT, 2023). The alarming decline in Atlantic sailfish catches along Ghana's coast underscores the urgent need for immediate implementation and strict adherence to management measures in alignment with international bodies, such as ICCAT. Without science-based intervention, the continued depletion of this species could have severe ecological and economic consequences, threatening marine biodiversity and the livelihoods of coastal communities.

Despite the reduction in catches, there is a lack of scientific information on the exploitation status of *I. albicans* on Ghana's coast, which limits policymakers' ability to implement effective management strategies (ICCAT, 2023). The lack of scientific information poses a serious risk of overexploitation, potentially leading to the collapse of the fishery and causing further socio-economic challenges (FAO, 2022). This study aimed to assess the population parameters of *Istiophorus albicans* in Ghanaian waters. The findings will provide preliminary information for the sustainable management of the sampled species from the coast of Ghana.

Materials and Methods

Study Site

The study was conducted in two coastal communities, including Dixcove and Tema fishing communities, which are hotspots for sailfish fisheries in Ghana. The artisanal fishing community of Dixcove, located in Ghana's Western Region (N 04.79368°, W 01.94612°), comprises of three landing beaches: Upper Dixcove, Lower Dixcove, and Urom, with over 1,081 fishermen (Dovlo et al., 2016). Tema has two landing beaches, namely Ashamang and Awudun, with over 5167 fishermen (Dovlo et al., 2016) (see Figure 1).

Figure 1. Map showing the sampling sites

Data Collection

Fish samples were collected randomly from local fishermen in Dixcove and Tema fishing communities, where the dominant fishing gears used include drift gill nets (DGNs) and longline gear, over twelve months (once a month) from November 2023 to October 2024. However, no data was obtained in July 2024 due to the closure of artisanal fisheries fishing season in Ghana. Identification of fish species to the species level was done using keys by Fischer et al. (1981) and Kwei and Ofori-Adu (2005). In-situ identified samples were analysed for total length to the nearest centimetre using a 500 m tape measure.

Growth Parameters

Growth parameters that follow the von Bertalanffy Growth Function (VBGF) were estimated using the Electronic Length Frequency Analysis (ELEFAN) option of the FiSAT II Tool. The von Bertalanffy Growth Function (VBGF) is given as follows:

$$L_t = L_\infty [1- e^{-K(t- t_0)}] \quad \text{(Pauly, 1984)}$$

Where Lt is the average length at time (or age) t, L∞ is the asymptotic length, K is the growth rate, and t represents the age when the average length was zero.

Estimation of longevity (Tmax) of the species was done using the formula:

$$3/K \quad \text{(Anato, 1999)}$$

The growth performance index was calculated using the formula:

$$2\log L_\infty + \log K \quad \text{(Munro \& Pauly, 1984)}$$

The theoretical age at length zero (t_0) followed the equation:

$$\log_{10} (-t_0) = -0.3922 - 0.2752 \log_{10} L_\infty - 1.038 \log_{10} K \quad \text{(Pauly, 1979)}$$

Length at First Capture

The ascending left part of the length converted catch curve was used to estimate the probability of length at first capture (Lc50), in addition to the length at both 25% and 75% capture, which correlates with the cumulative probability at 75% and 95%, respectively (Pauly, 1984).

Length at First Maturity

The length at first maturity (Lm_{50}) was estimated as

$$\log_{10} Lm_{50} = 0.8979 * \log_{10}(L_\infty) - 0.0782 \quad \text{(Froese \& Binohlan, 2000)}$$

Mortality Parameters

The natural mortality rate of Atlantic Sailfish was computed from the Then et al. (2015) formula:

$$M = 4.118K^{0.73} * L_\infty^{-0.333},$$

where M is natural mortality in a given stock, and the value of L∞ is the asymptotic length calculated from the sample.

The fishing mortality coefficient (F) was calculated as:

$$F = Z - M \quad \text{(Qamar et al., 2016)}$$

The exploitation rate (E) was calculated using the formula:

$$E = F/Z \quad \text{(Georgiev \& Kolarov, 1962)}$$

Recruitment Pattern

Recruitment patterns were analysed using the FISAT II program in the recruitment pattern subprogram to construct a time series of recruits from length frequencies, thereby determining the relative peak recruitment per year (Pauly, 1984).

Virtual Population Analysis (VPA)

Input parameters including L∞, K, M, F, constants of length-weight relationship ($a = 0.0001$ and $b = 3.0$), and annual catch based on single cohort during the exploited phase was used to calculate the abundance and fishing mortality rates of the cohort in each year were used as inputs to VPA analysis for the sampled species (Fry, 1949; Gulland, 1965).

Relative Yield Per Recruit (Y/R)' and Relative Biomass Per Recruit (B/R)'

Relative yield per recruit (Y/R)' and relative biomass per recruit (B/R)' were estimated using the model of Beverton and Holt (1957). The data of probability of capture file M/K values were used to estimate both (E_{max}), which represents the optimum exploitation that may maximize the yield per recruitment and ($E_{0.5}$) the exploitation level at which the biomass is reduced to 50% of the unexploited stock.

Yield Isopleth

Yield contours that indicate stock status were identified as the intersection of the exploitation rate (E) and the critical length ratio (Lc50/L∞) (Gayanilo et al., 2005).

Data Analysis

The length frequency data was pooled into groups with 20 cm length intervals. The data was then analysed using the FiSAT

II (FAO-ICLARM Stock Assessment Tools) software (Gayanilo et al., 2005).

Results and Discussion

Length Distribution

Overall, a total of 713 samples of *I. albicans* were analysed for growth and exploitation rates from the coast of Ghana. The annual length frequency ranged from 134 to 305 cm, with a mean of 252.5 ± 0.77 cm (Figure 2). The length frequency is illustrated in Figure 2. The mid-length range of 210-230 cm exhibited the highest rate, approximately 70.7%.

Growth Parameters

The results of von Bertalanffy's calculation yielded an asymptotic length ($L\infty$) of 325.5 cm TL, a growth coefficient value (K) of 0.64 per year, and a theoretical length at zero age (t_0) of -0.13 cm/year. The longevity (T_{max}) was estimated at 4.7 years. Figure 3 illustrates the reconstructed length-frequency distribution, along with growth curves (Figure 3).

Length at First Capture and Maturity

From the analysis of probability of capture of each length class, the estimated length at first capture was 186.15 cm TL (Figure 4). The length at 25 % and 75 % was 178.40 cm TL and 193.90 cm TL, respectively. The estimated length at first maturity was 150.6 cm TL.

Mortality Parameters

According to the current study, the estimated total mortality rate (Z), natural mortality (M), and fishing mortality (F) were 3.05 per year, 0.44 per year, and 2.61 per year, respectively. Based on the Z and F values, the exploitation level was 0.86 (Figure 5).

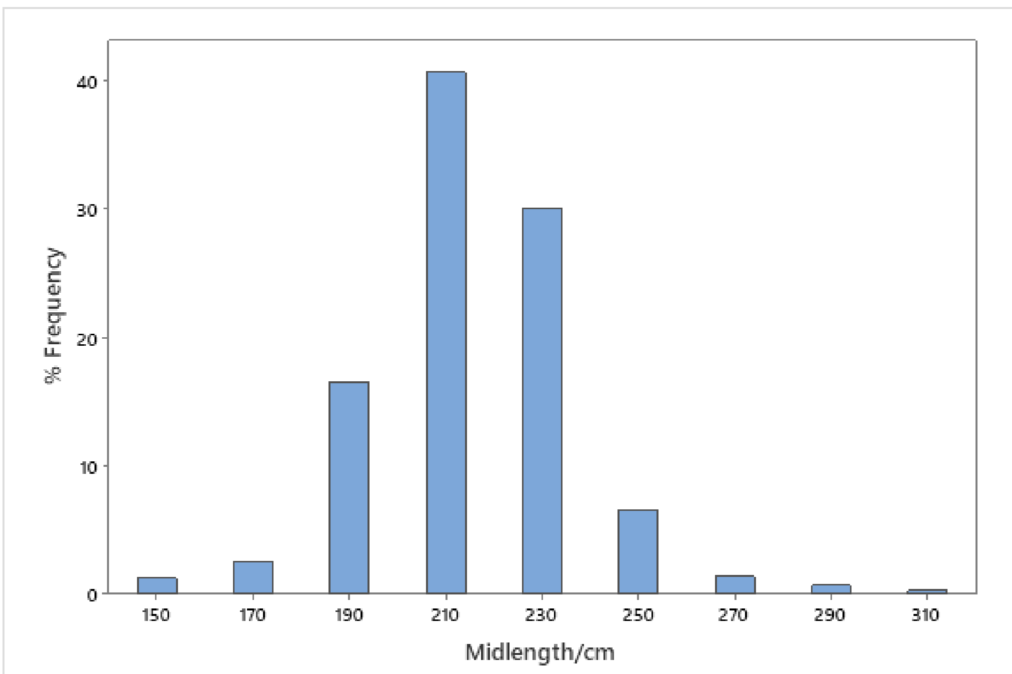

Figure 2. Annual total length frequency distribution of *Istiophorus albicans* on the coast of Ghana

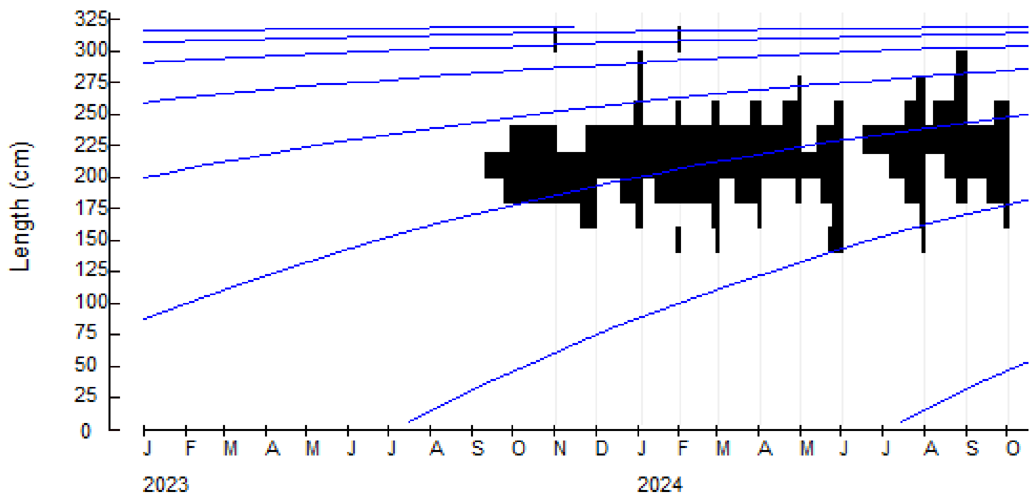

Figure 3. Reconstructed length frequency with growth curves for the Istiophorus albicans

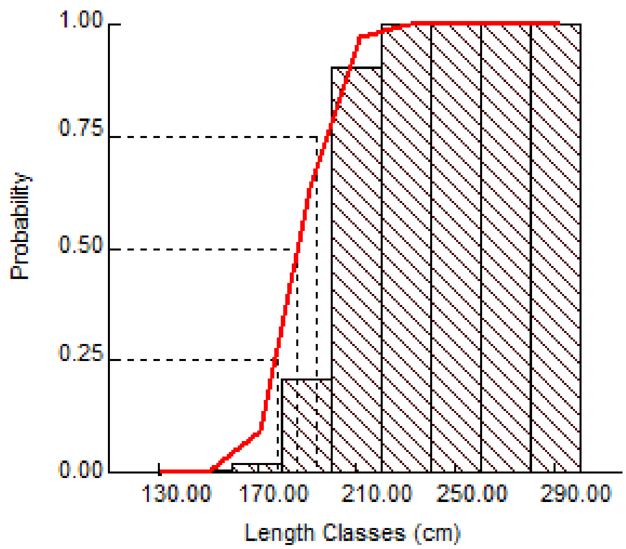

Figure 4. Probability of capture of Istiophorus albicans on the coast of Ghana

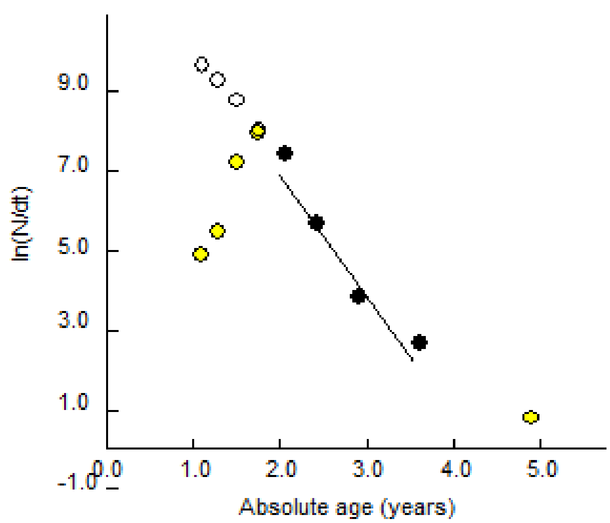

Figure 5. Linearised length-converted catch curve of Istiophorus albicans off the coast of Ghana

Recruitment Pattern

The plot shows a single central pulsed recruitment peak per year in a continuous recruitment pattern (Figure 6). The highest recruitment period occurred from April (10.4%) to September (10.6%), with a prominent peak in June, accounting for 15.8%.

Virtual Population Analysis (VPA)

The virtual population analysis revealed that the minimum and maximum fishing mortalities were recorded for mid-lengths of 150 cm and 230 cm, respectively, at rates of 0.051 and 3.97 per year (Figure 7). The highest fishing mortality for the exploited mid-length fish (230 cm) was 3.97 per year. The catches of fish were highest between mid-lengths of 210 cm and 230 cm.

Yield Per Recruit Analysis

The relative yield per recruit analysis, incorporating probabilities of capture, estimated the exploitation at maximum sustainable yield, at 10% and 50% of yield, as 0.702, 0.614, and 0.392, respectively (Figure 8).

Yield Isopleth

The isopleth yield map of the assessed fish species fell in the third quadrant with a critical length at capture (Lc) of 0.57 and an exploitation rate of 0.86 (Figure 9).

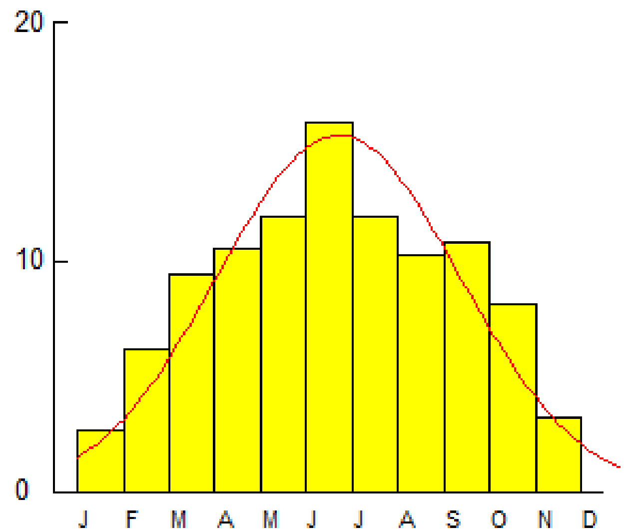

Figure 6. Recruitment of Istiophorus albicans from the coast of Ghana

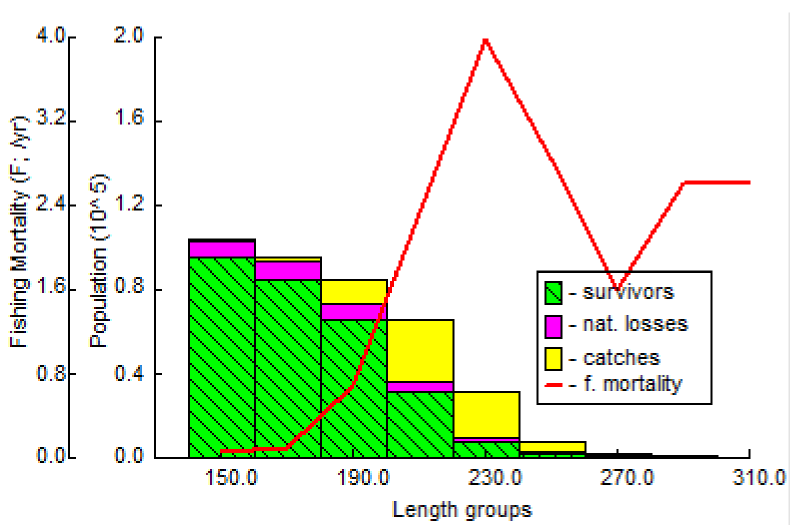

Figure 7. VPA of Istiophorus albicans from the coast of Ghana

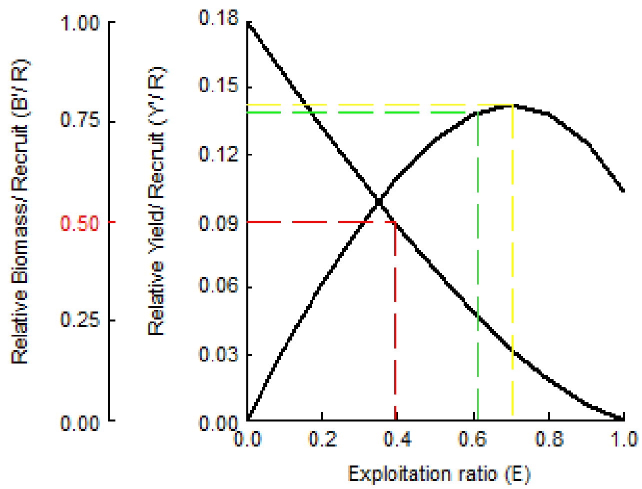

Figure 8. Relative yield per recruit of Istiophorus albicans off the coast of Ghana

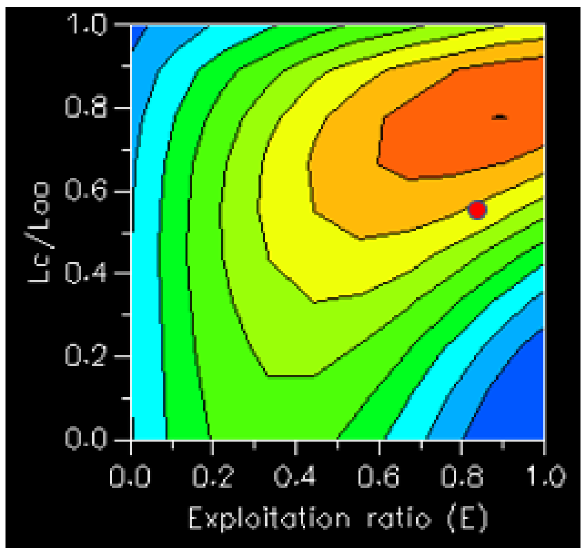

Figure 9. Isopleth plot of Istiophorus albicans from the coast of Ghana

The length interval of the sampled species from the present study varied with estimates from other studies in different locations (Table 1). The use of fishing gears with different mesh sizes across various geographical jurisdictions, differences in fishing intensities, as well as sampling size and methods, and the type of fish length recorded, could be the reason for the varying length estimates between the current study and the aforementioned studies (Amponsah et al., 2021).

The growth rate, theoretical age at length zero (t0), and L∞ values estimated in the current study differed from those estimated by other researchers (Table 2). Variations in sampling techniques, length type recorded, geographic locations, the range of sizes used in the analysis, genetic structure, and density of food may have accounted for the observed differences in growth parameters (Chiang et al., 2004; Morales-Azpeitai et al., 2013; Darvishi et al., 2018).

The recruitment pattern from the study exhibited a continuous pattern, similar to the one documented by Agnissan et al. (2014). This may suggest that environmental conditions of the Gulf of Guinea are conducive for recruitment and functioning of Atlantic sailfish. The highest peak of recruitment for the species occurred in July, which falls within the significant upwelling period in Ghana, characterised by an abundance of feed items.

According to the virtual population analysis (VPA), surviving individuals of the sampled species exhibited a declining trend as the length increased. This observation could be attributed to combined effects of natural and fishing activities. Some scholars (e.g., Agnissan et al., 2014) have noted similar implications. The fishing mortality rate has been widely used to estimate the abundance of commercial fish stocks since its development. Similarly, some scholars (Agnissan et al., 2014) have reported high fishing mortality on large-sized individuals, which may affect future recruitment potential of the species.

The length at first capture was higher than the estimates documented by Agnissan et al. (2014), which may be attributed to the mesh size of the fishing gears used, the sex of the harvested catch, environmental conditions, and the computation procedure applied (Amponsah et al., 2016). Comparatively, the length at capture was higher than the length at maturity, suggesting that the recruitment of the species may be unaffected, as individuals of the sampled species have the opportunity to spawn before becoming vulnerable to the fishing gears (Udoh et al., 2009). However, the size class obtained and the computational procedures applied may have affected the length at first maturity from the study as compared to estimates by Agnissan et al. (2014). The (L_c/L_∞) ratio estimated from the present study was 0.57, which is relatively above 0.5, indicating that the catch marginally comprised matured individuals of *I. albicans* (Pauly & Soriano, 1986). According to Sala et al. (2018), the type of fishing gear used can significantly affect the length and maturity composition of the catch. Hence, enforcing existing fishing gearregulations could help maintain the reproductive capacity of the sampled species.

The fishing mortality rate (F) of the sampled species from the current study was higher than that recorded by other scholars (Table 3), suggesting that *I. albicans* on the coast of Ghana is experiencing high fishing pressure. The exploitation rate estimated was 0.86, which is higher than the optimal exploitation rate of 0.5 (Gulland, 1971), indicating that the species is overexploited. Agnissan et al. (2014) and Surya et al. (2023) have recorded similar implications for the sampled species.

Table 1. Previous records on the minimum, maximum and mean of Atlantic sailfish

References	Location	Min. Length/cm	Max. Length/cm	Mean length/cm
Agnissan et al. (2014)	Ivory Coast	80	220	-
Surya et al. (2023)	Indian	96	284	179.0
This study	Ghana	134	305	252.5

Table 2. Previous records on growth parameters and phi-prime index of Atlantic sailfish

References	Location	K/per year	L∞/cm	Phi prime	to
Agnissan et al. (2014)	Ivory Coast	0.48		4.41	0.49
Surya et al. (2023)	Indian	0.40	231.6		-0.05
This study	Ghana	0.64	325.5	4.831	-0.13

Table 3. Previous records on the exploitation rate, length at capture and length maturity of Atlantic sailfish

References	Location	M/ per year	F/ per year	Z/ per year	E
Agnissan et al. (2014)	Ivory Coast	0.63	0.93	1.56	0.60
Surya et al. (2023)	Indian	0.32	0.69	1.01	0.68
This study	Ghana	0.44	2.61	3.05	0.86

The E_{max} was lower than the current exploitation rate (E), which was in variance with findings from the Ivorian waters, where the current E was lower than the maximum exploitation rate (Table 3). This suggests the species on the coast of Ghana is highly overexploited. In addition, at the eumetric stage, any efforts to increase yield per recruit will lead to reduced stock biomass and potential recruitment failure (Pauly & Soriano, 1986; Abobi et al., 2019). Therefore, it is crucial to reduce fishing pressure to levels closer to the E_{max}, to ensure maximum sustainability of *Istiophorus albicans* fishery and prevent further decline in its population.

Conclusion

This study offers preliminary insights into the population dynamics of *Istiophorus albicans* along Ghana's coast. The sampled species exhibited signs of rapid growth, a strategy used to compensate for the high fishing mortality rate. The recruitment pattern was continuous, with a significant peak in June. The sampled species was placed in the eumetric stage, characterised by a high exploitation rate on matured individuals. Science-based management measures by ICCAT, including gear modification, systematic data collection, catch reduction, and enforcement of minimum landing size, must be mandated and strictly enforced by policymakers in Ghana. These regulations are critical for protecting and ensuring the sustainability of sampled species.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: The data will be made available upon request from the author.

Funding disclosure: -

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Disclosure: -

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Temporal and spatial status of environmental variables and pollutants in Çardak Lagoon (Çanakkale Strait)

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ABSTRACT

The objective of this study was to analyse the correlation between domestic pollutants, specifically organic matter, anionic detergent, and suspended solids, and environmental variables such as temperature, salinity, and sediment grain size in Çardak Lagoon on a seasonal basis. Samples were obtained using 5 L Nansen bottles at a depth of 1 m from the surface waters across 6 stations within the lagoon, as well as from 1 station situated on the seaward side. The collection period for the latter was from October 28, 2018, to June 28, 2019. The study demonstrated that the primary indicators of water pollution, namely NH^{+}_{4} and anionic detergents, were consistently found at very low levels throughout the year in the study area.

Keywords: Domestic pollutants, Environmental variables, Çardak Lagoon, Turkish traits system

Introduction

Coastal lagoons are of ecological significance as they represent a distinct transition zone between the terrestrial and marine environments (Tagliapietra et al., 2009). However, the water quality of coastal lagoons worldwide has deteriorated owing to large discharges of nitrogen and phosphorus from domestic and industrial wastewater, as well as urban drainage. Anthropogenic stress, stemming from coastal development (including domestic and industrial development, as well as agriculture), is a salient feature of the coastal areas of lagoons (Vitousek et al., 1997; Abidi et al., 2018). Eutrophication has been identified as the most significant impact observed in coastal lagoons, particularly in those with inland water inputs and limited exchange with the sea (Soria et al., 2022). The increase in nutrients observed in coastal ecosystems has been linked to primary eutrophication, accumulation of organic matter at the bottom, and loss of biota (Arévalo et al., 2013). The excess nutrient content in lagoon waters, the primary stress factor, was predominantly attributable to agricultural, industrial, and domestic waste inputs. The chemical and biological factors that cause pollution include nutrient enrichment and the excessive accumulation of organic carbon, pathogens, and heavy metals (Crossland et al., 2005). The high percentage of dissolved organic nitrogen in the lagoon water is likely due to the recycling of nitrogen, which is presumably attributable to the recycling of organic matter in both the water column and sediment (Christian et al., 1998). Hypoxia in water causes anthropogenic eutrophication, and hypoxic conditions are temporally and spatially variable (dependent on both chemical and physical variables). Under hypoxic conditions, there is a known increase in nutrients, accompanied by changes in trophic levels and an increase in habitat degradation (Kennish & Paerl, 2010; Cloern, 2001). The development of a hypoxic environment can be attributed to the accumulation of organic matter, which exceeds the system's carrying capacity. The impact of environmental factors on fisheries and aquaculture yields, as well as the degradation of ecosystems, has been extensively researched (Karydis, 2009).

Coastal ecosystems are generally categorised through the utilisation of trophic indices, which predominantly rely on the concentrations of nutrients and chlorophyll-*a* (Cotovicz et al., 2013). Photophilous algal blooms, coupled with anoxic conditions and elevated chlorophyll-*a* levels in aquatic environments, serve as indicators of nutrient enrichment. The degradation of lagoonal regions within the Mediterranean Sea is chiefly attributable to the release of untreated domestic wastewater (Mozetic et al., 2008). The introduction of domestic wastewater into the coastal zones of the Mediterranean Sea has been shown to induce eutrophication, with potentially severe repercussions for the lagoon ecosystem when the standard thresholds for eutrophication are exceeded. Natural stressors, such as elevated temperatures and storms, can accelerate eutrophication in lagoons and decrease dissolved oxygen levels in deeper waters. Coastal lagoons are aquatic systems characterised by diminished regeneration capabilities, attributed to their restricted freshwater inflow and their interconnection with marine environments. Significant freshwater influxes coupled with minimal volumes result in diminished salinity levels. However, these salinity levels can experience sudden fluctuations due to storm activity at sea (Soria et al., 2022). Sea surface temperature and precipitation also significantly modify the lagoon environment (Anthony et al., 2009). The coastal areas of such lagoons are rich in organic matter due to their hydrological and trophic status (Mesnage & Picot, 1995; Lardicci et al., 1997, 2001). Numerous studies have focused on understanding the spatial and temporal patterns of all variables closely related to trophic state and water quality in lagoons (Karydis, 1983; Pérez-Ruzafa et al., 2005; Gikas et al., 2006; Coelho et al., 2007; Souchu et al., 2010; Lucena-Moya et al., 2012; Abidi et al., 2018; Acquavita et al., 2015; Cotovicz et al., 2013; Diamantopouloua et al., 2018). Recent studies (Specchiulli et al., 2010; Ponti et al., 2011; Molinaroli et al., 2014; Acquavita et al., 2015; Amos et al., 2017) on the lagoonal areas of the Mediterranean Sea were predominantly conducted along the Italian coast. Studies on this subject have been conducted along the Spanish coastline of the Mediterranean Sea. For instance, Menendez et al. (2004) examined the sediment characteristics of the Tancada Lagoon in eastern Spain. Additionally, Perez-Ruzafa et al. (2005) investigated the temporal and spatial variation of environmental variables, including nutrients and chlorophyll-*a*, in the Mar Menor Lagoon (southern Spain). In the southern Mediterranean, the lagoon ecosystems of the eastern Moroccan coast have been studied by Ruiz et al. (2006) and Bloundi et al. (2008) for their susceptibility to anthropogenic impacts. About the Tunisian Coast, Ayache et al. (2009) addressed the impact of environmental pressures on three lagoon sites (Merja Zerga, Morocco; Ghar El Melh, Tunisia; Manzala, Egypt) on the Tunisian Coast. Primary studies on this subject in Greek Lagoons were performed by Gikas et al. (2006), Diamantopoulou et al. (2008), and Avramidis et al. (2013, 2017). A few studies have been previously conducted on the Turkish Traits system. Noteworthy among these are the investigations by Topçuoğlu et al. (1999), who studied the effects of domestic pollution in Küçükçekmece Lagoon (northern Marmara Sea), and by Altun et al. (2009), who investigated physico-chemical variables and domestic pollution factors in the same area. Çardak Lagoon was designated a "first degree natural protected area" by the decision of the

Edirne Cultural and Natural Heritage Protection Board dated 06.08.1996 and numbered 3298. A rich natural habitat surrounds the lagoon. Çardak Lagoon, situated on the Anatolian north-eastern coast of Çanakkale Strait, is one of the 12 lagoon areas that collectively comprise the Turkish Straits System. This lagoon boasts a shoreline length of 4.3 km, an area of 1.2 km^2 and an average depth of 1.3 m.

Materials and Methods

Sampling Area

The Study area was the Çardak Lagoon in the northeast of the Çanakkale Strait (GPS coordinates; İst. 1; 40˚22´906″ N, 26˚43´103″ E, station. 2; 40˚23´053″ N, 26˚43´264″ E, ist. 3; 40˚23´203″ N, 26˚43´491″ E, ist. 4; 40˚23´345″ N, 26˚43´399″ E, ex. 5; 40˚23´278″ N, 26˚42´988″ E, ist. 6; 40˚23´236″ N, 26˚42´800″ E, ist. 7 (reference); 40˚22´931″ N, 26˚42´768″ E), and one reference station outside the lagoon at depths between 1-1.8 m (Figure 1).

Water Quality Analysis

The lagoon's quality variables, namely temperature, salinity, pH, and dissolved oxygen solubility, were assessed in situ using the YSI 600 QS Multiprobe System (Yellow Springs Instruments) at designated sampling points throughout the study (Figure 2). Concurrently, nutrient concentrations were evaluated by collecting water samples with a 5 L Nansen sampler.

Concentrations of nutrients such as NO^{-}_{2}, NO^{-}_{3}, NH^{+}_{4}, PO^{-}_{4}-P, and SiO^{-}_{2} were then quantified using a Jasco brand UV spectrophotometer using the appropriate chemical and biological analytical methods established by Strickland and Parsons in 1972. To quantify the chlorophyll-*a* concentrations, 5-litre water samples were carefully collected from each site using a 5-litre water sampler. Each sample was then subjected to in-situ vacuum filtration using a 7 mm GF/F filter, which was subsequently wrapped in aluminium foil and frozen in preparation for analysis. Spectrophotometric analysis, following extraction with 90% acetone, was conducted by the methodology outlined by APHA (1995). The total suspended solids (TSS) were quantified gravimetrically after filtration of the water samples through GF/C filters, as articulated by Clesceri et al. (1998). In the context of total phosphorus (inorganic and organic phosphate) analysis, it is imperative to note that all forms of phosphorus undergo a chemical reaction, resulting in the conversion to orthophosphate. (TIN = Total Nitrogen - Organic Nitrogen) (Water on Web, 2009). Analysis of chemical oxygen demand (COD) was performed using the open reflux method in conjunction with established protocols for assessing water and wastewater, as detailed by Eaton and Franson (2005). Spectrophotometric analysis for the quantification of anionic surfactants was performed using a standardised protocol, as described by APHA in 1995, which employed methylene blue.

Anionic Detergent Analysis

Anionic detergents were determined in 500 mL samples taken from the lagoon water (Figure 4). The solution was alkalised by the addition of 1 N NaOH, using phenolphthalein as an indicator. A volume of 10 mL of chloroform and 25 mL of methylene blue was used. Measurements were made using a UV spectrophotometer operating at a wavelength of 652 nm.

Redox Potential Measurements

In situ measurements of redox potential (Eh) were conducted using a pH meter equipped with a redox potential probe, immediately after sediment sampling across the autumn, winter, spring, and summer seasons.

Sediment Organic Matter and Granulometric Analysis

Sediment characteristics were analysed using a 393 cm^{-3} acrylic sediment core (Figure 3,4). This was used to determine the organic matter and particle size in the soft sediments of the lagoon. A total of 28 core samples were taken from the soft sediments at each sampling point during each sampling period for both analyses. The particle size analysis of the sediment was conducted by the methodology outlined by Allen (1997). The organic matter percentage (OM%) content was quantified by calculating the difference between the dry weight of the sediment (dried at 80°C for 24 hours) and the residue after combustion at 450°C for 2 hours (Parker, 1983). The analysis is based on the oxidation of organic substances in water using permanganate in acidic media. Potassium permanganate was utilised as the oxidising agent in this procedure. Following the oxidation process, the organic matter content of the sample was determined in terms of oxygen by back titration of the solution.

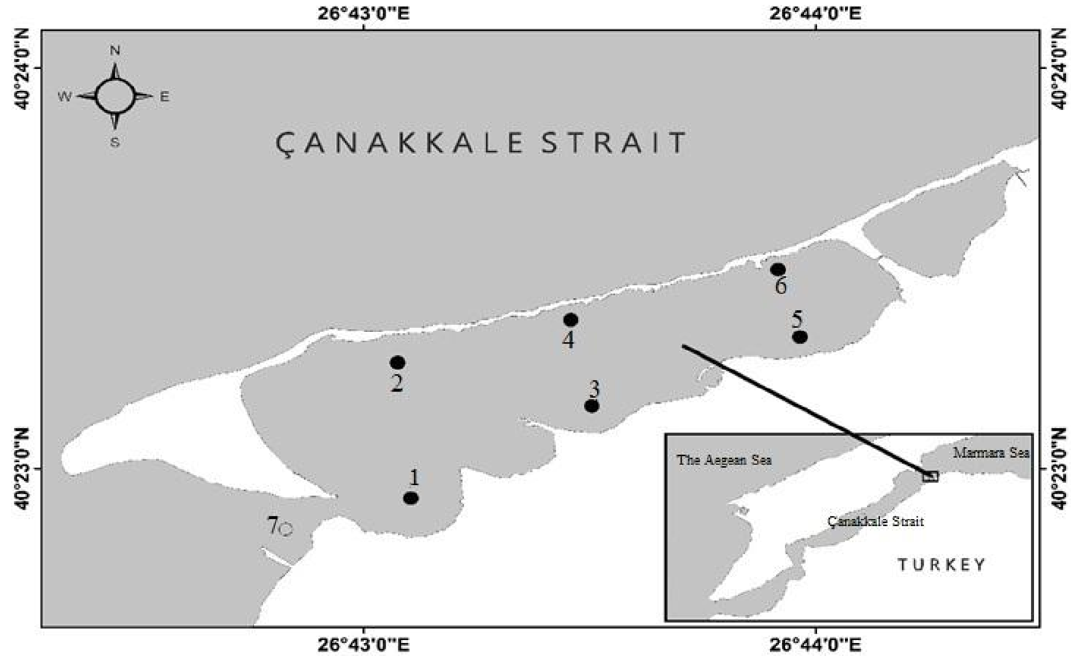

Figure 1. Map of the study area showing the sampling points

Figure 2. Measurements of water quality variables *in situ*

Data Analysis

All variables were analysed using multiple regression and MANOVA (Multiple Analysis of Variance) tests, facilitated by the appropriate statistical software. The analysis of environmental variables in the seawater samples collected from the specified sampling stations was conducted using one-way analysis of variance and Tukey's HSD tests to identify any significant differences between the stations. Additionally, correlation analysis was employed to investigate the relationships between the environmental variables examined in the lagoon water. Pearson's correlation coefficient (r) was used to calculate the correlations between environmental variables and pollutant variables using the PAST 4.02 software. Correlations between environmental and pollutant variables were determined statistically according to principal components analysis (PCA) (Jolliffe, 2002).

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Figure 3. Sediment samplings

Figure 4. Anionic detergent and granulometric analyses.

Results and Discussion

Water Quality, Nutrients, Chlorophyll-a, Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), and Anionic Surfactants

Water quality variables demonstrated seasonal variations, with temperatures ranging from 7.57°C to 27.34°C, with the highest value recorded in the summer of 2019. Salinity varied between 20.17‰ and 24.40‰, whereas pH levels remained within the characteristic ranges of seawater, oscillating from 7.78 to 8.56. DO levels showed variability, ranging from 5.90 mg/L to 9.51 mg/L, whereas NH^+_4 levels remained consistently minimal, below 0.01 mg/L at all stations throughout the study period. The PO_4-P values varied between 0.01 and 0.03 mg/L, while the total phosphate values exhibited variation between 0.02 and 0.17 mg/L. The maximum total nitrogen value recorded was 0.99 mg/L. The highest recorded NO_2+NO_3 value (0.195 mg/L) was observed in the autumn of 2018. The highest Chl-*a* value (12.85 µg/L) was observed in summer 2019, while the highest TSS value (71.33 mg/L) was recorded in winter 2019. Correlation analysis revealed a significant negative linear relationship ($p < 0.001$) between Chl-*a* and TSS values, and total suspended solids (TSS) values, suggesting that the system's load was predominantly terrestrial rather than phytoplanktonic. Furthermore, COD values (mg/L) were higher in autumn and winter than in other seasons, whereas anionic surfactant values (mg/L) were lower in autumn and winter than in other seasons. The mean values of all the environmental variables throughout the year during the sampling period are presented in Table 1.

Table 1 shows the descriptive statistics of the seasonal values of the water quality variables assessed in the lagoon water within the study area. Variance analysis revealed no statistically significant differences in water temperature variations among the stations (Table 1, $p > 0.05$). The analysis revealed no statistically significant differences in salinity values among the stations, regardless of seasonal variation (Table 1, $p > 0.05$). Similarly, no substantial seasonal variation in pH was observed. Nevertheless, a comparative analysis of the stations indicated that Station 1 demonstrated significantly lower values than its counterparts (Table 1, $p < 0.05$). The dissolved oxygen levels measured at Station 1, situated in close geographical proximity to the reference station, surpassed those observed at the other stations. This observation aligned with the results noted at the reference station (station 7). The oxygen levels recorded at alternative stations within the lagoon were found to be inferior. Nonetheless, the variance analysis indicated that there were no statistically significant differences in DO values among the stations (Table 1, $p > 0.05$).

The results of the analysis of variance, which show the differences in nutrient levels between stations obtained during the study, are presented in Table 1. NH^+_4 values, which are one of the indicators of water quality pollution, are at very low levels (less than 0.01 mg/L) in all stations and seasons during the study. Contrarily, the PO^-_4-P values, another indicator of pollution, demonstrated a range of 0.01-0.03 mg/L, with the lowest values recorded during the autumn and winter periods, and the highest values recorded during the spring and summer periods. Nevertheless, the inter-station variation in PO^-_4-P levels between stations was not statistically significant. Analysis of variance revealed no statistically significant differences between stations for the change in TP (Table 1, $p > 0.05$). Although there were differences between the stations in terms of the TN values, these differences were not statistically significant. Silicate levels recorded during the summer months were significantly higher than those recorded during the autumn, winter, and spring periods. However, statistical analysis revealed no statistically significant differences in silicate levels among the different stations (Table 1, $p > 0.05$). The NO^-_2+NO^-_3 values recorded during the autumn months were higher than those recorded during the other seasons. However, when the difference between the stations was analysed, it was evident that the NO^-_2+NO^-_3 values of the reference station were higher than those of the other stations, on average. However, this difference was not statistically significant. The TIN: P ratios recorded during the autumn and winter periods were notably higher than those recorded during other periods. A comparative analysis of the data reveals that the TIN:P ratio at station 4 is lower than that observed at the other stations, and the TIN: Si ratio at station 6 is significantly higher than that at the other stations. (Table 1, $p > 0.05$).

Table 1. Spatiotemporal variability in water chemistry, nutrients, chlorophyll-*a* (Chl-a), total suspended solids (TSS), chemical oxygen demand (COD), and anionic surfactant (AS) values were measured in the study. The one-way analysis of variance results for water quality variables are presented based on Tukey's HSD test

Variable	Station	Mean±SD	Min.	Max.	Variable	Station	Mean±SD	Min.	Max.
T (°C)	St. 1	15.59±6.77	8.46	24.75	pH	St. 1	7.97±0.19	7.78	8.20
	St. 2	16.74±8.07	7.86	27.34		St. 2	8.29±0.08	8.18	8.37
	St. 3	15.61±7.21	7.69	25.20		St. 3	8.18±0.09	8.05	8.26
	St. 4	15.53±6.61	7.96	24.07		St. 4	8.32±0.09	8.23	8.43
	St. 5	15.89±7.85	7.57	26.31		St. 5	8.25±0.15	8.07	8.40
	St. 6	16.33±7.88	7.68	26.54		St. 6	8.34±0.20	8.14	8.56
	St. 7, ref.	16.19±7.03	8.72	25.46		St. 7, ref.	8.25±0.09	8.12	8.35
S (‰)	St. 1	21.91±1.69	20.29	23.92	DO (mg/L)	St. 1	8.18±1.49	6.11	9.51
	St. 2	22.01±1.37	20.78	23.92		St. 2	7.76±1.19	6.85	9.50
	St. 3	21.79±1.20	20.76	23.00		St. 3	7.21±1.08	6.15	8.17
	St. 4	22.04±1.37	20.77	23.69		St. 4	7.07±1.01	5.99	8.27
	St. 5	22.04±0.88	20.80	22.85		St. 5	7.18±1.12	6.16	8.47
	St. 6	21.87±0.84	20.74	22.74		St. 6	7.08±0.81	5.90	7.69
	St. 7, ref.	22.04±1.93	20.17	24.40		St. 7, ref.	7.76±0.37	7.41	8.20
PO^{-}_4 (mg/L)	St. 1	0.02±0.012	0.01	0.03	NH^{+}_4 (mg/L)	St. 1	0.01±0.00	0.01	0.01
	St. 2	0.015±0.006	0.01	0.02		St. 2	0.01±0.00	0.01	0.01
	St. 3	0.013±0.005	0.01	0.02		St. 3	0.01±0.00	0.01	0.01
	St. 4	0.015±0.006	0.01	0.02		St. 4	0.01±0.00	0.01	0.01
	St. 5	0.01±0.00	0.01	0.01		St. 5	0.01±0.00	0.01	0.01
	St. 6	0.015±0.01	0.01	0.03		St. 6	0.01±0.00	0.01	0.01
	St. 7, ref.	0.01±0.00	0.01	0.01		St. 7, ref.	0.01±0.00	0.01	0.01
TP (mg/L)	St. 1	0.048±0.026	0.02	0.07	TN (mg/L)	St. 1	0.480±0.206	0.30	0.74
	St. 2	0.029±0.013	0.02	0.04		St. 2	0.230±0.126	0.10	0.37
	St. 3	0.026±0.009	0.02	0.04		St. 3	0.427±0.170	0.25	0.66
	St. 4	0.035±0.018	0.02	0.05		St. 4	0.162±0.047	0.10	0.20
	St. 5	0.051±0.066	0.02	0.15		St. 5	0.498±0.379	0.20	0.99
	St. 6	0.065±0.073	0.02	0.17		St. 6	0.498±0.379	0.20	0.99
	St. 7, ref.	0.052±0.072	0.02	0.16		St. 7, ref.	0.262±0.075	0.20	0.35
NO_2+NO_3 (mg/L)	St. 1	0.083±0.04	0.04	0.13	SiO_2 (mg/L)	St. 1	0.367±0.241	0.15	0.60
	St. 2	0.071±0.059	0.04	0.13		St. 2	0.563±0.442	0.20	1.20
	St. 3	0.071±0.059	0.02	0.13		St. 3	0.292±0.225	0.05	0.55
	St. 4	0.036±0.02	0.01	0.06		St. 4	0.575±0.561	0.20	1.40
	St. 5	0.088±0.074	0.03	0.20		St. 5	0.555±0.632	0.17	1.50
	St. 6	0.088±0.063	0.03	0.18		St. 6	0.186±0.156	0.02	0.40
	St. 7, ref.	0.094±0.041	0.05	0.14		St. 7, ref.	0.487±0.477	0.20	1.20
Chl-*a* (μg L^{-1})	St. 1	3.64±3.84	1.61	9.39	TSS (mg/L)	St. 1	11.00±4.62	6.80	17.60
	St. 2	2.64±1.52	1.06	3.96		St. 2	8.07±2.74	4.30	10.20
	St. 3	3.39±2.27	1.09	5.75		St. 3	22.1±22.5	3.80	54.40
	St. 4	2.96±2.26	0.97	6.19		St. 4	9.35±4.85	4.00	15.60
	St. 5	2.59±2.13	1.31	5.76		St. 5	19.1±24.8	3.20	56.00
	St. 6	4.47±5.61	1.07	12.85		St. 6	28.4±29.7	7.20	71.30
	St. 7, ref.	1.49±0.44	1.05	1.98		St. 7, ref.	15.98±8.36	7.70	24.40
COD (mg/L)	St. 1	127.9±52	76.00	198	AS (mg/L)	St. 1	0.045±0.022	0.02	0.07
	St. 2	152.1±102.8	76.00	295		St. 2	0.027±0.016	0.02	0.05
	St. 3	105.0±55.2	40.00	158		St. 3	0.032±0.014	0.02	0.05
	St. 4	87.5±64.1	40.00	181		St. 4	0.040±0.014	0.02	0.05
	St. 5	120.5±111.8	40.00	277		St. 5	0.034±0.011	0.02	0.05
	St. 6	148.3±98.1	53.00	277		St. 6	0.035±0.012	0.02	0.05
	St. 7, ref.	101.5±79.6	40.00	207		St. 7, ref.	0.032±0.016	0.02	0.05

The results of the analysis of variance showing the differences between the stations in the values of Chl-*a* and suspended solids are presented in Table 1. Chl-*a* levels are higher in summer and autumn than at other times. A check was made to determine if the difference between the stations was significant; however, the reference station had a lower Chl-*a* value than the other stations. Nonetheless, this difference was not large enough to be considered significant (Table 1, $p > 0.05$). Correlation analysis showed a significant negative linear relationship ($p < 0.001$) between Chl-*a* and suspended solids, indicating that the loading to the system is terrestrial rather than phytoplanktonic. However, analysis of variance shows that the differences in suspended solids between stations are not statistically significant. Analyses of Chemical Oxygen Demand (COD) and anionic detergent concentrations were conducted on a seasonal basis, with the results of the analysis of variance illustrating the differences between stations as shown in Table 4. After a thorough analysis of the COD values at the different stations, it was found that both the reference station (station 7) and the other station had lower values compared to their counterparts; however, the observed differences were not statistically significant (Table 1, $p > 0.05$).

Anionic detergent values are also presented in Table 1. When comparing the anionic detergent values between the stations, it was found that the values recorded for Stations 1 and 4 were slightly higher than those recorded for the other stations; however, these differences were not statistically significant (Table 1, $p > 0.05$). Additionally, the recorded COD values were higher in autumn and winter compared to the other seasons. In comparison, the anionic detergent values were lower in fall and winter compared to the other seasons.

Granulometry

Sediment composition in the sampling area was as follows: 71.59% sand, 20.16% gravel + shell, and 8.19% mud (clay + silt). The highest sand ratio was observed at station 6, with a value of 92%. Conversely, the highest levels of mud (clay+silt) content (16.71%) and the highest gravel+shell ratio (25.64%) were recorded at station 5 (Table 2).

The Amount of Organic Matter in Water and Sediment

As presented in Figure 5, the quantity of organic matter present in the water and sediment varies with the time of year.

Seasonal Correlations Between Environmental and Pollutant Variables in the Study Area

Relationships between environmental and pollutant variables measured seasonally in the lagoon water are presented in Table 3. Based on Pearson's correlation coefficient, the strongest positive correlations (r = 0.96, 0.94; $p < 0.05$) are observed between PO4 in water and TP and SiO_2 content. Conversely, the lowest correlations (r=0.01; r=0.02, $p < 0.05$) are found between anionic detergent concentration and the total nitrogen (TN), and suspended solids (SSM) and coarse sand and shell fraction in the sediment. The variable with the highest positive correlation (r=0.89, $p < 0.05$) with Chl-*a* levels, which is the determinant of primary production in the water, was the lagoon water temperature.

Principal component analysis (PCA) biplot analysis was employed to summarise the variance structure of the multivariate environmental data and to visualise the relationships between variables (Figure 6). The analysis showed that the first and second principal components (PC1 and PC2) accounted for a significant proportion of the total variation (PC1: 28.44% and PC2: 24.65%). For example, environmental factors such as temperature, salinity, and pH showed positive and significant loadings along the PC1 axis. This illustrates the relationship between temperature and salinity. On the other hand, pollutant variables such as silt+clay percentage, NO_2+NO_3, TP, and ACM had significant effects on the PC2 component, suggesting a connection with the features of nutrients and sediment. The angles formed between the vectors illustrate the degree to which the variables are related. A positive correlation is indicated by variables that are positively related, while a negative correlation is indicated by variables that are inversely related. For example, there is a positive correlation between OM% and TP. As a result, the PCA analysis effectively highlighted the most significant differences between the environmental variables. Of particular importance were the physico-chemical variables and nutrients, which played a key role in distinguishing the samples. These results are crucial for understanding the primary factors driving the observed environmental variation within the study area.

Table 2. Mean seasonal percent granulometry ratios recorded at stations

	Particle type %							
Factor	**Clay+silt**	**Very fine sand**	**Fine sand**	**Medium sand**	**Coarse sand**	**Very coarse sand**	**Gravel and shell**	**Coarse gravel and shell**
Stn, 1	14.16	13.89	18.04	9.43	8.91	11.46	14.74	9.3
Stn, 2	3.17	10.96	15.93	19.57	**16.49**	15.72	10.47	7.65
Stn, 3	13.24	7.25	7.92	9.85	12.82	**17.3**	**19.72**	11.83
Stn, 4	5.27	20.08	**43.68**	7.1	8.91	6.42	4.12	4.36
Stn, 5	**16.71**	**23.66**	9.27	7.17	7.75	9.76	11.85	**13.79**
Stn, 6	3.29	13.67	43.94	**25.06**	6.16	3.91	2.59	1.38
Stn, 7, ref,	1.49	3.1	15.26	17.55	15.88	**17.3**	16.11	13.25
Mean	8.19±6.27	13.23±7.06	22.00±15.32	13.67±4.04	10.98±4.08	11.69±5.34	11.37±6.24	8.79±4.65

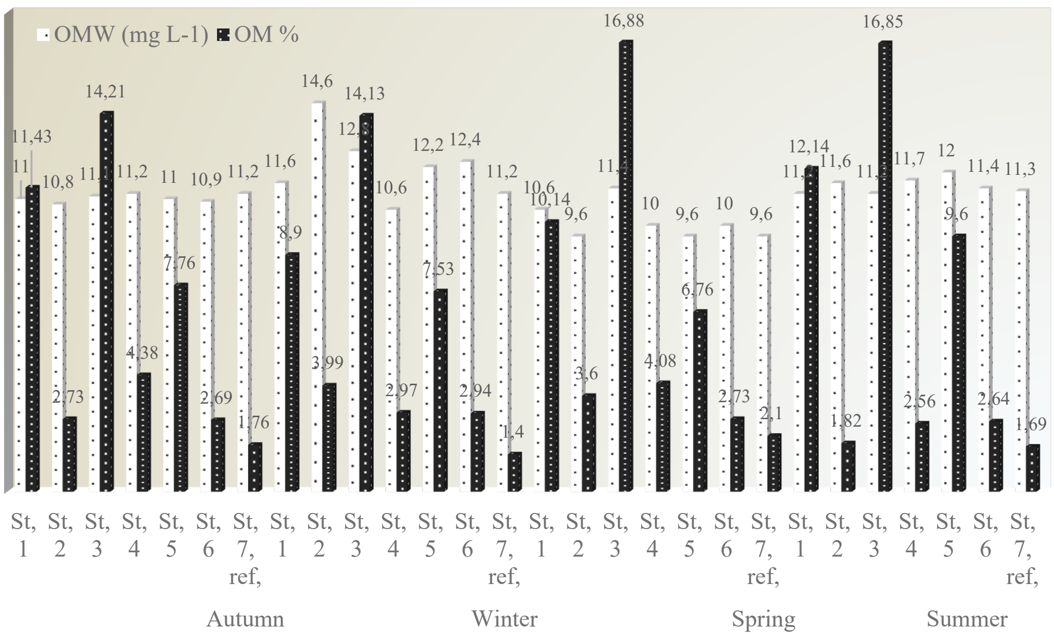

Figure 5. Spatio-temporal amount of organic matter in the water and sediment measured in the Çardak Lagoon

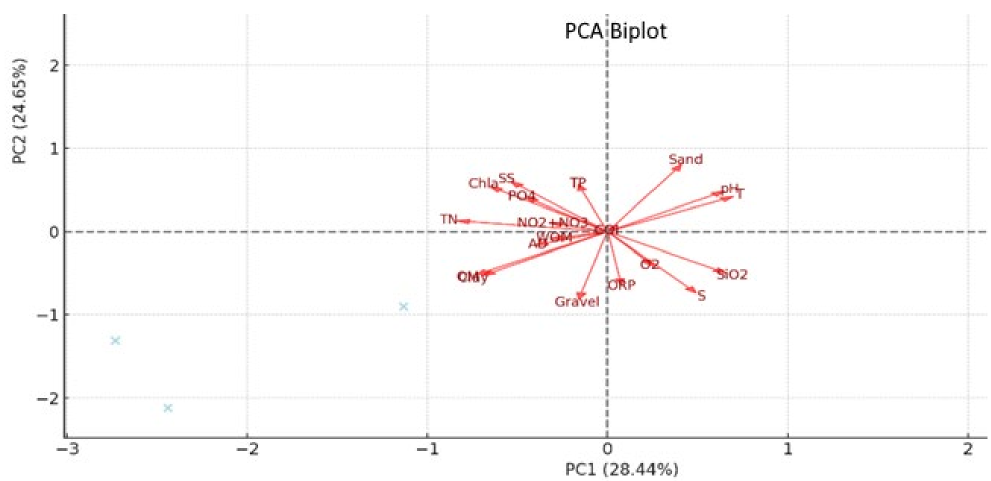

Figure 6. PCA ordination diagram showing the correlations between environmental variables and pollutants

Table 3. Seasonal correlations between environmental and pollutant variables in Çardak Lagoon
OM %: Oragnic matter in sediment, SOM: Organic matter in water (mg/L), C+S: Clay+Silt, T: Temperature, S: Salinity,
DO: Dissolved Oxygen (mg/L), Chl-*a*: Chlorophyll-*a* (($\mu g\ L^{-1}$), TP: Total phosphate (mg/L), TN: Total Nitrogen (mg/L), NO2+NO3: Nitrate+Nitrogen (mg/L),
TSS: Total Suspend Solid (mg/L), COD: Chemical Oxygen Demand,
AD: Anionic Detergent (mg/L), PO-4: Phosphate mg/L), S: Sand, CS+Sh.: Coarse Sand + Shell
(Pearson correlation r, p<0.05).

Variables	OM %	SOM	C+S %	T (ºC)	S (‰)	DO	ORP	pH	Chl-*a*	SiO_2	TP	NO_2+NO_3	TN	TSS	COD	AD	PO_4	S %	CS+sh%
OM%		-0,62	-0,97	0,91	-0,67	-0,08	-0,12	0,78	0,71	0,36	0,76	-0,58	0,08	-0,92	-0,76	0,42	0,63	0,30	0,36
SOM	-0,62		0,42	-0,24	0,25	0,57	-0,12	-0,57	-0,01	0,50	-0,03	0,08	-0,55	0,60	0,44	-0,79	0,21	0,04	-0,36
C+S %	-0,97	0,42		-0,97	0,78	-0,16	0,05	-0,66	-0,87	-0,56	-0,83	0,55	-0,02	0,93	0,67	-0,18	-0,76	-0,47	-0,19
T (ºC)	**0,91**	-0,24	-0,97		-0,71	0,22	-0,19	0,66	**0,89**	0,72	0,93	-0,66	-0,18	-0,83	-0,70	0,09	0,89	0,41	0,23
S (‰)	-0,67	0,25	0,78	-0,71		-0,62	-0,55	-0,07	-0,90	-0,44	-0,41	-0,06	-0,46	0,88	0,06	0,29	-0,48	-0,91	0,45
O2	-0,08	0,57	-0,16	0,22	-0,62		0,56	-0,58	0,63	0,59	0,09	0,35	0,13	-0,19	0,51	-0,93	0,35	0,84	-0,82
ORP	-0,12	-0,12	0,05	-0,19	-0,55	0,56		-0,66	0,19	-0,27	-0,52	0,86	0,88	-0,27	0,72	-0,47	-0,40	0,77	-0,88
pH	0,78	-0,57	-0,66	0,66	-0,07	-0,58	-0,66		0,24	0,19	0,73	-0,87	-0,34	-0,49	-0,99	0,77	0,50	-0,35	0,86
Chl.-*a*	0,71	-0,01	-0,87	**0,89**	-0,90	0,63	0,19	0,24		0,78	0,73	-0,29	0,03	-0,80	-0,29	-0,34	0,81	0,77	-0,24
SiO2	0,36	0,50	-0,56	0,72	-0,44	0,59	-0,27	0,19	0,78		0,81	-0,55	-0,57	-0,30	-0,32	-0,47	0,94	0,38	-0,03
TP	0,76	-0,03	-0,83	**0,93**	-0,41	0,09	-0,52	0,73	0,73	0,81		-0,87	-0,54	-0,57	-0,81	0,12	0,96	0,12	0,45
NO_2+NO_3	-0,58	0,08	0,55	-0,66	-0,06	0,35	**0,86**	-0,87	-0,29	-0,55	-0,87		0,74	0,24	0,93	-0,45	-0,74	0,39	-0,82
TN	0,08	-0,55	-0,02	-0,18	-0,46	0,13	**0,88**	-0,34	0,03	-0,57	-0,54	0,74		-0,39	0,46	0,01	-0,55	0,52	-0,56
SSM	-0,92	0,60	**0,93**	-0,83	**0,88**	-0,19	-0,27	-0,49	-0,80	-0,30	-0,57	0,24	-0,39		0,46	-0,18	-0,50	-0,62	0,02
COD	-0,76	0,44	0,67	-0,70	0,06	0,51	0,72	-0,99	-0,29	-0,32	-0,81	**0,93**	0,46	0,46		-0,68	-0,61	0,34	-0,86
AD	0,42	-0,79	-0,18	0,09	0,29	-0,93	-0,47	0,77	-0,34	-0,47	0,12	-0,45	0,01	-0,18	-0,68		-0,17	-0,62	0,83
PO_4	0,63	0,21	-0,76	**0,89**	-0,48	0,35	-0,40	0,50	0,81	**0,94**	**0,96**	-0,74	-0,55	-0,50	-0,61	-0,17		0,28	0,22
Sand %	0,30	0,04	-0,47	0,41	-0,91	0,84	0,77	-0,35	0,77	0,38	0,12	0,39	0,52	-0,62	0,34	-0,62	0,28		-0,78
CS+sh %	0,36	-0,36	-0,19	0,23	0,45	-0,82	-0,88	**0,86**	-0,24	-0,03	0,45	-0,82	-0,56	0,02	-0,86	0,83	0,22	-0,78	

This study clarifies the temporal and spatial interactions between domestic pollutants and environmental factors recorded in different areas of the Çardak Lagoon, located in the charming settlement of Çardak Town, within the Laspseki district, adjacent to the Çanakkale Strait. The importance of geographical and temporal variation is crucial in the examination of ecological systems. Nonetheless, it is often not possible to assess both types of variability at the same time. This problem is similar in studies of aquatic ecosystems, tend to be ephemeral in time or area. A large number of comparative studies are needed to clarify the spatial and temporal variations observed in lagoon areas. These studies are essential for understanding models that can be formulated for the efficient management of lagoons (Acquavita et al., 2015). Coastal lagoons are highly productive eutrophic ecosystems (Specchiulli et al., 2008) and play a significant role in regulating human impacts on coastal areas (Arévalo et al., 2013). However, such ecosystems are often subject to eutrophication, leading to a deterioration in their overall quality (Arévalo et al., 2013; Arima et al., 2013; Acquavita et al., 2015; Coelho et al., 2007; Arvamidis et al., 2013; Amos et al., 2017).

Recent studies show that the enrichment of nutrients and sediment particle size in lagoons have a significant impact on the stress levels observed in biota (Lardicci et al., 1997; Specchiulli et al., 2010). The main obstacles identified in lagoons are summer denitrification, increased surface temperatures, relatively low water flow, increased consumption of dissolved oxygen due to an excess of organic matter, and an increase of photophilous algae and bacterial communities (Sylasios & Theocharis, 2002). The temporal variability of water chemistry in coastal lagoons is a widely recognised characteristic of these ecosystems, and represents a major challenge in the efforts to accurately define them. Therefore, relying on isolated measurements of water chemistry may not be sufficient for the comprehensive characterisation of these lagoonal areas (Lucena-Moya et al., 2012).

Table 4 outlines the values of physico-chemical variables recorded in previous studies within the lagoon areas of the Mediterranean and northeast Atlantic coastlines. Environmental factors, such as dissolved oxygen levels, temperature variations, and salinity influence the complexity of biotic communities and numerous biogeochemical cycles (i.e., naturally occurring substances involved in the cycling of elements such as sulfur and nitrogen) within lagoon environments (Rosenberg et al., 2001). The differences in the measurements collected from different areas of the Mediterranean, together with the figures reported in this study, show minimal variations. Temperature and salinity are the most variable parameters of water quality. The highest temperature, reaching an impressive 39.0‰, was recorded in the lagoon of Bizerte, Tunisia, while the lowest temperature of 0.27‰ was observed in the lagoon of Klisova, located in western Greece. The lowest water temperature recorded was 3.4°C in Homa Lagoon, found in the eastern Aegean Sea, while the highest was 32.2°C, noted in Myrtari Lagoon, situated in western Greece. The decomposition of organic matter in the sediment leads to an increased concentration of nutrients in the water column (Arvamidis et al., 2017; Kennish & Paerl, 2010).

The hydrological dynamics of coastal lagoons, known for their high biomass and productivity (Reizopoulou et al., 1996), combined with their limited connectivity, result in a marked accumulation of organic matter in their sediments. It is generally accepted that the level of organic matter in sediments is closely related to sediment particle size (Tyson, 1995). However, the organic matter concentration in coastal lagoons is typically higher than that observed in coastal marine ecosystems (Tyson, 1995; Afli et al., 2009a). In addition, human activities can increase this percentage to about 2% (De Falco et al., 2000). Several previous studies have assessed the organic matter concentration in sediments of different Mediterranean lagoons (Khedhri et al., 2016). In this study, the values ranged from a low of 1.26% to a high of 29.1% during the summer season in the Boughrara lagoon (eastern Tunisia). The study found that the lowest amount of organic matter in the sediment (1.4%) was observed in February 2019, while the highest amount (16.88%) was found in April and June 2019 at the reference site.

Sediment particle dimensions are an important environmental factor in the analysis of lagoon areas (Avramidis et al., 2013). Several research efforts have focused on the sediment particle dimensions across the Mediterranean lagoons (Table 5). This research has shown that the highest sand proportion (97. 29%) and lowest sand proportion (6. 75%), percentage of sand in the sediment, as well as the highest (21. 59%) and lowest (21.59%) percentages of mud proportion in the sediment, were observed during the autumn and winter seasons. Coastal lagoons with high light penetration are characterised by elevated nutrient concentrations. Phosphorus and nitrogen inputs lead to eutrophication within the lagoons. Many detailed studies have been carried out on the extent of anthropogenic stress on these other Mediterranean lagoons. The different levels found in these studies are shown in Table 6. The median values of nutrient concentrations in the Çardak Lagoon were found to be lower than those observed in other Mediterranean lagoons. It is recognised that human activities are the main factor leading to spatial variability in water chemistry. The mean annual concentration of NO^{-}_{2}+NO^{-}_{3} in the lagoon water was found to be 0.076 mg/L.

Table 4. Physico-chemical variables recorded in coastal lagoons at different locations in the Mediterranean Sea and the North-east Atlantic

Locality	Values	T (°C)	S (‰)	DO (mg/L)	pH	Reference
Güllük Lagoon (Eastern Aegean Sea)	Ort.	19.53±3.48	10.65±0.88	7.32±0.69	8.00±0.08	Egemen et al. (1999)
Homa Lagoon (Eastern Aegean Sea, Türkiye)	Min./Max.	3.4-26.6	33.1-61.6	2.3-9.6	8.0-8.8	Can et al. (2009)
Bizerte Lagoon (North Tunisia)	Min./Max.	10.0-30.6	21.4-38.4	4.3-9.3	-	Boukef et al. (2012)
Myrtari Lagoon (Western Greece)	Min./Max.	9.6-32.2	2.45-19.5	5.9-11.1	7.51-8.47	Avramidis et al. (2013)
Marano and Grado Lagoons (Italy)	Mean	6.32-26.0	17.2-28.7	7.0-10.8	-	Acquavita et al. (2015)
Almargem (A) and Salgados (S) Lagoons (Southern Portugal)	Mean	S.; 20.9±3.49/A.; 21.8±4.59	S.; 8.2±5.25/A.; 13.1±7.96	S.; 10.2±5.07/A.; 8.9±1.61	S.; 8.6±0.61/A.; 8.3±0.39	Coelho et al. (2015)
Saros Bay Lagoons (Northeastern Aegean Sea)	Min./Max.	18-27.7	0.8-57.9	-	7.9-8.9	Barut et al. (2015)
Klisova Lagoon (Western Greece)	Min./Max.	7.6-28.1	0.27-13.63	0.42-9.08	7.5-9.2	Avramidis vd. (2017)
Çardak Lagoon (Turkish Straits System	Mean.	15.98±7.01	21.95±0.85	7.46±1.12	8.22±0.09	This study

S: Salinity T: Temperature DO: Dissolved oxygen

Table 5. Sediment particle values recorded in coastal lagoons at different localities in the Mediterranean and northeastern Atlantic

Locality	Sand %	Clay+silt %	Coarse sand +Shell %	Reference
Thau Lagoon (Southern France)	23.8-91.6	8.0-76.2	0.2-0.6	De Casabianca et al. (1997)
Cadiz Lagoon (Southern Spain, Atlantic)	3.68	96.32	-	Drake & Arias (1997)
Tancada Lagoon (Eastern Spain)	Mean: 23.35	Mean: 76.55	-	Menendez et al. (2004)
Cabras Lagoon (Western Sardinia Island)	Mean: 5.11	Mean: 94.87	-	Magni et al (2005)
Obidos Lagünü (Batı Portekiz, Atlantik)	-	4-98.40	-	Carvalho et al. (2005)
Venice Lagoon (Northern Adriatic)	Mean: 19	Mean: 0.3	-	Molinaroli et al. (2014)
Bizerte Lagoon (Tunisia)	20-80	17-80<	<15	Afli et al. (2009b)
Karavasta Lagoon (Albania, Adriatic)	Mean 6.11	Mean. 93.88	-	Munari et al. (2010)
Myrtari Lagoon (Western Greece)	42-72.81	18.87-62.10	-	Avramidis et al. (2013)
Saros Bay Lagoons (Northeastern Aegean Sea)	2.71-86.29	0.73-96.18	0.51-36.32	Barut et al. (2015)
Homa Lagoon (Eastern Aegen Sea, Türkiye)	15-45	48-85	-	Can et al. (2009)
Almargem and Salgados Lagoons (Southern Portugal, Atlantic)	Mean A.; 81.4/S.; 71.0	Mean A.; 15.6/S.; 19.6	-	Coelho et al. (2015)
Çardak Lagoon	46.75-97.29 Mean: 71.59	0.27-21.59 Mean: 8.19	2.15-48.11 Mean: 20.16	This study

Although no significant irregularities are noted in the annual nutrient values in the study area, which is characterised by shallow water (mean 1.3 m), the NH^{+}_4 value remains below 0.01 mg/L throughout the year. Nonetheless, the peak levels of total nitrogen reached 0.99 mg/L and total phosphorus at 0.17 mg/L. Ligorini et al. (2023) found that the highest nutrient levels were recorded in winter in three lagoons located on Corsica Island. Nutrient levels were also found to be low during the winter season in this study. About 83% of the phosphorus pollution detected in marine and lagoon ecosystems is attributed to industrial and wastewater discharges. Detegent accounts for 32-70% of phosphates in wastewater (Sarı, 2005). Detergent concentrations in surface water samples collected from lagoons have been examined in prior studies. Among these studies, Stora & Arnoux (1983) reported detergent concentrations ranging from 31.19 to 62.90 mg/L in the Etang de Berre lagoon (southern France). In Türkiye, anionic detergent levels were found to remain below 0.50 mg/L throughout the year in the Güllük lagoon (eastern Aegean Sea) (Egemen et al., 1999).

Topçuoğlu et al. (1999) recorded the highest detergent concentration as 0.228 mg/L at the surface (0 m) in the Küçükçekmece lagoon area in April. The average seasonal concentration of anionic detergents in the study area is 0.035 ±0.015 mg/L, while a notably higher level of 0.116 mg/L was found in Küçükçekmece lagoon. The presence of suspended solids in water serves as a significant source of physical pollution, leading to various detrimental effects, including increased turbidity, elevated toxicity levels, and reduced dissolved oxygen content (Tavora et al., 2019). In this context, Carvalho et al. (2011) reported a minimum suspended concentration of 21 mg/L during the winter season, with a maximum of 153 mg/L reached during the spring season within the Obidos Lagoon of western Portugal. The Çardak lagoon showed an average seasonal concentration of suspended solids of 16.29 mg/L.

Relationships Between Environmental and Pollutants Variables

Relationships between environmental factors in Mediterranean lagoons have been analysed in many studies. As noted by Kormas et al. (2001), Logarou, Rodia, and Tsoukalio found that among the environmental variables evaluated in the lagoons of the Amvrakikos Gulf in western Greece, salinity and temperature had the strongest positive correlation ($r = 0.73$; $p < 0.001$).

Despite the presence of a weak negative correlation between the chlorophyll-*a* values and the salinity/temperature metrics recorded in these three lagoons, they have relatively high nutrient contents (excluding PO^{-}_4) compared to the Amvrakikos Gulf. This study found a robust positive correlation ($r = 0.88$; $p < 0.05$) between lagoon water temperature and oxidation-reduction potential (ORP). Specchiulli et al. (2008) reported a positive correlation ($r = 0.83$; $p < 0.001$) between chlorophyll-a levels and temperature, and a significant negative correlation ($r = -0.93$; $p < 0.001$) between temperature and dissolved oxygen concentrations in the Orbetello Lagoon, located on the northern Adriatic coast. The researchers identified the strongest positive correlation between NH^{+}_4 and temperature ($r = 0.75$; $p < 0.001$) and the weakest negative correlation between NO^{-}_2 and TP ($r = -0.55$; $p < 0.001$) in the Varano lagoon of the Adriatic Sea. The present study has revealed a robust positive correlation ($r = 0.89$, $p < 0.05$) between seasonal chlorophyll-*a* concentrations and water temperature, as observed within the Orbetello Lagoon. Furthermore, a modest positive correlation was observed ($r=0.22$; $p < 0.05$), which contrasts with the correlation coefficient observed between water temperature and dissolved oxygen levels in the Orbetello Lagoon. Souchu et al. (2010) studied the relationships between environmental variables in a total of 20 lagoons in the eutrophicated south of France and the island of Corsica in the western Mediterranean Sea between 1998 and 2002. The highest correlation ($r = 0.92$; $p = 0.0001$) between the environmental variables measured in these 20 lagoons was found between TN and TP. In a study by Pérez-Ruzafa et al. (2005) conducted in the Mar Menor lagoon (south-eastern Spain), which is one of the largest lagoons in the western Mediterranean, the strongest correlations among the environmental variables of the lagoon water were observed between Chl-*a*+2 and temperature (r=0.93), salinity (r=0. 93), and NO^{-}_2 (r=0.81). In another study, Cañedo-Argüelles et al. (2012) identified a moderate negative correlation ($r = -0.68$) between pH and NO_3 in the Remolar lagoon (northeastern Spain). Conversely, there exists a strong negative correlation ($r = -0.87$, $p <0.05$) between the summer months and the other periods of sampling for NH^{+}_4, dissolved oxygen saturation, and Chl.-*a* recorded in the waters of the Ria Formosa Lagoon (southern Portugal), located in the northeast Atlantic outside the Mediterranean ecosystem. Nevertheless, markedly significant differences ($p \leq 0.01$) were found between the annual means of most water variables in Almargem and Salgados lagoons, situated in southern Portugal (Coelho et al., 2015).

Furthermore, only the concentration of Chl-*a* in the water showed considerable variability, while the variability between the annual means of sediment variables in the same lagoons was not significant. The strongest positive correlation ($r = 0.96$, $p < 0.05$) was observed between the concentrations of PO^{-}_4 and TP in the water. Avramidis et al. (2013) reported that the amount of PO^{-}_4-P in Myrtari Lagoon (western Greece), which was recorded between <0.01-0.03 mg/L throughout the year, decreased significantly in 2010 and 2011 compared to previous years (especially the phosphate increase observed in 2001-2002 with a maximum of 1.79 mg/L). They explained this situation in terms of the increased dissolved oxygen concentrations in the water of Myrtari Lagoon.

In shallow lagoons, the movement of pollution or its dilution is mainly influenced by the interaction between rainfall and the intense wave action caused by strong winds. The accumulation of organic matter, mainly due to domestic pollution, has been observed to occur mainly during the summer and autumn seasons, which are characterised by lower wind speeds (1-12 km/h) and little wave action. The water discharged from the outfall has a lower specific mass than that of the lagoon water, but wave action can promote horizontal stratification and subsequent dispersion away from the outfall, all while avoiding direct contact with the surface. Regarding the lagoon's water quality, variability and inconsistency in the measurement of certain environmental variables (e.g. %OM in sediment, organic matter in water, chlorophyll-a, nutrients, suspended solids, COD, anionic detergents), especially in the seaward regions, may be misleading. The effects of the wind and the intensity of the strong waves observed in the study area may contribute to a decrease in pollution. The dynamic tides of the currents and waves, primarily influenced by the strong north and southwest winds, facilitate a significant transfer of pollutants, especially from the lagoon, to the shallow water environment of the study area, with an average depth of 1.3 meters. Peak %OM values of 12.1% and 16.88% were recorded during the summer season, correlating with wind speeds of 1 km/h. In contrast, the percentage of organic matter (%OM) decreased to 8.90% and 1.13% during the winter sampling period, which was characterised by wind speeds between 39 and 49 km/h. Nevertheless, the seasonal mean of %OM in the sediment at the seaward stations of the lagoon varied between 2.7% and 3.9%.

The observed variation in the physico-chemical properties of the lagoon water can be attributed to the waves and currents induced by the strong winds in the Çanakkale Strait. In contrast to other seasons, the elevated salinity levels observed in February 2019 suggest that the study area is more influenced by the Çanakkale Strait current system during the winter months. The water quality data collected in this study are in close agreement with the results of previous studies conducted in the region; the observed discrepancies are likely due to seasonal variations and terrestrial inputs (Uzundumlu & Büyükateş, 2019).

The concentrations of NH_4^+, a crucial parameter in evaluating water quality, were consistently low across all monitoring stations and throughout all seasons. This is in marked contrast to the findings of multiple studies conducted at various times during the study period in a region adjacent to the area under investigation (Büyükateş et al., 2017). The levels of phosphate (PO-P) and silicate (SiO_2), critical indicators of water pollution, are markedly diminished compared to the findings of previous study conducted by Büyükateş et al. (2017) in the Çanakkale Strait, notable variations in the concentration of suspended solids in the lagoon water were observed both across different stations and throughout various seasons within the study area. These fluctuations may be indicative of the lagoon being influenced by both terrestrial inputs and the dynamic impacts of substantial wave action and strait currents. The concentration of phosphate (PO) in aquatic environments is intricately connected to the presence of anionic detergents. Levels of anionic detergents and phosphates in surface waters increase during the summer months, primarily due to heightened cleaning activities, in contrast to other seasons. This study observed a notable consistency in the concentrations of detergents and phosphates in the surface water of the lagoon across various seasons. The peak Chlorophyll-a levels observed during the summer months can be attributed to two main factors: an increase in air temperature and the subsequent phosphate run-off from the terrestrial surroundings of the lagoon. The analysis of chlorophyll-a and suspended solids measurements recorded in the study area indicates that the observed system load is mainly due to terrestrial inputs. According to the findings of Kanarska and Maderich (2008), the mean concentration of suspended solids in the water column of the Dardanelles was measured to be 1.65 mg/L. Despite the influence of erosion, transport processes, and wave dynamics, significantly elevated values can be observed within the study area and its neighbouring coastal regions. The annual mean concentration of suspended solids in the study area, which ranges from 8.07 to 28.1 mg/L, can be attributed to the interaction of transport dynamics with vigorous wave activity.

Table 6. Nutrient levels recorded in lagoon areas in the Mediterranean and eastern Atlantic

Locality	NH^{+}_{4} (µM)	NO^{-}_{2} (µM)	NO^{-}_{3} (µM)	Chl.-*a* (µg L^{-1})	PO^{-}_{4} (µM)	Reference
Güllük Lagoon (Western Türkiye)	14.66±4.09	0.61±0.21	3.67±2.79	5.5±3.40	0.08±0.07	Egemen et al. (1999)
Vistonis Lagoon (Northeastern Aegean Sea)	Mean: 25.5	-	Mean: 125.4	Mean: 39.6	Mean: 104.5	Gikas et al. 2006
Lapalme Lagoon (Southern France)	2.85	48±11.8	0.15±0.1	1.9±0.7	0.31±0.0	Carlier et al. (2008)
Bizerte Lagünü (Kuzey Tunus)	-	-	-	3.3- 5.0 (ort.min./maks.)	-	Boukef et al. (2012)
Marano and Grado Lagoons (It-aly, Northern Adriatic)	0.04–26.4 (3.97)	0.03–12.2 (1.00)	0.12–368 (59.0)	0.06–111 (1.31)	0.05-522 (5.44)	Acquavita et al. (2015)
Klisova Lagoon (Western Greece)	0.22-13.48	Low throughout the year	0.026-0.42	-	Low throughout the year	Avramidis et al. (2017)
Senillar de Moraira Lagoon (Eastern Spain)	Mean: 3.0 µmol L^{-1}	-	50-180 µmol L^{-1}	1-5 µmol L^{-1}	0.6-9.2 µmol L^{-1}	Camacho et al. (2012)
Almargem (A) and Salgados (S) Lagoons (Southern Portugal)	S.; 56.0±123.0/A.; 3.2±3.0	S.; 5.7±7.79/A.; 0.7±0.61	S.; 17.9±29.82/A.; 26.8±42.41	S.; 158.5±177.95/A.; 3.0±3.43	S.; 65.2±42.04/A.; 1.3±0.76	Coelho et al. (2015)
Çardak Lagoon	<0.01	NO_2+NO_3 0.015-0.135		0.97-12.85	<0.01-0.03	This study

Conclusion

The designated protection zone within the study area experienced a progressive decrease in depth over time, which was attributed to the accumulation of organic matter in the sediment. The sediment of the lagoon showed robust populations of photophilic algae, particularly during the spring and summer months. The increased growth rate of these photophilic algal populations suggests a significant influx of nutrients into the ecosystem. Excessive nutrient inputs have been shown to act as a catalyst for lagoon pollution. Robust wave dynamics, generally associated with strong wind conditions, enhance the considerable dispersion of pollutants throughout the study area. Although the prevailing winds in the lagoon are expected to mitigate the effects of the discharge, significant pollution indicators have been detected in the vicinity of the discharge sites. The majority of Mediterranean lagoons have eutrophic trophic status, with the most pristine examples being Mar Menor in Spain, Amvrakikos in Greece and Mare Piccolo in Italy. To mitigate the risks to these regions, it is imperative to adopt robust resource management strategies that ensure the protection of pristine estuaries.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: The data will be made available upon request from the author.

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Research Article

Utilising water hyacinth (*Eichhornia crassipes*) in Nile tilapia (*Oreochromis niloticus*) diets: Effects on growth, digestibility, and optimal inclusion rate

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ABSTRACT

This study investigates the potential of water hyacinth (*Eichhornia crassipes*) leaves as a protein-rich dietary alternative for *Oreochromis niloticus* (Nile tilapia). Water hyacinth is an invasive aquatic plant with promising nutritional properties, including moderate protein content and low levels of antinutritional factors. The digestibility of WH leaves was assessed, and their optimal incorporation level in fish feed was evaluated. Apparent digestibility coefficients (ADCs) for WH leaf were 77.49% (dry matter), 69.58% (crude protein), and 59.14% (energy). Six experimental diets containing 0–12% WH leaf were formulated and fed to *O. niloticus* fingerlings over 45 days. Growth performance, feed utilisation, and carcass composition were analysed. Results showed that inclusion levels up to 6% did not significantly affect growth, feed conversion ratio, or protein efficiency; however, inclusion levels above 6% led to reduced zootechnical performance and a lower nutritional quality of fish flesh. Survival rates remained unaffected across all treatments. The study concludes that WH leaves can be safely included in tilapia diets at a maximum of 6% without adverse effects on growth, feed efficiency, or flesh quality, offering a sustainable feed alternative in aquaculture.

Keywords: Incorporation, Digestibility, Tilapia diet, Water hyacinth

Introduction

Water hyacinth (WH) (*Eichhornia crassipes*) is considered a pest in several tropical and semi-tropical countries because it disrupts river transport, irrigation and aquatic ecosystems (Edwards et al., 1985). It is a highly invasive aquatic plant native to South America and has spread throughout the world, especially in Africa and Asia (Dagno et al., 2007; Tchiaze & Priso, 2016). Several studies have been carried out on this plant for its eradication, given the damage caused by its proliferation in aquatic ecosystems (Tchiaze & Priso, 2016; Fox et al., 2008). Despite the damage caused by Water Hyacinth, some studies have been able to transform the proliferation of water hyacinth into an opportunity and find practical uses for this plant (Hassan et al., 2015; Fox et al., 2008). Water hyacinth is an aquatic plant rich in mineral elements and possesses several significant properties. By examining its biochemical composition, we note that it contains interesting nutrients for animal feed. Indeed, it contains the vitamins (Hasan et al., 1990) and amino acids (Mahmood et al., 2016) necessary for animal growth, especially in herbivores, or it is used as a protein supplement in the diets of ruminants and rabbits. It is also used in the production of biofuel (Shanab et al., 2018). In addition, water hyacinth contains, on a dry basis, between 12 and 35% protein and has a very low or almost non-existent level of antinutritional factors, such as tannins (Lareo & Bressani, 1982). It also plays a purifying role in wastewater treatment plants (Fox et al., 2008), and its leaves are recently used for the production of black soldier fly larvae (Vodounnou et al., 2024).

In aquaculture, some studies have explored its use (Hontiveros & Serrano, 2015; Saha & Ray, 2011) to reduce production costs associated with feed. In fact, in sub-Saharan Africa, the primary issue related to aquaculture is the reliance on imported fish feed, which is significantly more dependent on feed from developed countries (FAO, 2020). This situation hinders the profitability of aquaculture production. To remedy this, aquaculture operators and research institutions are seeking alternatives to reduce the cost of feed, which can account for up to 75% of aquaculture operating expenses. These alternatives include the use of various local animal by-products (Vodounnou et al., 2025; Alofa et al., 2023; Agbohessou et al., 2021; Ng et al., 2001) or plant (Chabi et al., 2015; El-Saidy & Gaber, 2003) origin as sources of protein, energy, and others. The use of macrophytes, such as ferns and aquatic plants, is also an alternative due to their nutritional value. Examples include the use of azolla in fish feed (Abou et al., 2007) and the use of water hyacinth as a feed supplement in aquaculture (Hontiveros & Serrano, 2015; Saha & Ray, 2011).

Given the abundance of this plant and its nutritional value, and considering the disadvantages and damages associated with its eradication through chemical control, it is appropriate to seek ways to enhance the value of this macrophyte for the production of animal proteins. It is for this purpose that the present study aims at the incorporation of this plant in the diet of *O. niloticus*. However, incorporating an ingredient into feed requires information on its bromatological composition, the optimal rate of incorporation, and its digestibility. These elements are crucial in assessing the ability of the ingredient to be valued by an animal organism in terms of its availability and utilisation of nutrients (Liu et al., 2009; Md Mostafizur et al., 2016). Digestibility depends not only on the animal species but also on the animal's diet (Hien et al., 2010). *Oreochromis niloticus* is an omnivorous species with a tendency towards herbivory that is widespread globally. It is a popular species with high economic value. Its production is approximately 6 to 7 million tonnes per year and is the most widely produced fish species in the world (FAO, 2020), which justifies its inclusion in this study.

Materials and Methods

Study Area

The research was conducted at the Aquaculture and Fisheries Management Research Unit (URAGeP) of the Aquaculture School (EAq) at the National University of Agriculture (UNA) in the Republic of Benin. URAGeP is located in Adjohoun, Ouémé Department in southern Benin (6° 46' 18.73" N | 2° 30' 2.32" E).

Choice of Part of Water Hyacinth to be Valued

To gain a comprehensive understanding of the nutritional characteristics of water hyacinth (WH) plants, various components, including roots, stems, and leaves, were carefully analysed using the methods outlined by the Association of Official Analytical Chemists (1990). The parts were carefully separated and dried prior to analysis. Key parameters, including dry matter protein content, ash content, lipid content, fibre content, and nitrogen-free extract (NFE) content, were then measured and recorded for each component, as detailed in Table 1.

Table 1 Nutritional composition of water hyacinth from the Ouémé River, Benin

Parameter	Plant part (%)		
	Leaf	Root	Stem
Dry Matter	83.99	90.04	89.99
Lipid	0.36	0.15	0.30
Ash	11.66	39.33	20.56
Protein	14.31	6.39	6.46
Fiber	15.03	10.72	22.75
NFE	42.62	33.44	39.92

Preparation of Diets for the Digestibility Test

The nutritional requirements of *O. niloticus* (Mugo-Bundi et al., 2015) were taken into consideration when formulating the reference diet (Table 2). To conduct the digestibility test, the reference diet was combined with the WH leaf, resulting in a test diet that consisted of 70% reference diet by weight and 30% WH leaf (Sklan et al., 2004). Chromic oxide (Cr_2O_3) was used as the inert marker at a rate of 1% in the reference diet. The various ingredients of the reference diet and WH leaf were ground, weighed, and mixed before being extruded through a 2 mm mesh. The diets (Table 2) were then packaged and stored in a refrigerator at 5 °C.

Fecal Collection

The acclimatisation period lasted for 7 days to allow the fish to adjust to their new conditions before the start of the study. To ensure complete evacuation of the digestive contents of the feed consumed during acclimatisation, faecal collection occurred 3 days after the study began (Koprucu & Ozdemir, 2005). Twice a day, at 7 a.m. and 6 p.m., faecal samples were collected manually through stripping. Immediately after collection, the samples were stored in a freezer at -20 °C.

Chemical Analyses of Diets and Faecal Samples

Proteins, lipids, and dry matter were analysed in all diets and faeces. Protein and lipid determinations were performed in triplicate according to standard methods (Association of Official Analytical Chemists, 1990). To determine the dry matter content of the samples, 10 g of the sample was dehydrated in an oven at 105 °C overnight. The method described by Furukawa and Tsukahara (1966) was used to determine the amount of chromium oxide in the diet and faeces.

Experiment on the Digestibility Study

The digestibility study was conducted using six rectangular aquaria, each with a water volume of 60 litres. The experimental device consisted of a randomised Fisher block with two treatments and three repetitions each. *O. niloticus* fingerlings with an average individual weight of 4.46 ± 0.2 g were selected for the experiment, with a density of twenty fish per tank (one fish per 2 L). The fingerlings were fed three times daily for 30 days. Physicochemical parameters of the water, such as temperature, pH (WTW 340i/SET-2E30-101201FB), and dissolved oxygen (WTW Oxi 340i/SET-2B30-0017FB), were measured twice daily (8 am and 5 pm).

Table 2: Diets for the digestibility test

Ingredient	T0 (%)	T1 (70% T0 + 30% WH)
Fish meal	30	-
Soybean meal	20	-
Wheat bran	15	-
Corn flour	20	-
Moringa leaf	3	-
Starch	2	-
Methionine	2	-
Lysine	2	-
Dicalcium phosphate	1	-
Premix (Vit. + Min.)*	1	-
Palm oil	3	-
Cr_2O_3	1	-
T0	-	70
Water hyacinth leaf	-	30
Total (%)	100	100
Protein (%)	35.88	29.40
Lipid (%)	10.75	7.63
Carbohydrate (%)	30.43	25.81
Energy (kcal/kg)	3619.9	2895.1

T0: reference diet (A0 with Chromic oxide (Cr_2O_3); T1: test diet
* premix (vitamin – mineral) contains (‰): vitamin A, 4,000,000 U.I.; vitamin D, 800,000 IU; vitamin E, 40,000 IU; vitamin K3, 1600 mg; vitamin B1, 4000 mg; vitamin B2, 3000 mg; vitamin B6, 3800 mg; vitamin B12, 3 mg; vitamin C, 60,000 mg; biotin, 100 mg; inositol, 10,000 mg; pantothenic acid, 8,000 mg; nicotinic acid, 18,000 mg; folic acid, 800 mg; choline chloride, 120,000 mg; colbat carbonate, 150 mg; ferrous sulphate, 8000 mg; potassium iodide, 400 mg; manganese oxide, 6000 mg; copper, 800 mg; sodium selenite, 40 mcg; lysine, 10,000 mg; methionine, 10,000 mg; zinc sulfate, 8000 mg

Digestibility Parameters

Apparent digestibility coefficients (ADCs) for dry matter, protein, lipids, and energy of the WHs were determined using the equation of Cho et al. (1985).

$$\text{ADC of dry matter} = [1 - \frac{\%\ \text{dietary chromic oxide}}{\%\ \text{feces chromic oxide}}] \times 100$$

$$\text{ADC of nutrient} = [1 - \frac{\%\ \text{feces nutrient}}{\%\ \text{dietary nutrient})} \times \frac{(\%\ \text{dietary chromic oxide}}{\%\ \text{feces chromic oxide}}] \times 100$$

The ADCs of the test ingredients were calculated based on the digestibility of the reference diet and test diets using the equation by Cho et al., 1985:

$$\text{ADCI} = \text{ADCTD} + (\text{ADCTD} - \text{ADCRD}) \times \frac{0.7\text{x DRD}}{0.3 \text{ x DI}}$$

where ADC_I = ADC of the test ingredients

ADC_{TD} = ADC of the test diet

ADC_{RD} = ADC of the reference diet

D_{RD} = % nutrient of the reference diet

D_I = % nutrient of the test ingredients.

Diet Formulation for the Optimal Rate of Incorporation of Water Hyacinth Leaves

The nutritional requirements of *O. niloticus* (NRC, 2011; Mugo-Bundi et al., 2015) were taken into consideration when formulating the diets (Table 3). A total of six isoproteins, isolipids and isoenergetic diets were formulated for the study. Four of these diets contained varying amounts of WH leaves (3%, 6%, 9%, and 12%), while one diet served as a control without the addition of WH leaves. The final diet was a reference diet composed of imported commercial feed (Gouessant ®). The ingredients were ground and mixed before being extruded through a 2 mm mesh. The diets were then stored in boxes in a refrigerator at a temperature of 5°C. The protein, lipid, carbohydrate, ash, and dry matter contents of the manufactured diets were analysed according to AOAC (2005) (Table 4). Gross energy was calculated according to the method of El-Sayed and Tashima (1992).

Table 3 Feed formulations containing WH leaves in the diet of *O. niloticus* fingerlings.

Ingredient	Incorporation Level (%)				
	A_0 (0%)	A1 (3%)	A2 (6%)	A3(9%)	A4(12%)
Fish meal	30	30	32	32	32
Soybean meal	20	20	20	20	22
Wheat bran	15	13	10	10	9
Corn flour	20	20	18	14	10
Moringa leaf	3	2	2	1	1
Water hyacinth leaf	0	3	6	9	12
Starch	2	2	2	2	2
Methionine	2	2	2	3	3
Lysine	2	2	2	3	3
Dicalcium phosphate	1	1	1	1	1
Premix (Vit. + Min.) *	2	2	2	2	2
Palm oil	3	3	3	3	3
Total (%)	100	100	100	100	100

A_0: Diet without WH leaf, A_1: Diet with 3% WH leaf, A_2: Diet with 6% WH leaf, A_3: Diet with 9% WH leaf, A_4: Diet with 12% WH leaf

* premix (vitamin–mineral) contains (‰): vitamin A, 4,000,000 U.I.; vitamin D, 800,000 IU; vitamin E, 40,000 IU; vitamin K3, 1600 mg; vitamin B1, 4000 mg; vitamin B2, 3000 mg; vitamin B6, 3800 mg; vitamin B12, 3 mg; vitamin C, 60,000 mg; biotin, 100 mg; inositol, 10,000 mg; pantothenic acid, 8,000 mg; nicotinic acid, 18,000 mg; folic acid, 800 mg; choline chloride, 120,000 mg; colbat carbonate, 150 mg; ferrous sulphate, 8000 mg; potassium iodide, 400 mg; manganese oxide, 6000 mg; copper, 800 mg; sodium selenite, 40 mcg; lysine, 10,000 mg; methionine, 10,000 mg; zinc sulfate, 8000 mg

Table 4: Nutritional composition of the diets

Nutritional composition of diets based on formulation						
		A0 (0%)	**A1 (3%)**	**A2 (6%)**	**A3(9%)**	**A4 (12%)**
Protein (%)		35.88	35.39	35.35	35.18	35.07
Lipid (%)		10.75	10.19	10.1	10.1	10.06
Carbohydrate (%)		30.43	30.5	30.62	30.28	30.14
Energy (kcal/kg)		3619.9	3552.7	3547.8	3527.4	3513.8
Chemical analysis of constituted diets based on analyses						
	At (commercial feed)	A0 (0%)	A1 (3%)	A2 (6%)	**A3(9%)**	**A4 (12%)**
Dry matter (%)	95.88	89.11	88.04	88.25	88.14	87.14
Protein (%)	35.4	33.12	33.05	32.89	32.94	32.66
Lipid (%)	13.82	11.4	10.84	10.75	10.32	10.06
Carbohydrate (%)	33.4	30.04	31.42	31.12	32.08	32.74
Ash (%)	11.14	10.12	9.94	9.47	8.54	8.31
Energy (kcal/kg)	3995.8	3552.4	3554.4	3527.9	3529.6	3521.4

Experimental Conditions for Water Hyacinth Incorporation

Eighteen 60-litre aquaria were used during the experiment, each arranged in a Fisher random block design with six treatments and three repetitions. *O. niloticus* fingerlings with an average initial weight of 4.46 ± 0.2 g were used, with a density of twenty fingerlings per tank or one fish per 2 L. The fingerlings were fed three times daily for 45 days, and the physicochemical parameters of the water (temperature, dissolved oxygen, and pH) were recorded twice daily to monitor water quality. Control fishing occurred weekly, and the water was renewed at a flow rate of 2 l/min. To evaluate feed performance, zootechnical and feed utilization parameters such as the survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), feed conversion rate (FCR), feed efficiency (FE), protein efficiency ratio (PER), and protein production value (PPV) were calculated. The quality of the fish flesh was evaluated by assaying the proximate composition of protein, lipid, ash, and dry matter content before and after feeding, according to the Association of Official Analytical Chemists (1990.

$$SR(\%) = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{DWG (g/day)} = \frac{\text{body mass gain (g)}}{\Delta T}$$

Δt: the duration of the experiment in number of days

$$\text{SGR (\%/day)} = \frac{\ln \text{(Final Biomass Weight)} - \ln \text{(Initial Biomass Weight)}}{\Delta T} \times 100$$

ln: natural logarithm

$$\text{FCR} = \frac{\text{dry feed fed (g)}}{\text{body mass gain (g)}}$$

$$\text{FE} = \frac{1}{\text{FCR}}$$

$$\text{PER} = \frac{\text{wet body mass gain}}{\text{crude protein fed}}$$

$$\text{PPV} = \frac{\text{body protein gain}}{\text{dietary protein fed}}$$

The determination of the optimal incorporation rate of WH leaves in the diet of *O. niloticus* was based on the analysis of the specific growth rate and the feed efficiency.

Statistical Analysis

All data are expressed as means ± standard deviation. Data were analysed for homogeneity of variance by Levene's test. Differences were considered significant when P < 0.05. Data were analysed using a one-way analysis of variance (ANOVA) and the Tukey test. All statistical analyses were performed using the STATVIEW version 5.01 software, and graphs were created with Microsoft Excel.

Results and Discussion

Physicochemical Parameters of the Water

The physicochemical parameters of the water were recorded throughout the experiment. The average temperature during the experiment was 27.52 ± 0.34 °C, with a range of 27.30 to 27.85 °C. The average pH was 6.82 ± 0.14, with a range of 6.62 to 7.12. Average dissolved oxygen was 6.47 ± 0.22 mg/L, ranging from 6.25 to 6.71 ± 0.12 mg/L.

Digestibility Parameters

The apparent digestibility coefficients (ADCs) of dry matter, protein, lipids, and energy in the reference diet were 90.66%, 88.45%, 89.47%, and 80.14%, respectively. The ADC values for the same parameters in the test diet were lower, with values of 82.38%, 81.17%, 82.41%, and 75.54%, respectively. After calculation, the ADC values for water hyacinth leaves were determined to be 77.49% for ADCDM, 69.58% for ADCCP, 76.47% for ADCCL, and 59.14% for ADCE (Table 5).

Zootechnical and Feed Utilisation Parameters

The final biomass (FB) ranged from 118.46 ± 1.46 to 205.72 ± 2.53 g, with A4 having the lowest FB and At having the highest (Table 6). The evolution of biomass over time differed significantly among the treatments (Fig. 1). Diets with more than 6% WH leaf incorporation were significantly different ($p < 0.05$) from those with less than 6% WH leaf incorporation. The daily weight gain (DWG) varied from 0.65 ± 0.15 g (A4) to 2.6 ± 0.12 g (At). The DWG drops considerably beyond an incorporation of 6%. The same trend was observed for the feed conversion rate (FCR) and protein efficiency ratio (PER), which ranged from 5.02 ± 0.14 (A4) to 1.21 ± 0.08 (At) and from 0.61 ± 0.05 (A4) to 2.32 ± 0.15 (At), respectively. For the survival rates (SR), no significant differences ($p > 0.05$) were detected among the treatments, with the SR ranging from 85.75% (A4) to 95.33% (A0). For the protein productive value (PPV), no significant differences ($p > 0.05$) were detected among the treatments with PPV ranging from 0.67 ± 0.05 (A4) to 0.78 ± 0.09 (A1).

Table 5. Apparent digestibility coefficient of diets evaluated for *O. niloticus* fingerlings fed a diet based on WH leaves

ADC (%)	T0	T1
	Reference	Test
DM	90.66	82.38
CP	88.45	81.17
CL	89.47	82.41
E	80.14	75.54
	WH leaf	
DM	-	77.49
CP	-	69.58
CL	-	76.47
E	-	59.14

Table 6. Zootechnical and feed utilisation performance of *O. niloticus* fingerlings fed the experimental diets.

Parameters	At	A0 (0%)	A1(3%)	A2 (6%)	A3 (9%)	A4 (12%)	*P-Value*
IBW (g)	88.41 ± 0.15^a	90.20 ± 0.48^a	89.5 ± 0.50^a	89.7 ± 0.66^a	89.35 ± 0.25^a	88.6 ± 0.75^a	0.82
FBW (g)	205.72 ±2.53^a	190.4 ±2.12^{ab}	179.8 ±1.75^b	176.4 ±1.5^b	138.8 ±1.2^c	118.46 ± 1.46^c	< 0.0001
DWG (g/day)	2.6 ± 0.12^a	2.23 ± 0.15^a	2.01 ± 0.2^a	1.92 ± 0.19^a	1.11 ± 0.18^b	0.65 ± 0.15^b	< 0.0001
FCR	1.21 ± 0.08^a	1.34 ± 0.05^a	1.42 ± 0.09^a	1.49 ± 0.04^a	2.91 ± 0.04^b	5.02 ± 0.14^c	< 0.0001
PER	2.32 ± 0.15^a	2.24 ± 0.12^a	2.13 ± 0.14^a	2.02 ± 0.12^a	1.04 ± 0.07^b	0.61 ± 0.05^b	< 0.0001
SR (%)	90.66 ± 2.00^a	95.33 ± 2.66^a	90.33 ± 2.66^a	95.66 ± 2.00^a	90.33 ± 2.66^a	85.75 ± 2.33^a	0.65
PPV	0.70 ± 0.05^a	0.74 ± 0.04^a	0.78 ± 0.09^a	0.76 ± 0.04^a	0.69 ± 0.04^a	0.67 ± 0.05^a	< 0.0001

At: "Gouessant ®" commercial *O. niloticus* feed, A_0: Diet without WH leaf, A_1: Diet with 3% WH leaf, A_2: Diet with 6% WH leaf, A_3: Diet with 9% WH leaf, A_4: Diet with 12% WH leaf.
Initial Body Weight (IBW), Final Body Weight (FBW), Daily Weight Gain (DWG), Feed Conversion Rate (FCR), Protein Efficiency Ratio (PER), Survival Rate (SR), and Protein Productive Value (PPV).
The values are expressed as the means ± standard deviations. Values with the same alphabetical letters in the same row are not significantly different at $p > 0.05$.

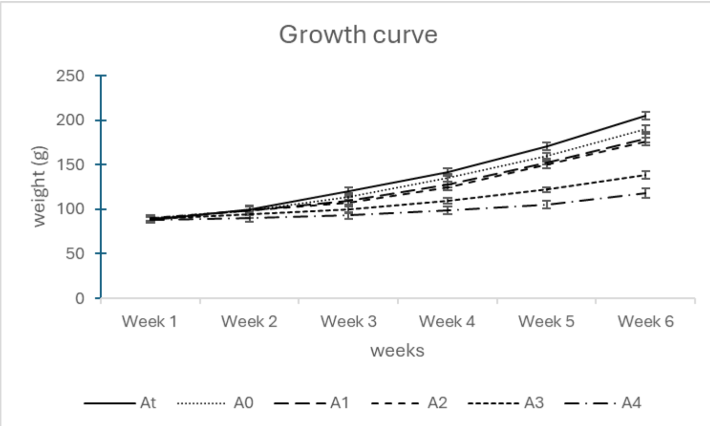

Figure 1. Growth evolution of *O. niloticus* fingerlings during experimentation

Optimal Rate of Incorporation of WH Leaves Into the Diet of O. niloticus

Based on the analysis of the specific growth rate (Fig 2) and feed efficiency (Fig 3) of the different diets, it appears that there is no significant difference ($p > 0.05$) between diets that have undergone an incorporation rate of less than 6% (A0, A1, and A2). The same observation is made with those that have undergone an incorporation rate greater than 6% (A3 and A4). However, there was a significant difference between the diet groups that had incorporated less than 6% WH leaves and those with an incorporation greater than 6%.

Nutritional Values of Fish Carcasses Fed Diets Containing WH Leaves

The experimental diets given to the fish resulted in higher protein, lipid, and ash contents than did the initial fish flesh (Table 7). The lipid levels ranged from 9.05 ± 0.21% in A4 to 14.19 ± 0.21% in At, while the protein levels varied from 26.4 ± 0.66% in A4 to 30.42 ± 0.89% in At. The ash content also varied from 24.3 ± 0.68% in A1 to 29.4 ± 0.33% in A2. The difference in ash, protein, and lipid content of the fish carcasses fed diets incorporated at a rate higher than 6% was found to be significant ($p < 0.05$).

The water parameters, including temperature (27.52 ± 0.34 °C), pH (6.82 ± 0.14), and dissolved oxygen (6.47 ± 0.22 mg/l-1), are within the acceptable ranges for *O. niloticus* (Abo-State et al., 2014; Mugo-Bundi et al., 2015).

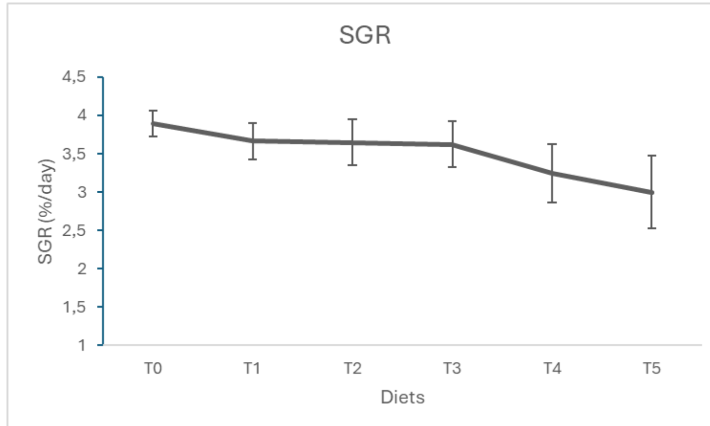

Figure 2. Variation in specific growth depending on diet based on WH leaves

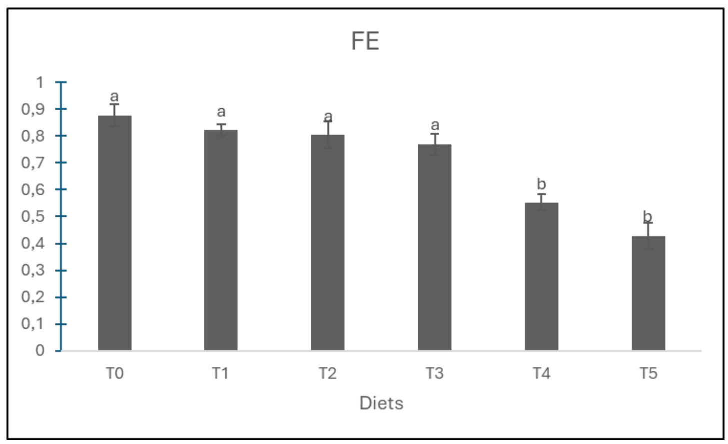

Figure 3. Variation in feed efficiency depending on diet based on WH leaves

Table 7. Proximate composition of *O. niloticus* fingerlings fed the experimental diets

Composition	Initial	At	A0 (0%)	A1(3%)	A2 (6%)	A3 (9%)	A4 (12%)	*P-Value*
Dry matter (%)	90.41	89.4 ± 1.48^{a}	88.5 ± 1.54^{a}	87.4 ±1.09^{a}	88.4 ±1.15^{a}	87.1 ± 1.07^{a}	82.47 ± 1.12 ^{a}	0.81
Ash (%)	30.14	21.4 ± 0.42^{a}	22.2 ± 0.54^{a}	22.3 ± 0.68^{a}	23.4 ± 0.33^{b}	23.74 ± 0.84^{b}	24.47 ± 0.26 ^{b}	< 0.0001
Protein (%)	25.17	30.42 ± 0.89^{a}	28.4 ± 0.75^{b}	27.98 ± 0.82^{b}	27.88 ± 0.54^{bc}	26.8 ± 0.78^{bc}	26.4 ± 0.66 ^{c}	0.009
Lipid (%)	8.9	14.19 ± 0.21^{a}	10.58 ± 0.38^{b}	10.47 ± 0.45^{b}	10.41 ± 0.18 ^{b}	9.1 ± 0.14 ^{b}	9.05 ± 0.21 ^{b}	< 0.0001

The values are expressed as the means ± standard deviations. Values with the same alphabetical letters in the same row are not significantly different at the threshold of $p > 0.05$.

WH leaves have a reasonably interesting nutritional profile that could be used in aquaculture feed. Its protein content (14.30%) was greater than that of corn bran (12.6%) and rice bran (14.10%) and was similar to that of wheat bran (15.10%). However, this percentage is slightly lower than that of sweet potato leaves (16.6%) and cassava leaves (22.30%) (Da et al., 2013). The protein content was also lower than that of *Moringa oleifera* leaves (27.7%) (Djissou et al., 2019). Several studies have examined the use of water hyacinth in animal and fish feeds. However, they did not focus on its digestibility or the optimal rate of incorporation into aquaculture feed. Therefore, this study aimed to determine the digestibility and optimal rate of incorporating water hyacinth into *O. niloticus*. The inclusion of water hyacinth leaves in the tilapia diet had an impact on the digestibility of the feed. The apparent digestibility coefficient of the reference diet changed for the same parameters when water hyacinth leaves were introduced into the feed. A study conducted by Da et al. (2013) indicated that the digestibility coefficients of dry matter, protein content, and energy obtained from sweet potato leaves were 79.3%, 71.8%, and 78.9%, respectively. The same parameters for cassava leaves were 4%, 63.6%, and 76.7%, respectively, for *Pangasianodon hypothalamus*. These results are similar to those of a study conducted on WH leaves, which showed that the digestibility coefficients of dry matter, protein content, and energy were 77.49%, 69.58%, and 59.14%, respectively. Thus, it can be inferred that WH leaves can be used in fish diets in the same way as cassava and sweet potato leaves. Like duckweed, WH is a type of aquatic plant. However, the digestibility of proteins in duckweed (*Lemna sp*.) is much greater (81.7%) than that in WH leaves (Mbagwu & Adeniji, 1988). This difference in digestibility can be attributed to the fact that duckweed has a more balanced amino acid composition than WH leaves (Mbagwu & Adeniji, 1988). A balanced amino acid composition can improve the digestibility of some ingredients. This is also the reason why ingredients of animal origin are more digestible than those of plant origin, due to the balance of amino acids (Sklan et al., 2004; Panini et al., 2017; Nor et al., 2019).

It is evident that the use of WH leaves in the diet of *O. niloticus* can have a notable impact on zootechnical parameters such as DWG, FCR, PER, and SGR. As the rate of WH leaf incorporation increases, there is typically a slight decrease in zootechnical performance beyond a 6% rate. However, when the incorporation rate exceeded 6% (9% and 12%), there was a considerable decrease in the zootechnical parameters. This result may be due to the imbalanced amino acid profile of WH leaves (A-Rahman Tibin et al., 2012; Sayed-Lafi et al., 2018). Additionally, the presence of antinutritional factors, such as phytates, in most plant-origin proteins may also limit their incorporation into fish feed (Francis et al., 2001). In contrast, *Moringa oleifera* leaves and *Azolla filiculoides* have lower levels of antinutritional factors; therefore, incorporating 14% of *Moringa oleifera* leaves and 20% of *Azolla filiculoides* in the diet of *O. niloticus* did not adversely impact zootechnical performance (Djissou et al., 2017; Djissou et al., 2019). Overall, it appears that incorporating more than 6% of WH leaves into the diet of *O. niloticus* could lead to a decrease in the specific growth rate and feed efficiency. However, the incorporation of WH at levels of up to 12% did not negatively affect survival rates, which remained at approximately 90% on average.

Regarding the nutritional value of the flesh of the fish fed with WH leaves, there was a significant difference ($p < 0.05$) in ash, protein and lipid content between the treatments. The differences observed relate to diets with an incorporation rate greater than 6%. This result indicates that incorporating more than 6% of WH leaves can impact the nutritional quality of fish.

Conclusion

This study demonstrated that WH leaves can be incorporated into *the diets of O. niloticus*. A maximum of 6% is recommended for the *O. niloticus* diet to avoid affecting the digestibility, zootechnical parameters and nutritional quality of the fish. However, even a substitution of up to 12% of water hyacinth leaf in the diet of the O. niloticus did not affect the survival rate.

Compliance with Ethical Standards

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

Ethics committee approval: This research was conducted by Framework Law No. 2014-19 of August 7, 2014, on Fisheries and Aquaculture in the Republic of Benin, and Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Data availability: The data will be made available upon request from the author.

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Growth and condition of Indian major carp (*Catla catla* Ham., 1822) in perennial small water bodies of south Gujarat (India)

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ABSTRACT

Fish growth is a significant factor in fish production and is directly related to the income of fish farmers. Thus, in the present study, it is aimed to determine the growth status of the Indian major carp (*Catla catla* Ham. 1822) at Keliya reservoir (Gujarat). The morphometric measurements (total length and weight) were taken from 597 fish specimens that were randomly collected from commercial catches between November 2021 and March 2022. During the study, the total length ranged from 25.00 to 46.00 cm (34.41 ±0.16 cm), and the weight ranged from 206.00 to 1525.00 g (528.29 ±8.62 g) of fish was recorded. The length frequency of fish shows that the population was dominated by the length group 30-35 cm (307), followed by the length group 35-40 cm (170), 25-30 cm (75), 40-45 cm (41) and 45-50 cm (4). The total length and weight variables were used to calculate the length-weight relationship and condition factor. The value of the correlation coefficient (r^2) was 0.906, showing a positive relationship between the variables, and the regression coefficient (b, 3.164±0.041 at 0.05 CI, which fulfilled the 'cube law' and indicates positive allometric growth of the studied fish in earthen ponds. The mean value of condition factor (K) for length groups A (25-30 cm), B (30-35 cm), C (35-40 cm), D (40-45 cm), E (45-50 cm) and pooled population were 1.26±0.02, 1.3 ±0.01, 1.25 ±0.01, 1.33 ±0.02, 1.39 ±0.09 and 1.24±0.01 respectively, which indicated that the condition of fish was good. The aquatic environment was conducive for fish survival in the studied water body.

Keywords: Keliya reservoir, Fish growth, Length-weight relationship

Introduction

The relationship between morphological characteristics of fish is imperative from various perspectives, including behaviour, ecology, conservation, evolution, and aquatic resource management (Ujjania et al., 2012; Ujjania et al., 2013; Başusta et al., 2014; Kalhoro et al., 2015). Information on morphometric characteristics (total length and weight) plays a crucial role in fishery biology. It is used to assess the general well-being (Froese et al., 2011), comparative growth studies (Moutopoulos & Stergiou, 2002), biomass (Adarsh & James, 2016), ecosystem modelling (Kulbicki et al., 2005), reproductive history (Kara & Bayhan, 2008), life history (Shah et al., 2013; Ferdaushy & Alam, 2015), and growth and condition of fish (Froese, 2006; Ujjania et al., 2022). Similarly, these reflect the physiological condition of the fish and are influenced by gonadal development, organic reserves, the presence or absence of food in the gut, and food availability, as well as environmental variability in the surrounding water body (Nikolsky, 1969). The length and weight of fish are commonly used in fisheries sciences to estimate the mean weight of a fish stock from the known length of the fish group in a given water body (Gupta & Banerjee, 2015), by establishing a precise mathematical equation between length and weight. Moreover, the statistical relationships among morphometric measurements i.e. length and weight of fishes are significant for fish biologists (Mustafa & Brooks, 2008) and fish taxonomists (Simon et al., 2010) that is helpful too to determine some aspects of fish population dynamics (Adeyemi et al., 2009) like age structure and growth pattern i.e., allometric or isometric (Quist et al., 2012). When the fish growth was isometric, that implies the length and weight are growing to follow the cube law (i.e. value of 'b' equal to 3), while it was allometric that depicted either the length or weight of fish is not followed the cube law (i.e. value of 'b' more than 3). The condition factor (K) is an indirect morphological indicator commonly used by fish growers and biologists to estimate fish growth. It is an index of feeding intensity and growth (Fagade, 1979), revealing information on the biological state of fish, including their well-being (Abowei, 2010). Moreover, it also reveals the conduciveness of the water body for fish growth and survival (LeCren, 1951). It has also been noted that the condition factor helps to evaluate significant changes in body shape among different fish species (Froese, 2006). Studies on the length-weight relationship and condition factor of Indian major carps, including Calta, were carried out by Singh and Kaur (2015), Rajput and Wast (2021), and Ujjania et al. (2023). Significant linear relationship between the total length and weight of catla from Sukhna Lake, Chandigarh (Johal & Tandon, 1983). Although, catla contributing major portion of the fish catch in these small water bodies but such essential information on the length-weight relationship and the condition factor of this fish from various large water bodies of different geographical regions of the world is available but data is scarce on a small perennial aquatic environment, which may be used for comparative growth assessment of fish and useful for fisheries stakeholder including fish biologist and fish farmers. Thus, the present study was conducted to describe the length-weight relationship and condition factor of the Indian major carp (Catla catla, Ham., 1822) from a small, perennial water body in south Gujarat.

Materials and Methods

Keliya reservoir was constructed across the River Kharera, a tributary of the River Kaveri, near the village Keliya, Taluka Vansda, district Navsari of Gujarat state (India). It is a freshwater artificial reservoir with the primary purpose of irrigation and flood control. Additionally, the local people use it for fish culture. The maximum height of the dam wall is 28.10 m, and the maximum length is 814.00 m (Table 1 and Figure 1).

The morphometric data, including length and weight of 597 fish specimens, which were randomly collected from the commercial catches during the fishing year 2021-22 (November 2021 to March 2022). The total length (snot tip to caudal fin end) was measured by measuring the tail, whereas the body weight (WT) of the fish was measured with the help of a single pan balance. The total length and weight of fish are used for further calculations, such as the length–weight relationship and condition factor. The length-weight key and parabolic equation ($W = a\,L^b$) of Froese (2006) and ($\log w = \log a + b \log L$) of Pauly (1983) were used for the length-weight relationship from log-transformed data of length and weight (W = weight of fish, L = length of fish, a = intercept and b = exponent). The condition factor (K) was calculated using the following equation ($K = w/L^3$, where w is the weight and L is the length, in meters, and the exponent is 100, as per Fulton (1902). The graphical presentation and statistical analysis were performed using MS Excel 2010.

Table 1. Morphometric details of the studied water body (Keliya reservoir) in south Gujarat

S.N.	Description	Details
1.	State	Gujarat
2.	District	Navasri
3.	Taluka	Vansda
4.	Village	Keliya
5.	River	Kharera tributary of River Kaveri
6.	Name of reservoir	Keliya reservoir
7.	Type of Dam	Earthen dam
8.	Construction year	1983
9.	Latitude	20° 68' 98" to 20° 71' 18" N
10.	Longitude	73° 27' 78" to 73° 29' 73" E
11.	Catchment area	27.58 Sq. km
12.	Area (FRL)	3.18 Sq. km
13.	Depth at FRL	113.40 m
14.	Minimum water level	98.10 m
15.	Maximum water level	115.79 m
16.	Average annual rainfall	1970 mm
17.	Length of Dam	814 m
18.	Height of Dam	28.1 m

Results and Discussion

In the present study, the total length minimum (25.00 cm), maximum (46.00 cm) and mean (934.41 ±0.16 cm), while the weight minimum (206.00 g), maximum (1525.000 g), and mean (528.29 ±8.62 g) of the studied fish were measured (Table 2). The length data of the fish were distributed in different length groups, as described in table 2 and figure 2. which show that length group B was the most dominant (307, 51.4%), followed by length group C (170, 28.5%), A (75, 12.5%), D (41, 6.9%), and E (4, 0.7%). Figure 3 shows a straight line, with a correlation coefficient (r^2) of 0.91. The correlation coefficient depicted a strong, positive, and significant linear relationship between the total length and weight of the studied fish. The value of the growth coefficient (b) was 3.16 ±0.041 at 0.05 CI (Figure 3), which is more than 3.0 and resulted in positive allometric growth of the studied fish in Keliya reservoir. Findings depicted that either the length or weight of fish does not follow the cube law, i.e. weight is slightly more than the required normal weight of the fish. Froese (2006) also reported that the value of 'b' is less than 3.0, and the body shape of the fish specimen is elongated. A similar growth coefficient for Catla catla was reported as 3.2 from Mahi Bajaj Sagar reservoir (Ujjania et al., 2012), 3.22 from Pakistan (Ishtiaq & Naeem, 2016) and 3.2 from Harike wetland, Punjab (Singh & Kaur, 2015), that are in agreement with the findings of the present study. Rajput and Wast (2021) reported very high value of growth coefficient (5.0) for catla in Morvan dam (Madhya Paresh) while negative allometric growth (1.5-2.17) for catla was reported by Sachidanandmurthy et al. (2013) in Mysure Lake and Negi (2013) was reported isometric growth for Indian major carp rohu (3.0) from fishpond Roorkee, Uttarakhand (India).

Table 2. The length, weight and condition factor measurements of catla in the studied water body

Length group (TL, cm)		n	Total length (cm)				Weight (g)				Condition factor (K)			
			Min.	Max.	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE
25-30	A	75.00	25.00	30.00	28.63	0.14	206.00	403.00	296.84	6.05	0.94	1.64	1.26	0.02
30-35	B	307.00	30.50	35.00	32.92	0.08	222.00	756.00	437.85	4.55	0.76	2.10	1.22	0.01
35-40	C	170.00	35.50	40.00	37.39	0.10	314.00	940.00	654.55	7.43	0.67	1.60	1.25	0.01
40-45	D	41.00	40.50	45.00	42.38	0.21	807.00	1521.00	1014.41	22.95	1.15	1.67	1.33	0.02
45-50	E	4.00	45.50	46.00	45.88	0.13	1185.00	1525.00	1338.00	87.67	1.23	1.57	1.39	0.09
Pooled		597.000	597.00	25.00	46.00	34.41	0.16	206.00	1525.00	528.29	8.62	0.67	2.10	1.24

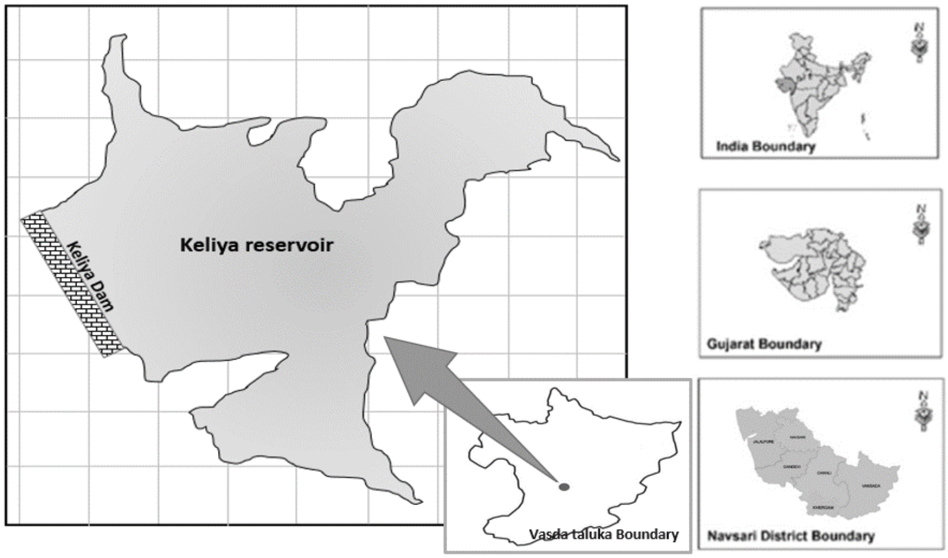

Figure 1. Map of study area (Keliya reservoir, Gujarat)

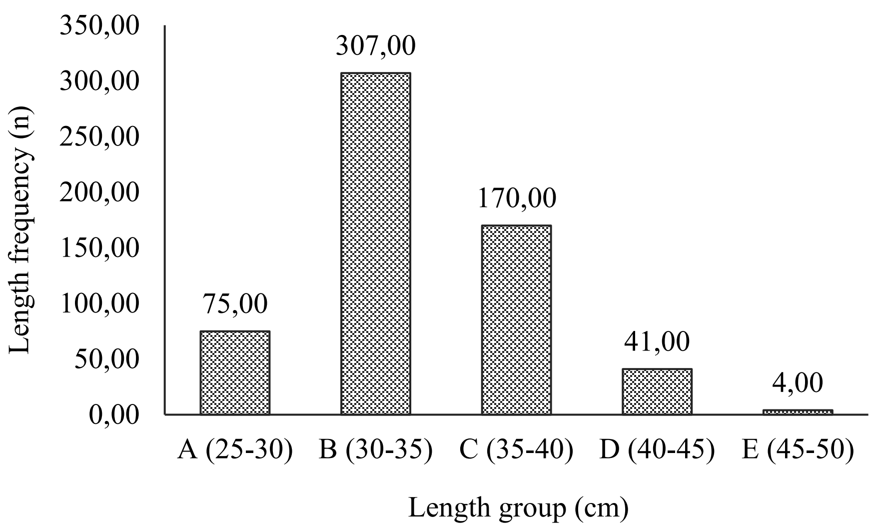

Figure 2. Length frequency distribution of catla in the studied water body

The range and mean of condition factor (K) for length group A (0.94-1.64 and 1.26 ± 0.02), for length group B (0.76-2.10 and 1.22 ±0.01), for length group C (0.67-1.60 and 1.25 ±0.01), for length group D (1.15-1.70 and 1.33 ±0.02), for length group E (1.13-1.57 and 1.39 ±0.09) and 0.67-2.10 and 1.24 ±0.01) for pooled population of studied fish was noted (Table 2). The condition factor (K) for different length groups and the pooled population was noted to be greater than 1.0, indicating the good condition of the fish in the studied aquatic environment. Similar recommendations were also given by Carlander (1977) and Williams (2000). The condition factor decreases with an increase in length (Bakare, 1970; Fagade, 1979) and affects the reproductive cycle in fish (Welcome, 1979). The value of condition factor (>1.0) was reported by Ujjania et al. (2012), Ujjania et al. (2013), Singh et al. (2015), Ishtiaq and Naeem (2016), Balai et al. (2017), Khalid et al. (2020) and Ujjania et al. (2023) for catla, which concluded good condition and conducive environment of the water body.

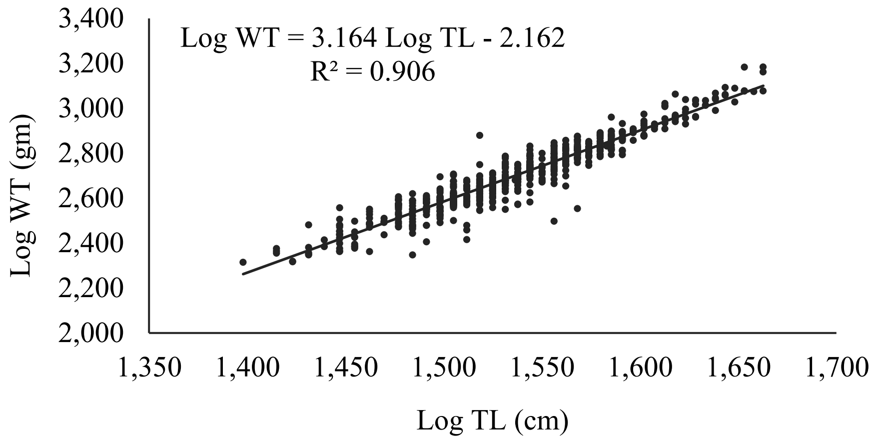

Figure 3. Length-weight relationship of catla

Conclusion

The present study elucidates the length-weight relationship and condition factor of *Catla catla* (Hamilton, 1822) from Keliya Reservoir, a small perennial water body. The findings of the present study suggest that a significant, linear, and positive relationship exists between the length and weight variables. The growth of fish was positive allometric (b = 3.16 ±0.041 at 0.05 CI), and the high value of the condition factor indicates better dwelling conditions and the optimum environmental condition of Keliya reservoir for fish growth and survival. Thus, it is suggested that we explore and manage the water body to enhance fish production quality, potentially. The findings of this study may serve as a baseline for understanding the growth status and condition of catla, which would be helpful for management and conservation practices in fisheries.

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: Ethical approval was not required for this study.

Data availability: The data will be made available upon request.

Funding disclosure: -

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Disclosure: -

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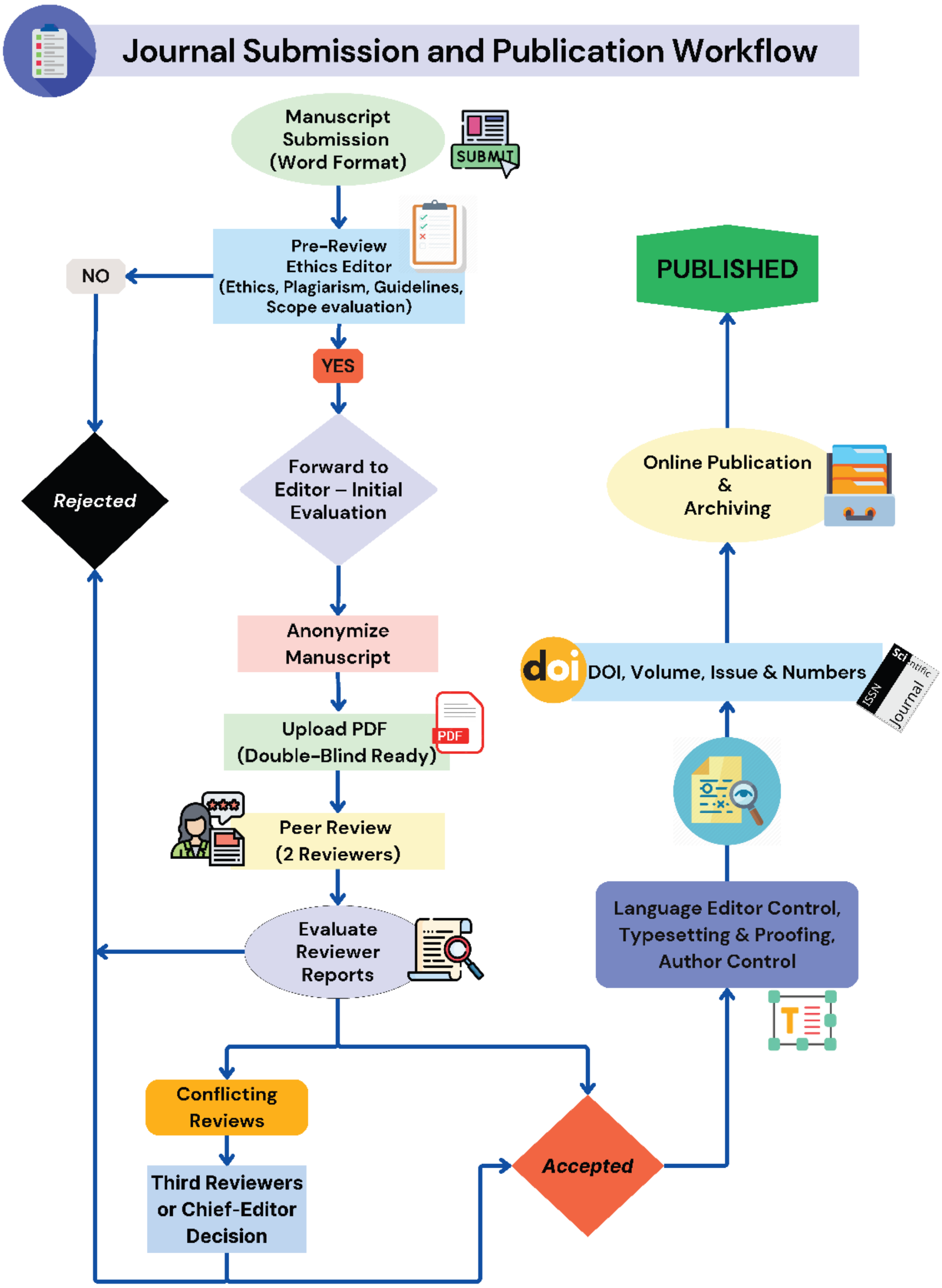

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All authors submitting their works to the "**AQUATIC RESEARCH**" journal for publication as original articles attest that the submitted works represent their authors' contributions and have not been copied or plagiarised in whole or in part from other works. The authors acknowledge that they have disclosed all actual or potential conflicts of interest related to their work or its partial benefits. Similarly, the "**AQUATIC RESEARCH**" journal is committed to conducting objective and fair double-blind peer review of submitted works for publication and to preventing any actual or potential conflicts of interest between the editorial and review personnel and the reviewed material.

Journal Publisher Policy

1. Aims and Scope

"Aquatic Research" journal aims to contribute to the world of science with high-quality publications based on scientific research on aquatic ecosystems. The journal focuses on a wide range of topics, including aquaculture, sustainable water resources management, aquatic biology, marine ecology, and articles covering all fields of aquatic sciences. The journal's publication language is English or Turkish.

2. Scientific Quality and Objectivity

The journal evaluates and publishes research articles and reviews, upholding the highest standards of scientific excellence. Adhering to the principle of impartiality, it strictly complies with ethical rules to prevent conflicts of interest among editors, referees, and authors.

3. Open Access

The journal adheres to an open-access policy, promoting unrestricted access to information. This aims to increase access to scientific knowledge in society by making science more accessible to a broader audience.

Open-access articles in the journal are licensed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.

4. Ethical Standards

The journal maintains a rigorous attitude towards upholding ethical standards among authors and reviewers. The processes of evaluating the effects of research on humans, animals and the environment are carried out in full compliance with national and international ethical rules.

5. Peer Review

The journal employs a double-blind referee system. Referees are selected from among experts and experienced individuals in their respective fields. The peer review process involves subjecting articles to rigorous review in terms of scientific content, methodology and ethics.

6. **Author Rights and Licensing**

The journal respects the property rights of authors and grants appropriate licenses to articles. It allows articles to be freely shared and used by others, provided they are used by appropriate licensing models, such as Creative Commons licenses.

7. **Diversity and Inclusion**

The journal encourages diversity among authors, editors, and reviewers. It fights against inequalities in the scientific world, taking into account factors such as gender, geographical origin, discipline, and other elements of diversity.

8. **Communication and Transparency**

The journal fosters open communication among authors, reviewers, and readers. Publisher policies, article evaluation processes and other important information are transparently published on the journal's website.

9. **Archiving**

Journal archiving is conducted by the Republic of **Türkiye Ministry of Industry and Technology, TÜBİTAK Turkish Academic Network and Information Centre (ULAKBİM), and by the** "DergiPark" publication policy (LOCKSS).

Publication Ethics

1. **Scientific Neutrality and Objectivity:**

All publications must reflect an impartial and objective perspective. If there are any conflicts of interest, authors must disclose these conflicts.

2. **Scientific Soundness:**

Articles should be based on a solid methodology and reliable results. The accuracy of statistical analyses should be a top priority.

3. **Ethical Standards:**

The journal supports the principles of the Basel Declaration (https://animalresearchtomorrow.org/en) and the guidelines published by the International Council for Laboratory Animal Science (https://iclas.org/). In this regard, the research must fully comply with the relevant ethical rules and standards. International ethics committees must conduct studies involving humans, animals, or the environment, and the journal's authors must confirm that these studies have been performed.

For research submitted to this journal, authors are advised to comply with the **IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on Trade in Endangered Species of Wild Fauna and Flora for research involving plants.**

4. **Use of Artificial Intelligence (AI) Tools:**

Aquatic Research adheres to international standards of publication ethics and requires complete transparency regarding the use of artificial intelligence (AI)-based tools (e.g., ChatGPT, Copilot, etc.) during manuscript preparation.

a. **Disclosure Requirement:** Authors must disclose the use of any AI tool in the manuscript, specifying the name of the tool, the stage(s) at which it was used, and its purpose. This text should be included in the "*Disclosure*". Authors must affirm that all scientific content is their own and that they retain full responsibility for any material generated with the assistance of AI tools.
b. **Content Limitation:** The use of AI tools must remain supplementary. If the proportion of AI-generated text content exceeds **20% of the total manuscript**, the submission will be considered

ineligible for review and rejected on ethical grounds.

c. **Responsibility:** AI tools cannot be listed as authors. All named authors are solely accountable for the content of the submission, including any material produced with AI assistance. Failure to disclose the use of AI tools or excessive reliance on them will be considered a breach of publication ethics.

5. **Originality and Plagiarism:**

Publications must be original, and appropriate attribution must be made when quoting other sources. In our journal, plagiarism is considered a serious crime. For this reason, all articles submitted to the "Aquatic Research" journal must undergo a preliminary evaluation. Advanced Plagiarism Detection Software tools will be used.

6. **Open Access:**

The journal adheres to open access principles, promoting free and open access to information, and complies with the **Budapest Open Access Initiative** (BOAI) definition of open access. Open-access articles in the journal are licensed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.

All journal processes are free of charge. No article processing, submission, or publication fee is charged for submitted or accepted articles.

Peer Review

1. **Confidentiality:**

The principles of double-blind refereeing should be followed in the peer review process. Reviewers and authors should not be aware of each other's identities.

2. **Expertise:**

Referees should be selected among experts and experienced people in relevant fields. Referees must be trusted to make an impartial and ethical assessment.

3. **Timely Evaluation:**

The peer-review process must be completed on time to ensure the timely publication of articles. Time limits should be set for referees to evaluate within a specific period.

4. **Open Communication:**

Reviewers should be encouraged to provide open and constructive feedback to authors and editors.

Author Guidelines

1. **Article Format:**

Authors must write in the article format determined by the journal. Sections such as title, abstract, keywords, introduction, method, findings, discussion and references should be included. All submissions are screened by similarity detection software. The similarity rate in the articles sent to the journal should be below 20%.

2. **Citations and Sources:**

Authors must properly cite the sources used in accordance with scientific standards.

3. **Submission Process:**

Authors must comply with the specified submission process when submitting their articles to the journal. This process should include evaluating, editing and publishing the article.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at **http://dergipark.gov.tr/journal/2277/submission/start**.

"**Aquatic Research**" journal requires corresponding authors to submit a signed and scanned version of the copyright transfer, ethics, and authorship contribution form (available for download at **https://dergipark.org.tr/en/download/journal-file/19583**)

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ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors). Download this form from http://www.icmje.org/conflicts-of-interest/, fill it out, and save it. Please send this to the journal along with your other files.

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 up to date 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.

   Evaluating articles by focusing on areas of expertise on the subject.

2. **Privacy and Reliability:**

   To protect the confidentiality of the article evaluation process.

   Provide reliable and constructive feedback to authors, journal editors, and other reviewers.

3. **Timely Evaluation:**

   Evaluating articles by the timelines determined by the journal.

   Informing editors promptly in case of delays.

4. **Compliance with Ethical Rules:**

   To ensure full compliance with ethical standards and journal policies.

   Clearly express conflicts of interest and withdraw from the evaluation process when necessary.

5. **Constructive Feedback to Writers:**

   Provide clear and constructive feedback to authors, suggesting improvements to the article as 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 main text document (Word 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

Materials 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 for 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 determine whether it requires approval.
- **Data availability:** The data availability statement/data access statement informs the reader where research data associated with an article is available and under what conditions the data can be accessed, and may include links to the dataset, if any.

  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 used is confidential.
  3. The authors do not have permission to share the data.
  4. Data will be made available on request.
  5. The author is unable to specify which data has been used or has chosen not to.
  6. Other (please explain; for example, I have shared the link to my data in the attached file).

- **Funding:** If available, the institutions supporting the research and the agreements with them should be listed here.
- **Acknowledgement:** Acknowledgements allow you to thank people and institutions who assist in conducting the research.
- **Disclosure:** Explanations about your scientific/article work that you consider ethically important, and generative AI and AI-assisted technologies in the writing process. Author(s) who have used generative AI or AI-assisted tools during the preparation of their manuscript are required to include a brief statement in this section. This statement must specify the name of the tool or service used and the purpose for which it was employed. Authors are advised to follow the example format below:

  During the preparation of this work, the author(s) used [NAME OF TOOL / SERVICE] to [PURPOSE]. After using this tool/service, the author(s) reviewed and edited the content as necessary and take full responsibility for the content of the publication.

References

Tables (all tables given in the main text)

Figures (all figures/photos shown in the main text)

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 is usually necessary to support conclusions. International statistical reporting standards require conducting 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 in accordance with the International System of Units (SI).

Review Articles: Reviews prepared by authors with extensive knowledge of a particular field and a scientific background that has been translated into a high volume of publications with high citation potential are welcome. The journal may even invite these authors. Reviews should describe, discuss, and evaluate the current state of knowledge on a research topic, and should also 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 educational 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. The data presented in the tables should not be a repetition of the data presented in the main text, but rather 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)."

All references, tables, and figures should be referred to within the main text and numbered consecutively in the order they are referred to within it.

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 required for the "Aquatic Research" journal are outlined below, as they pertain to the sources used by authors in their works.

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

The APA style requires three types 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 the 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 fo od industry: A case study. In Food Allergy Issues for t he 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 primary 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 evidence.