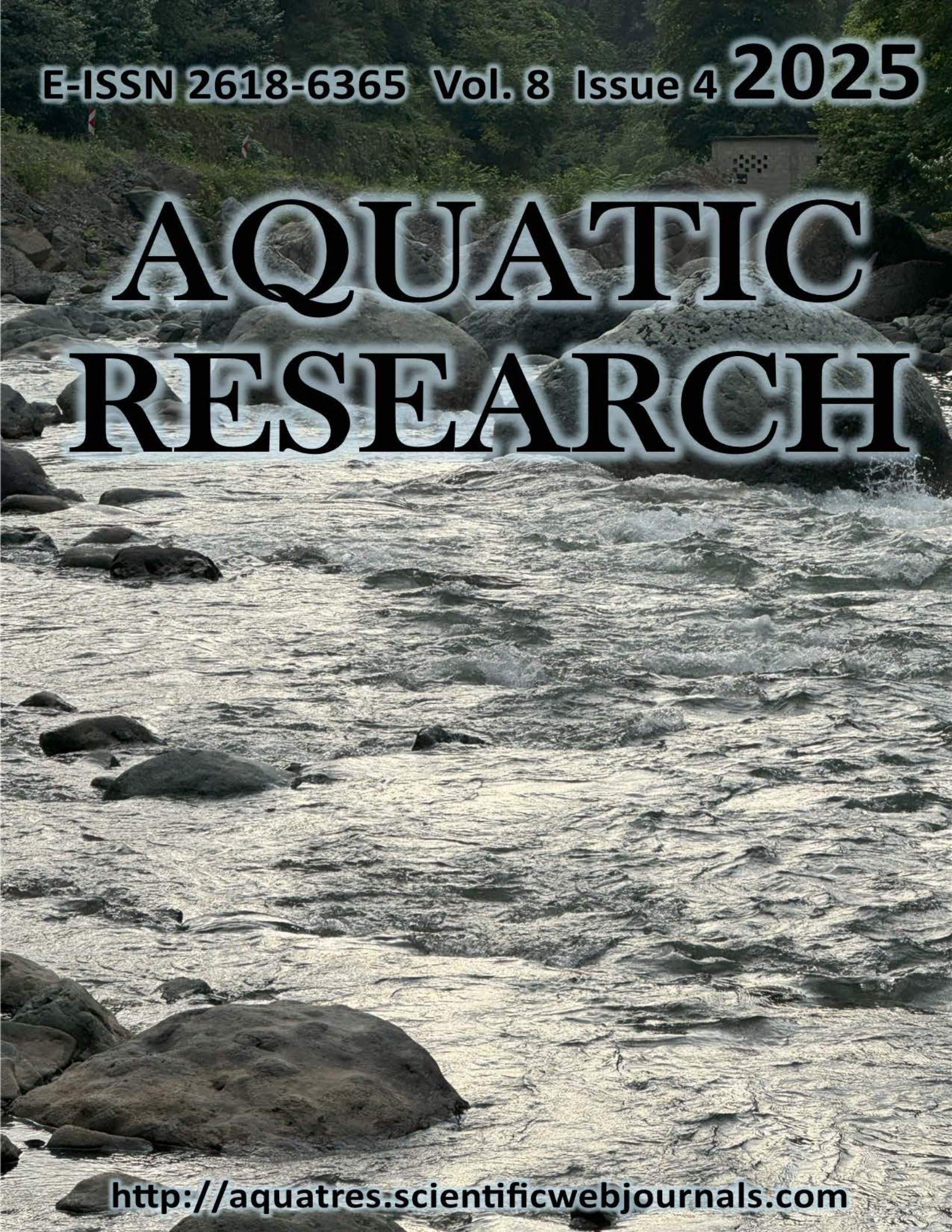
E-ISSN 2618-6365 Vol. 8 Issue 4 2025

AQUATIC RESEARCH



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AQUATIC RESEARCH
E-ISSN 2618-6365

Aims and Scope

AQUATIC RESEARCH

Abbreviation: **Aquat Res**

e-ISSN: 2618-6365

Journal published in one volume of four issues per year by

http://aquatres.scientificwebjournals.com web page

The "Aquatic Research" journal aims to contribute to the literature by publishing manuscripts at the highest scientific level in all fields of marine and aquatic sciences. The journal publishes original research and review articles prepared in accordance with ethical guidelines. The journal's publication language is English or Turkish, and it has been in continuous publication since 2018.

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AQUATIC RESEARCH
E-ISSN 2618-6365

Vol. 8 Issue 4 Page 225-294 (2025)

Content

Research Articles

AQUATIC RESEARCH
E-ISSN 2618-6365

Aquatic Research 8(4), 225-233 (2025) • https://doi.org/10.3153/AR25022 *Research Article*

Morphometric assessment of plastic deposition in a wind-driven marine corridor under bidirectional forces

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Cite this article as:
Gürses, R.K., Maghuyop, A.L., Roa, N., Büyükateş, Y., Ergün, S., Erenoğlu, R.C., Capangpangan, R.Y. (2025). Morphometric assessment of plastic deposition in a wind-driven marine corridor under bidirectional forces. *Aquatic Research*, 8(4), 225-233. https://doi.org/10.3153/AR25022

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Submitted: 02.07.2025
Revision requested: 09.07.2025
Last revision received: 14.07.2025
Accepted: 25.07.2025
Published online: 08.10.2025

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Available online at
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ABSTRACT

Plastic pollution poses a growing threat to coastal and marine ecosystems. This study examines the spatial dynamics of plastic accumulation in the coastal area of Kepez Cape, located within the Çanakkale Strait (Türkiye), a bidirectional marine corridor connecting the Black Sea and the Sea of Marmara to the Aegean and the Mediterranean. Due to its exposure to dominant wind regimes (NNE, NE, SW), Kepez Cape offers a valuable natural setting for investigating the interaction between wind and currents in shaping the deposition of plastic debris. Departing from traditional particle count metrics, the study introduces morphometric parameters, surface area and volume, as more ecologically relevant indicators of pollution load. Plastic debris was sampled from three shoreline sectors using standardised transects, and particle dimensions were analysed. Results revealed substantial spatial variability in plastic accumulation, with larger particles found in regions influenced by Black Sea surface currents. These findings highlight the importance of physical forces in shaping deposition patterns and advocate for incorporating surface area-based metrics into coastal plastic monitoring. The study contributes to the development of wind-informed, regionally coordinated monitoring strategies, offering a refined framework for assessing and ultimately reducing plastic pollution in dynamic marine corridors worldwide.

Keywords: Coastal zone pollution, Morphometric analysis, Plastic debris, Surface area of plastics, Wind-driven transport

Introduction

Plastic pollution has become a persistent and growing threat to marine environments. Since the mid-20th century, the rapid growth of plastic materials driven by their affordability, versatility, and durability has resulted in widespread environmental accumulation (Thompson et al., 2009; Gilbert, 2017). Recent global modelling studies estimate that over 53 million metric tons of plastic waste could enter aquatic environments annually by 2030 (Borrelle et al., 2020), highlighting the urgent need for mitigation strategies. The varying levels of plastic accumulation among different fish species reveal notable differences that should be carefully considered, along with necessary measures, for both the marine ecosystem and human health (Mondal et al., 2024). Once released, plastic debris undergoes fragmentation and dispersal influenced by a complex interaction of environmental factors, including wind, wave dynamics, precipitation, coastal morphology, and currents (Jambeck et al., 2015; Lebreton et al., 2018; van Sebille et al., 2020). While microplastics (<5 mm) and nano-plastics (<1 μm) receive considerable attention for their bioavailability and potential for trophic transfer (Gigault et al., 2018; Campanale et al., 2020), macroplastic fragments (>5 mm) remain ecologically significant due to their longevity and impacts mediated by surface area (Andrady, 2011; Delaeter et al., 2022). Coastal areas serve as both zones of deposition and points of re-entry for plastics, depending on the prevailing hydrodynamic forces (Corcoran et al., 2009; Dekiff et al., 2014). Coastal deposition patterns are highly site-specific, shaped by geomorphology, local wind exposure, and current interactions (Isobe et al., 2014; Vousdoukas et al., 2020). As a result, locally contextualised investigations are essential for understanding plastic fluxes and retention, particularly in dynamic transitional environments. The Strait of Çanakkale, part of the Turkish Straits System, connects the Black Sea to the Mediterranean through a stratified bidirectional current regime: surface flow from the Black Sea to the Aegean and a denser undercurrent flowing in the opposite direction (Beşiktepe et al., 1994; Öztürk & Öztürk, 1996). Kepez Cape lies at the centre of this corridor, exposed to three dominant regional wind directions, offering an ideal setting for examining plastic transport mechanisms in a bidirectionally driven marine system.

This study analyses plastic debris accumulation across three wind-exposed shoreline sectors at Kepez Cape in the Strait of Çanakkale. The novelty lies in the use of morphometric metrics, such as surface area and volume, as indicators of environmental plastic load, addressing a gap in traditional count-based assessments. The objectives of the present study are to (i) assess bidirectional transport and retention patterns, (ii) evaluate spatial variability in relation to wind and current regimes, (iii) characterise particle types and colours, and (iv) apply morphometric parameters to infer environmental risks.

Materials and Methods

Study Site and Mapping

The present study was conducted in the Strait of Çanakkale, the southern component of the Turkish Straits System, which serves as a dynamic conduit. Here, the upper current predominantly carries Black Sea waters towards the Aegean, while the lower counter-current transports Mediterranean waters in the opposite direction (Öztürk & Öztürk, 1996). This bi-directional flow plays a crucial role in shaping the distribution and accumulation of plastic debris not only within the strait but also directs the distribution of plastic in a vast water basin extending from the Black Sea to the Mediterranean. Additionally, the Strait's northeast-southwest topographical alignment channels the prevailing wind in the same direction, potentially enhancing the transport of floating debris along its axis.

The cartographic representations in this study were generated by integrating two distinct GIS applications to ensure both accuracy and visual clarity. Ocean Data View ODV version 5.1 (Schlitzer, 2023) was employed for geospatial visualisation and site-specific mapping, while Open Source QGIS (QGIS.org, 2023) was used for all geographical analyses and spatial data processing. To ensure consistency and interoperability, datasets created by QGIS were standardised using ESRI software (Environmental Systems Research Institute, http://www.esri.com). Additionally, coastline delineation was refined through data contributions from OpenStreetMap, enhancing the spatial accuracy of coastal boundary representations. A superimposed illustration of prevailing wind directions for the Çanakkale Strait, based on data retrieved from the Turkish State Meteorological Service (TSMS, 2012) and Arslan et al. (2020), is mapped and presented in Figure 1.

Reflecting the distinct hydrodynamic and ecological characteristics of both the Black Sea and the Mediterranean, the Çanakkale Strait is bordered by a relatively small city and features more extensive natural coastlines compared to the heavily urbanised Istanbul Strait. The Kepez Cape region was selected as the study area due to its location at the point where the Aegean and Mediterranean meet, thereby providing strategic significance for the investigation of marine plastic pollution. Its relative remoteness from dense urbanisation and the absence of significant touristic activities reduce direct anthropogenic inputs, making the study area a valuable site for assessing the transport and deposition of plastic debris.

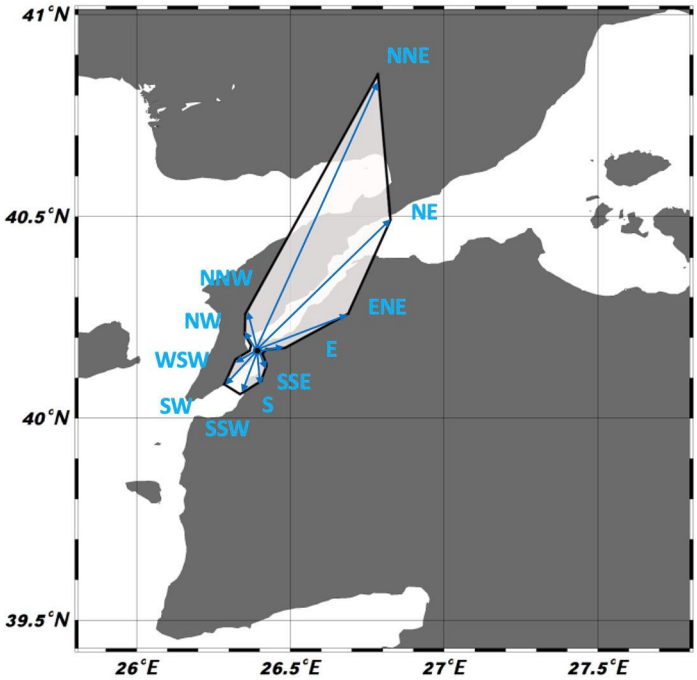

Figure 1. Superimposed illustration of prevailing wind directions in the Çanakkale Strait, produced by Ocean Data View. NNE: north-north-east, NE: north-east, SW: south-west

Sampling

Samples were collected from the Kepez Cape area (Çanakkale, Türkiye) between the geographical coordinates (WGS-84) 40°06'13.83" N and 26°22'14.30" E, and 40°06'14.75" N and 26°22'19.12" E, in April 2024. To ensure comprehensive spatial coverage, three different transects, each 50 m in length, were established along the three shores of the peninsula-like cape. A total of six sampling points were identified, two on each transect, and beach samples were collected by creating 30 x 30 cm quadrats at each sampling point. The Random Points feature in QGIS was employed to minimise potential selection bias (Figure 2).

The sampling locations at Kepez Cape were mapped to illustrate the spatial design of the study. Random sampling points were generated using QGIS along 50 m transects on three coastal sections, each aligned with one of the prevailing wind directions in the Çanakkale Strait, according to data provided by TSMS (2012) and Arslan et al. (2020): north-northeast (NNE, 22.5°), northeast (NE, 45°), and southwest (SW, 270°). This approach ensured representative coverage of the area, while accounting for local wind-driven influences on plastic deposition.

Sampling procedures were carefully designed to account for local wind conditions and minimise contamination risks. Following the collection, all samples were meticulously preserved to prevent contamination and subsequently transported to the Water Quality Laboratory of Çanakkale Onsekiz Mart University (Türkiye) for analysis.

Plastic Isolation

In the laboratory, samples were sieved using a 1.00 mm mesh stainless steel sieve to remove fine sediments. The retained fraction was transferred to metal trays, where tap water was added, and the mixture was left undisturbed for 48 h. This settling period allowed buoyant plastic particles to separate based on their lower specific gravity. Floating particles were retrieved using sterilised forceps, transferred to Petri dishes, and air-dried in a controlled environment to prevent contamination. Particle colour and morphology were recorded under daylight conditions.

Plastic Measurement and Classification

Isolated plastic particles were distinguished based on density differences and individually measured using a scale board, with their dimensions systematically recorded. As no spectrometric analysis was conducted, only plastic particles larger than 2.00 mm, which are sufficient for visual identification according to Isobe et al. (2019), were included in the analysis. Additionally, the type and colour of each particle were documented during the measurement process.

Volume and Surface Area of Plastics

To quantify the dimensions of plastic particles, ImageJ version 1.54 g (Wayne Rasband and contributors, National Institutes of Health, Bethesda, MD, USA; http://imagej.org) software (Schneider et al., 2012) was used to obtain their major and minor axes, along with additional parameters such as angle (the orientation of the primary axis relative to a line parallel to the x-axis of the plastic particle image) and area (the projected surface area in square pixels, later converted into metric units). These parameters, particularly the area, were incorporated into the "equivalent spherical diameter" (ESD) formula to approximate the spherical volume of each particle (Colas et al., 2018). The ESD formula is defined as:

$$ESD = 2 \times \sqrt{\left(\frac{Area}{\pi}\right)} \quad (1)$$

ESD: represents the equivalent spherical diameter
Area: corresponds to the surface area of the plastic particle

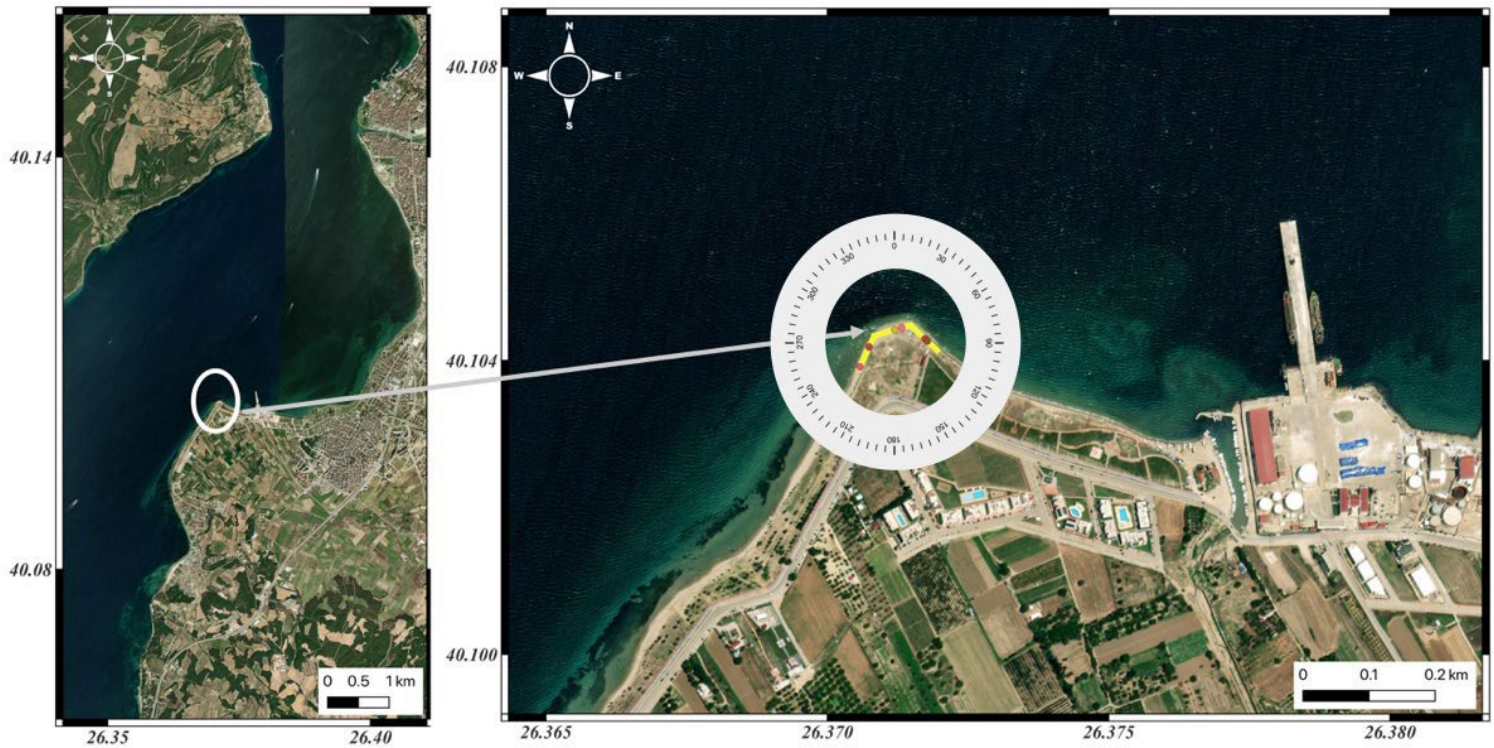

Figure 2. Illustration of the sampling area of Kepez Cape in the Çanakkale Strait, created by QGIS

Once the ESD of each particle was determined, its volume was calculated using the standard sphere volume formula. This approach provides a reasonable approximation of particle volume, despite the irregular morphologies of plastic particles, allowing for more consistent comparisons across samples.

Considering that two sampling points were selected along each 50-m transect, forming the three transects of 150 m in total, and plastic debris collected using 30 × 30 cm quadrats at each point, this will correspond to a total sampled area of 0.18 m² per transect (2 × 0.09 m²). Based on the quadrats at each sampling point, particle counts per surface area (items/cm2) have been calculated using the equation below:

$$\sum PC = \frac{item\#}{SA} \quad (2)$$

where,

∑PC: Total plastic count per area

item#: represents the number of plastic items

SA: corresponds to the sampled surface area

Statistical Analysis

To compare plastic pollution levels across the three directional coastal zone areas of the sampling stations, statistical analyses and data visualisations were carried out using R version 4.3.1 (R Core Team, 2023). The dataset comprised individual plastic particles collected from three coastal stations (NNE, NE, and SW), for which surface area (mm²) and volume (mm³) were measured. Prior to inferential testing, the Shapiro–Wilk test was applied to assess the normality of the distributions within each station. As the data significantly deviated from normality ($p<0.05$ in all cases), non-parametric tests were employed.

Differences in surface area and volume among stations were evaluated using the Kruskal–Wallis H test. Where significant effects were detected, pairwise comparisons were performed using Dunn's test with the Bonferroni correction to adjust for multiple comparisons. All tests were two-tailed, and results with $p<0.05$ were considered statistically significant.

Results and Discussion

Long-term meteorological records from the Çanakkale Strait indicate that the prevailing wind direction is north-northeast (NNE), followed by a secondary dominant wind from the northeast (NE). Further, a significant yet less frequent dominant wind is observed from the southwest (SW) (TSMS, 2012; Arslan et al., 2020).

A total of 74 plastic items were collected, specifically 32 from the NNE, 30 from the NE, and 12 from the SW. Surface area and volume were highest at NNE, followed by NE, with the lowest values obtained at SW (Table 1). These patterns suggest a higher deposition of larger debris along shorelines more directly exposed to Black Sea surface flows.

Table 1. Quantities, surface areas, and volumes of plastic particles were identified in the samples collected from different coastal zone locations in the Strait of Çanakkale

Station	Plastic particle (#)	Surface area (cm^2)[a]	Volume (cm^3)[b]
NNE	32	432.19	352.49
NE	30	411.75	747.24
SW	12	25.99	4.95
Total	74	869.94	1,104.69

[A, b] Surface area and volume of particles were dimensionally quantified using the Equivalent Spherical Diameter equation given as equation no 1. NNE: north-north-east, NE: north-east, SW: south-west

To contextualise these findings relative to sampling effort, surface area and volume were normalised per square meter. The NNE station exhibited the highest value of plastic counts (177.78#) for the surface area of 2400 cm², followed by NE (166.66#) with a coverage of 2280 cm², and SW (66.66#) with 140 cm² surface coverage, across a total sampled area (Table 2).

Table 2. Density-adjusted plastic particle metrics by stations (per cm²), selected along the coastal zone locations in the Strait of Çanakkale

Station	Particle count (#) *(items/cm²)*	Surface area (cm^2)*	Volume (cm^3)*
NNE	**177.78** ***(0.0741)***	**2400**	**1940**
NE	**166.66** ***(0.0731)***	**2280**	**4140**
SW	**66.66** ***(0.4761)***	**140**	**20**
∑SA	**411.1** ***(0.0851)***	**4820**	**6100**

* Surface area (SA) and volume of particles were dimensionally quantified using the Equivalent Spherical Diameter equation given as equation no 1. Particle counts per unit surface area (items/cm²) have been calculated using equation (2). NNE: north-north-east, NE: north-east, SW: south-west, $\sum_{SA}$ = total coverage of surface area

Kruskal-Wallis tests confirmed significant differences in surface area across stations ($p < 0.001$), with post-hoc analysis revealing that NNE had significantly higher values than NE. No significant difference was found between SW and the other stations.

The mean surface area per item was also greater at NE and NNE stations (1.3×10^{-3} m²/item) compared to SW stations (0.2×10^{-3} m²/item), suggesting that larger plastic fragments preferentially accumulate where Black Sea surface currents dominate. Conversely, the SW station, more influenced by Mediterranean inputs, retained smaller, more fragmented pieces of plastic (Figure 3). Surface area proved a more informative metric than particle counts. For instance, while NNE and NE had similar counts, NNE had nearly double the total volume, suggesting greater ecological impact due to larger debris size. These larger particles have greater potential to serve as vectors for invasive species, biofilm colonisation, and persistent organic pollutants (Zettler et al., 2013; Holmes et al., 2012). Moreover, surface area is a critical factor in the leaching behaviour of plastic additives such as bisphenol A and phthalates, which have known endocrine-disrupting properties (Hermabessiere et al., 2017).

When the Kruskal-Wallis test was conducted to assess the statistical difference in plastic surface area between sampling stations due to non-normality in the dataset, a significant difference was found among the three stations ($\chi^2 = 18.24$, df = 2, $p = 0.0001$), indicating that surface area values were not uniformly distributed across the study site. Post-hoc pairwise comparisons, using Dunn's test with the Bonferroni adjustment, provided further insight. Namely, a significant difference was found between the NNE and NE stations ($p = 0.00008$), while the difference between the SW and NE stations was marginally non-significant ($p = 0.06$). No significant difference was detected between the SW and NNE stations ($p = 1.0$).

These results highlight the NNE station as a central deposition zone, likely due to persistent wind exposure and alignment with south-westward currents. The minor elevation in surface area at SW compared to NE may reflect complex localised influences but lacks statistical support. Overall, the observed spatial variability supports the role of wind-driven transport and current directionality in shaping patterns of plastic accumulation along the coast (Figure 4).

Beyond quantitative metrics, particle morphology and colour were documented. Foam was the predominant type (51%), followed by fibre (43%), fragment (5%), and pellet (1%). The NE station showed the highest diversity of plastic types, whereas only foam, fibre, and fragments were noted in the NNE and SW stations.

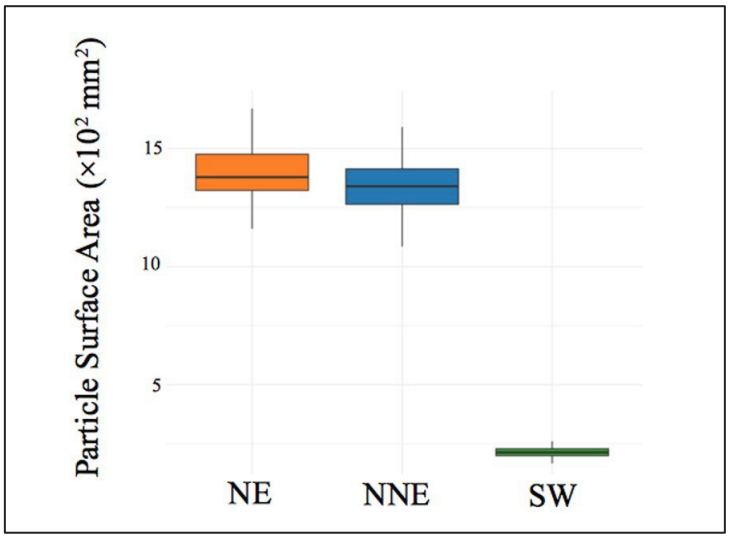

Figure 3. Distribution of individual plastic particle surface areas by station. NNE: north-north-east, NE: north-east, SW: south-west

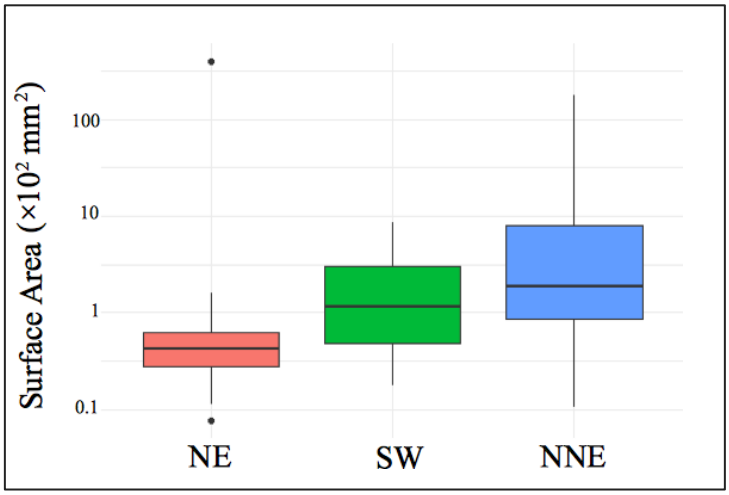

Figure 4. Total surface area of plastic debris collected from the three coastal stations of Kepez Cape, presented by station. NNE: north-north-east, NE: north-east, SW: south-west

Considering the colour properties found in the present study, white was the most common colour (38%), likely due to the use of titanium dioxide pigments, a compound widely used in the production of white plastics (Christie, 1998). The white colour was followed by green (26%) and grey (26%) at similar levels. The presence of green and grey fragments further reflects potential sources related to branding, packaging and fishing gear. Christie (1998) reported that green plastics are typically pigmented by using chromium (III) oxide (Cr_2O_3). Studies have shown that certain pigments contain heavy metals, including chromium (Cr), zinc (Zn), lead (Pb), cobalt (Co), titanium (Ti), and cadmium (Cd) (Campanale et al., 2020) which may be released into the environment during degradation (Christie, 1998), and particularly chromium (Cr), arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb) are reported as toxic and carcinogenic to both animals and human beings (Tchounwou et al., 2012), posing significant health concerns, particularly if absorbed into food webs or penetrating the sediment and groundwater systems.

The findings highlight the significant impact of wind and hydrodynamic forces on the transport and accumulation of plastic. The NNE station emerged as a depositional hotspot, potentially due to persistent wind exposure and alignment with Black Sea-derived surface currents. Similar dynamics have been observed in other transitional systems, such as the Strait of Gibraltar and the Istanbul Strait, where narrow topography and stratified flows enhance debris retention (Cózar et al., 2021; Isobe et al., 2019). This observation aligns with broader findings in marine environments, such as those reported in the Bay of Bengal, where plastic debris has also exhibited spatial and morphological variability in response to hydrodynamic forces (Mondal et al., 2024).

Like maritime traffic, winds play a significant role in transporting debris across coastal and marine environments. In recent decades, plastics, now the predominant component of marine litter, have also been shown to travel considerable distances under the influence of wind (Cózar et al., 2021), which is in agreement with findings in the present study, conducted along the Çanakkale Strait, a region characterised by its bi-directional strong wind regimes. The findings in this study highlight the importance of incorporating surface area as a key metric in assessing the environmental impact of coastal plastic pollution, offering a more nuanced understanding of transport mechanisms in complex marine systems. As recently underlined by Delaeter et al. (2022), additives such as phthalates and bisphenols, incorporated to enhance plastic properties during manufacturing, may leach over time into the marine environment, resulting in rates proportional to surface area (Delaeter et al., 2022). Therefore, surface area is a critical metric for understanding the impacts of plastic pollution on the water column and biota, as it governs interactions such as biofouling, contaminant adsorption, and the leaching of additives used in plastic production.

Addressing the targets for improving future work, this study highlights the importance of morphometric indicators, specifically surface area and volume, in evaluating the ecological significance of plastic debris accumulation. Building upon these findings, future work should aim to integrate morphometric data with spatial analysis tools such as Geographic Information Systems (GIS) and remote sensing. By georeferencing plastic deposition patterns and correlating them with wind, current, and shoreline morphology datasets, it will be possible to model plastic transport dynamics with greater precision.

The use of drone-based imagery and satellite-derived wind

and surface current data could enhance temporal monitoring and facilitate the detection of seasonal or event-driven variability in plastic accumulation. Such integration would allow for the creation of high-resolution spatial risk maps, which can inform local coastal management and policy-making.

Moreover, expanding this methodology to other regions of the Turkish Straits System and similar bidirectional marine corridors could help assess the generalizability of morphometric indicators in dynamic hydrodynamic contexts. Future studies may also incorporate machine learning algorithms for automated plastic classification and volumetric estimation from imagery, further improving data efficiency and standardisation in coastal plastic monitoring protocols.

Conclusion

This study demonstrates the value of incorporating morphometric parameters into assessments of plastic pollution. Surface area and volume provide a more ecologically meaningful understanding of pollution load than particle count alone. Wind-driven transport and current stratification have a significant influence on deposition patterns in narrow straits, such as the Çanakkale Strait. Results from the present study call for wind- and current-informed monitoring strategies and highlight the need to standardise morphometric measurements in coastal plastic surveys. Expanding this approach to other straits could enhance regional understanding of plastic dynamics across the Mediterranean basin. The conclusions drawn here are based strictly on the quantitative data collected during the study, thereby avoiding speculative interpretations.

Compliance with Ethical Standards

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

Ethics committee approval: No living organisms have been used in any part of this research; therefore, no ethics committee approval is required for this study.

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

Funding disclosure: -

Acknowledgements: We want to thank the Turkish State Meteorological Service (TSMS) for providing wind data.

Disclosure: -

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AQUATIC RESEARCH
E-ISSN 2618-6365

Aquatic Research 8(4), 234-242 (2025) • https://doi.org/10.3153/AR25023 *Research Article*

Determination of Ephemeroptera species diversity of Akçay Creek (Sakarya, Türkiye)

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Cite this article as:
Gider, C., Aydınlı, C. (2025). Determination of Ephemeroptera species diversity of Akçay cressk (Sakarya, Türkiye). *Aquatic Research*, 8(4), 234-242. https://doi.org/10.3153/AR25023

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Submitted: 25.07.2025
Revision requested: 15.09.2025
Last revision received: 16.09.2025
Accepted: 16.09.2025
Published online: 08.10.2025

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Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

The Marmara Region holds an important place in Türkiye's zoogeography and biodiversity, as the Macedonia-Thrace refuge, in which it is located, serves as an entranceway for European faunal elements to Anatolia. Although comprehensive studies have been conducted on the mayflies in some provinces of the Marmara Region, only four species have been reported from Sakarya province, which has many valuable aquatic habitats, and there is no detailed study.

This study, conducted in the Akçay Creek, aimed to address the deficiencies in the Marmara Region and provide more precise data on the Ephemeroptera fauna of the region. In this study, conducted in the Akçay Creek, 10 genera and 15 species belonging to five families were identified from the collected specimens. Thirteen of these species were reported for the first time from this area and are new records for Sakarya province.

Keywords: Mayflies, Nymph, Fauna, Marmara Region, Aquatic insects

Introduction

The order Ephemeroptera, one of the oldest and most primitive winged insect groups known, dates back to approximately 290 million years ago (late Carboniferous or Permian periods) (Edmunds & McCafferty, 1988; Brittain & Sartori, 2003). Unlike other insect groups, they have a winged stage called subimago before the adult stage. The order members, who spend most of their life cycle (99%) as nymphs in water, adapt to different habitats during this period, and morphological diversity occurs most frequently during this time (Barnard, 2011).

They are one of the important groups studied in zoogeographical studies as they are one of the oldest known insect groups, and due to factors that prevent their distribution, such as their nymphal stages being aquatic without exception, their ability to fly in adult stages, and their very short life span (Brittain, 1982; Kazancı, 2001; Ertorun & Tanatmış, 2004). Additionally, they are widely used as bioindicator organisms in studies assessing water quality (Barnard, 2011). Their sensitivity to organic pollution, presence in almost all freshwater systems, diverse feeding habits, ability to absorb toxic substances uniformly, and gradual and long-term response to environmental changes provide significant advantages in their use in studies on water quality (Landa & Soldan, 1995).

It is known that Ephemeroptera species, which can be found in almost all natural and clean waters, also constitute 10-25% of all macrozoobenthos in clean waters and make a significant contribution to secondary production in the food chain, especially in streams and stagnant waters (Brittain, 1982; Brittain & Sartori, 2003).

Since Ephemeroptera nymphs are highly sensitive to both long-term and short-term changes in the aquatic environment, they are often preferred as indicator organisms in various biotic indices, including the Chandler Biotic System (CBS), Biological Monitoring Working Party (BMWP), Trent Biotic Index (TBI), and Belgian Biotic Index (BBI), among others, for determining water quality. Some features of Ephemeroptera species make them suitable for use as bioindicators in determining water quality: Their long nymphal periods and spending this period in the aquatic environment, their nymphs being recognised taxonomically, being found in almost all aquatic ecosystems, reacting to changes in the environment gradually and for long periods, and having different feeding styles. (Alba-Tercedor et al., 1995; Kazancı et al., 1997; Landa & Soldan, 1995; Moog et al., 1997).

Many studies have been conducted to reveal the Ephemeroptera fauna of Türkiye and to determine the distribution of these species. To date, a total of 165 species (including four subspecies) belonging to 14 families and 33 genera of the Ephemeroptera order have been identified (Salur et al., 2016; Aydınlı & Ertorun, 2023).

Although studies have been conducted in the Marmara Region regarding the Ephemeroptera order, there are no studies covering the northeastern part of the region, where Kocaeli, Sakarya, and Yalova provinces are located (Salur et al., 2016). This region holds a critical position in zoogeographical terms, as it is situated on the Macedonia-Thrace line, which serves as the entrance and distribution route for Boreal European fauna elements into Anatolia.

The Akçay Creek, located in the Sapanca district of Sakarya Province, which is part of the Marmara Region, originates from the Eskiyayla region in the northwest of the Geyve district and merges with several small streams before flowing into the Sakarya River in the village of Adliye.

There is the Akçay Dam in the Eskiyayla region where the creek originates. In addition, there are trout farms, restaurants and marble processing facilities on the creek, especially around Adliye village, which is close to the Sakarya River (Baştürk, 2006; Baltacı & Aydın, 2016; Anonymous, 2018, 2023).

There is no detailed study on the Ephemeroptera order in Sakarya province, which contains important wetlands in the Marmara Region. To date, three studies have been conducted in this region, but only four species have been reported (Tanatmış, 1995; Taşdemir et al., 2008; Kazancı, 2013).

This study aims to collect samples of the Ephemeroptera order from Akçay Creek, located within the borders of Sakarya province, where the number of species that have not been studied extensively and have been identified within the provinces of the Marmara Region is relatively low, and to identify these species. At the same time, the identified species are intended to serve as a reference in future studies on biodiversity, biotic indices, and the determination and monitoring of water quality.

Materials and Methods

In this study, 308 larvae were collected and identified from six stations (Figure 1) on the Akçay Creek (Sakarya) in July, August, and September 2023, as well as in May 2024. During the field studies, sampling was conducted at each station from habitats with different ecological characteristics (stony or sandy ground structure, current velocity, presence or absence of aquatic vegetation, turbidity, etc.) (Figure 2).

The specimens were collected using a sieve (1mm mesh size), a water hand net, and forceps (leg. C. Gider). Samples were collected into bottles containing 70% ethyl alcohol, and data, including date, locality, elevation, and coordinates, were recorded (Table 1).

Based on their general body structures, the specimens were identified to the family and genus levels. Permanent slides were prepared by dissecting the mouthparts, forelegs, and gills to identify them to the species level. Slides and specimens were examined and identified using a Leica MZ12.5 stereomicroscope and a Leica DM LS2 light microscope. In the identification of the species, Grandi (1960), Müller-Liebenau (1969), Belfiore (1983), Malzacher (1984), Elliott et al. (1988), Harker (1989), Studemann et al. (1992), Kluge (1997), Bauernfeind (1995), Eiseler (2005), Bauernfeind & Soldán (2012) and Hrivniak et al. (2020) were used.

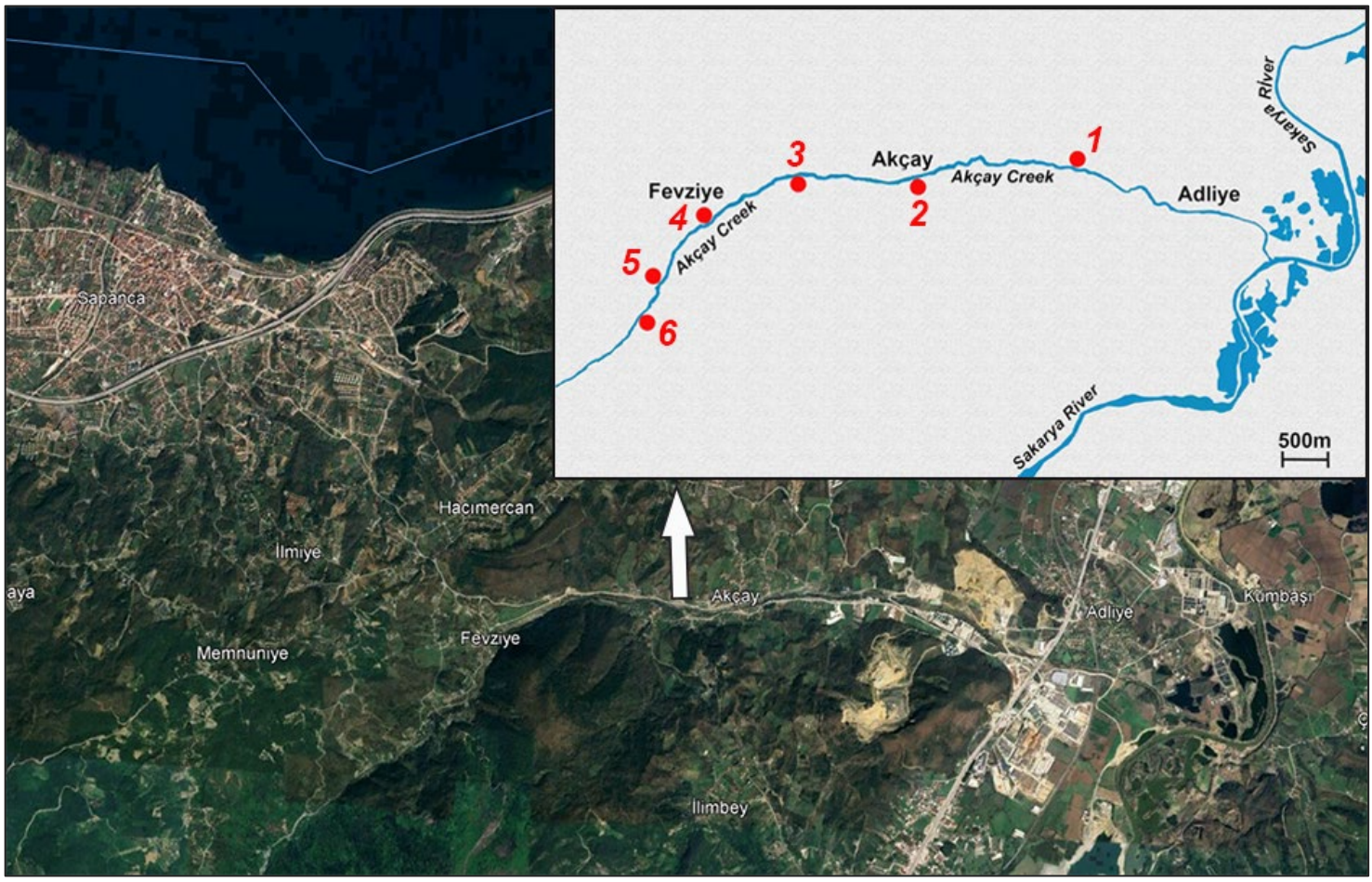

Figure 1. Map of the study area and location of sampling stations.

Figure 2. Photographs of sampling stations (st) (Photo: *C. Gider*).

Table 1. Information on the sampling stations.

Station	Station name	Coordinates (N, S)	Elevation (m)
1	Ahmediye	40°40'11.4", 30°20'21.9"	75
2	Akçay	40°40'05.7", 30°19'33.6"	106
3	Akçay-Fevziye yolu	40°40'06.0", 30°18'37.3"	144
4	Fevziye	40°39'46.7", 30°18'06.8"	173
5	Şükriye	40°39'17.9", 30°17'31.9"	224
6	Memnuniye	40°38'14.7", 30°16'29.3"	382

Results and Discussion

In this study, 308 specimens collected from 6 stations were examined, and 15 species belonging to 10 genera from 5 families were identified. The taxa list and sampling data for each species (date, number of individuals collected and station number, respectively) are given below:

Baetidae Leach, 1815

Baetis Leach, 1815

1. *Baetis (Baetis) buceratus* Eaton, 1870

   Material examined: 10.09.2023, 19 larvae (1); 10.09.2023, 1 larva (3).

2. *Baetis (Baetis) fuscatus* (Linnaeus, 1761)

   Material examined: 10.07.2023, 1 larva (1); 10.07.2023, 1 larva (4); 12.08.2023, 1 larva (3); 10.09.2023, 3 larvae (1); 10.09.2023, 4 larvae (3); 14.05.2024, 7 larvae (3); 14.05.2024, 1 larva (4).

3. *Baetis (Baetis) lutheri* Müller-Liebenau, 1967

   Material examined: 10.07.2023, 2 larvae (2); 10.07.2023, 1 larva (3); 12.08.2023, 1 larva (6); 10.09.2023, 2 larvae (1); 10.09.2023, 2 larvae (6); 14.05.2024, 5 larvae (3).

4. *Baetis (Baetis) vernus* Curtis, 1834

   Material examined: 12.08.2023, 1 larva (3); 10.09.2023, 7 larvae (1).

5. *Baetis (Rhodobaetis) rhodani* (Pictet, 1843)

   Material examined: 10.07.2023, 10 larvae (1); 10.07.2023, 15 larvae (2); 10.07.2023 13 larvae (3); 10.07.2023, 3 larvae (4); 10.07.2023, 1 larva (6); 12.08.2023, 28 larvae (3); 12.08.2023, 18 larvae (4); 12.08.2023, 6 larvae (5); 12.08.2023, 4 larvae (6); 10.09.2023, 3 larvae (1); 10.09.2023, 22 larvae (3); 10.09.2023, 1 larva (4); 10.09.2023, 8 larvae (5); 10.09.2023, 6 larvae (6); 14.05.2024, 20 larvae (3)

Procloeon Bengtsson, 1915

6. *Procloeon pennulatum* (Eaton, 1870)

   Material examined: 12.08.2023, 3 larvae (3); 10.09.2023, 7 larvae (3).

Cloeon Leach, 1815

7. *Cloeon dipterum* (Linnaeus, 1761)

   Material examined: 12.08.2023, 1 larva (2); 12.08.2023, 2 larvae (3).

Centroptilum Eaton, (1869).

8. *Centroptilum luteolum* (Müller, 1776)

   Material examined: 10.09.2023, 1 larva (3).

Heptageniidae Needham in Needham & Betten, 1901

Epeorus Eaton, 1881

9. *Epeorus (Caucasiron) znojkoi* (Tshernova, 1938)

   Material examined: 14.05.2024, 1 larva (3).

Electrogena Zurwerra & Tomka, 1985

10. *Electrogena affinis* (Eaton, 1886)

Material examined: 10.07.2023, 1 larva (4); 10.07.2023, 1 larva (5); 10.07.2023, 1 larva (6), 12.08.2023, 4 larvae (4), 12.08.2023, 3 larvae (6); 10.09.2023, 1 larva (5).

Leptophlebiidae (Banks, 1900).

Habrophlebia Eaton, 1881

11. *Habrophlebia lauta* McLachlan, 1884

Material examined: 10.07.2023, 7 larvae (4); 10.07.2023, 3 larvae (6).

Ephemerellidae Klapálek, 1909

Torleya Lestage, 1917

12. *Torleya major* (Klapálek, 1905)

Material examined: 14.05.2024, 1 larva (3).

Ephemerella Walsh, 1863

13. *Ephemerella (Serratella) ignita* (Poda, 1761)

Material examined: 10.07.2023, 1 larva (3).

Caenidae Newman, 1853

Caenis Stephens, 1835

14. *Caenis luctuosa* (Burmeister, 1839)

Material examined: 10.07.2023, 5 larvae (2); 12.08.2023, 6 larvae (2); 12.08.2023, 24 larvae (3).

15. *Caenis macrura* Stephens, 1836

Material examined: 12.08.2023, 9 larvae (1); 12.08.2023, 4 larvae (2); 12.08.2023, 2 larvae (4); 10.09.2023, 3 larvae (3).

To date, four species (*B. muticus, B. rhodani, C. dipterum, and Ephoron virgo*) have been reported from Sakarya Province (Salur et al., 2016). Thirteen of the 15 species identified in this study were reported for the first time from Sakarya province and are new records for this region (Table 2). When the distribution of species across sampling stations was examined, it was found that the first four stations had higher species diversity and a greater number of individuals collected than the others (Table 2). It is thought that since the ground at the fifth and sixth stations is covered with medium-to large-sized stones, the stream exhibits flood stream characteristics in these parts. Accordingly, the number of individuals and species diversity are low.

When the identified species were evaluated according to the sampling stations, it was determined that the pollution level increased as the Akçay Creek approached the Sakarya River. The presence of Heptageniidae species (which have low tolerance to pollution) and the higher species diversity in the third and sixth localities support this view. In addition, it is thought that the low species diversity in the first and second localities is related to the cumulative increase in waste from tourist hostels and trout farms along the Akçay Creek towards the first locality, as well as the presence of marble processing facilities in these localities.

Conclusion

Türkiye possesses a rich biodiversity potential due to its vast surface area, its regions exhibiting diverse ecological and geological characteristics, its availability of areas where faunal elements from different zoogeographic regions can spread, and its role as a gateway for species migrating from north to south and vice versa. The first study on the order Ephemeroptera in Türkiye was conducted by Ulmer in 1920. Until the 1980s, few studies by foreign researchers were available. In recent years, numerous new records and species have been identified, particularly in studies conducted by Turkish researchers, and the Ephemeroptera fauna of Türkiye is beginning to take shape. For all that, many areas remain to be researched, as Türkiye spans a large area. Studies have generally focused on large rivers and their basins, and the number of researchers working in this field is limited.

While studies have been conducted in the Marmara Region of Türkiye, there are no studies covering the northeastern part of the region, which includes Kocaeli, Sakarya, and Yalova provinces (Salur et al., 2016). The few studies conducted consist of samples collected from the Sakarya River. Considering that this region holds an important zoogeographical position due to its location on the Macedonia-Thrace line, which serves as the entry and distribution route for Boreal European fauna elements into Anatolia, it is expected that the study of other small streams and creeks in the region will also contribute to the fauna of Türkiye. In this context, this study, conducted in the Akçay Creek of the Sapanca district in Sakarya province, aimed to address these deficiencies in the Marmara Region and to provide more precise data on the Ephemeroptera fauna of the region.

Table 2. The number of individuals collected by species and station.

	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	Total
B. (B.) buceratus[*]	19	-	1	-	-	-	**20**
B. (B.) fuscatus[*]	4	-	12	2	-	-	**18**
B. (B.) lutheri[*]	2	2	6	-	-	3	**13**
B. (B.) vernus[*]	7	-	1	-	-	-	**8**
B. (R.) rhodani	13	15	83	22	14	11	**158**
P. pennulatum[*]	-	-	10	-	-	-	**10**
C. dipterum	-	1	2	-	-	-	**3**
C. luteolum[*]	-	-	1	-	-	-	**1**
E. (C.) znojkoi[*]	-	-	1	-	-	-	**1**
E. affinis[*]	-	-	-	5	2	4	**11**
H. lauta[*]	-	-	-	7		3	**10**
E. (S.) ignita[*]	-	-	1	-	-	-	**1**
T. major[*]	-	-	1	-	-	-	**1**
C. luctuosa[*]	-	11	24	-	-	-	**35**
C. macrura[*]	9	4	3	2	-	-	**18**
Total	**54**	**33**	**146**	**38**	**16**	**21**	**308**

[*] *Reported for the first time from Sakarya province*

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 article.

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

Funding disclosure: This research was financially supported by the Scientific and Technological Research Council of Türkiye, 2209-A-Research Project Support Programme for Undergraduate Students (Grant no: 1919B012308706) and the Scientific Research Projects Unit of Eskişehir Technical University (Grant no: 24LÖP082).

Acknowledgements: -

Disclosure: -

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AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 8(4), 243-252 (2025) • https://doi.org/10.3153/AR25024 *Research Article*

Preliminary study on the impact of mussel integration on qualitative changes of water in an aquaponic system

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Cite this article as:
Kapucuoğlu, B., Tunçelli, G., Memiş, D. (2025). Preliminary study on the impact of mussel integration on Qualitative changes of water in an aquaponic system. *Aquatic Research*, 8(4), 243-252. https://doi.org/10.3153/AR25024

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Submitted: 16.07.2025
Revision requested: 18.08.2025
Last revision received: 26.09.2025
Accepted: 28.09.2025
Published online: 08.10.2025

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Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

Freshwater resource sustainability is a growing concern in modern agriculture. Aquaponic systems, which integrate fish farming and soilless plant cultivation, offer a promising solution by enabling nutrient recycling within a closed water loop. However, the accumulation of organic waste and suspended solids can challenge system stability and water quality. This study investigates the potential of the freshwater mussel *Unio crassus* as a biological filter to enhance organic waste removal and water purification in aquaponic systems.

Two system configurations were tested: the Media Bed System and the Nutrient Film Technique (NFT) system, each receiving identical daily tilapia effluent dosing, and containing identical plant cohorts (lettuce and collard greens) and *U. crassus* mussels. Over 30 days, key water quality parameters—total suspended solids (TSS), dissolved oxygen (DO), pH, and ammonia (NH_3)—were monitored, alongside plant biomass development.

Results indicated that while the media bed system achieved significantly lower TSS concentrations (14.2 ± 2.1 mg L^{-1}), the NFT system maintained higher DO levels (5.8 ± 0.6 mg L^{-1}) and supported greater plant growth. Localised zones of improved clarity were observed near mussel clusters; however, quantitative mussel-specific contributions were not measured.

Within this scope, integrating *U. crassus* may offer ecological value while warranting controlled, quantitative verification. Moreover, this approach may contribute to species conservation through practical ex situ applications. The study highlights the potential of hybrid aquaponic systems utilising both design types and nature-based filtration for sustainable food production.

Keywords: Aquaponics, Freshwater mussel, Organic waste, Water quality, NFT system, Media bed, Sustainability

Introduction

The increasing scarcity and contamination of freshwater resources, resulting from urbanisation, agriculture, and industrial activities, have driven the development of sustainable and integrated food production systems. Aquaponics, a synergistic combination of aquaculture and hydroponics, has emerged as a promising method that allows the recirculation of water and nutrients in a closed-loop system, significantly reducing water usage while supporting the simultaneous cultivation of fish and plants (Goddek et al., 2019). This system enhances resource efficiency, minimises environmental impact, and contributes to circular economy models in agriculture (Tyson et al., 2011; Yeşiltaş et al., 2025).

Aquaponics utilises the nitrogenous waste products produced by fish, which are converted by nitrifying bacteria into nutrients that are accessible to plants. In turn, plants absorb these nutrients and help to improve water clarity by reducing suspended particles in the water before it is returned to the fish tanks (Rakocy et al., 2006; Kargın & Bilgüven, 2018). This mutualistic relationship is crucial to the sustainability of aquaponic systems, eliminating the need for synthetic fertilisers and reducing extensive water inputs.

Historically, integrated farming practices can be traced back to ancient civilisations such as the Aztecs, who practised Chinampa agriculture—a floating garden system that utilised nutrient-rich lake water for crop production (Yegül et al., 2020). Contemporary aquaponic systems build upon these principles while incorporating technological advancements such as automated monitoring, energy-efficient pumps, and modular system designs (Tunçelli & Memiş, 2024).

One of the critical challenges in aquaponic systems remains the efficient removal of organic particulates and suspended solids, which can accumulate and negatively impact plant growth and fish health (Tunçelli et al., 2023). While mechanical and biological filtration methods are commonly employed, there is a growing interest in nature-based solutions to improve water quality. In this context, freshwater mussels—particularly *Unio crassus*—present a unique opportunity.

Unio crassus is an endangered species (Lopes-Lima et al., 2024) of freshwater mussel known for its ecological role as a natural biofilter (Tomović et al., 2023). These organisms are filter feeders, capable of removing fine suspended particles and microorganisms from the water column, thus enhancing water clarity and reducing total suspended solids (TSS) (Bahrioğlu, 2017). Their incorporation into aquaponic systems has the potential to complement existing filtration strategies while also providing a model for ex situ conservation of threatened mussel species.

Unio crassus was chosen because (i) it is a locally occurring unionid with recognised suspension-feeding capacity and documented water-polishing potential; (ii) its environmental requirements (cool, well-oxygenated flow over sandy–gravel substrates) can be approximated in controlled aquaponic loops with stable temperature and gentle current; and (iii) non-lethal, permit-based husbandry can contribute to ex-situ conservation and outreach while testing nature-based filtration within food-production contexts. Husbandry conditions in our systems (acclimation, continuous flow, high dissolved oxygen, shelter within perforated containers) were aligned with these requirements. In this study, mussels were acclimated and maintained under stable temperature and flow, housed in mesh containers near the return line to maximise contact with suspended solids while minimising stress.

Recent investigations have highlighted the importance of microbial community dynamics in aquaponic systems, where the presence of beneficial bacterial taxa, including Bacillus, Pseudomonas, and Serratia, facilitates enhanced nutrient cycling and inhibits the growth of opportunistic pathogens. Tunçelli et al. (2025) reported that *Bacillus spp.* may inhibit pathogenic species, such as *Aeromonas sobria*, in koi carp–parsley systems, supporting the hypothesis that native microbial communities play a role in stabilising water quality.

Despite this ecological promise, the use of freshwater mussels in aquaponic systems has been rarely explored in the scientific literature. While some studies have examined the role of unionid mussels in natural or engineered water purification contexts, very few have assessed their functionality within closed-loop aquaponic configurations. Table 1 summarises selected studies that investigated various freshwater mussel species and their contributions to water treatment performance in aquaculture or wastewater systems. These findings provide a foundation for understanding the potential role of *U. crassus* in integrated aquaponic setups.

Building upon these insights, this study aims to evaluate the filtration capacity and practical integration of *Unio crassus* in two commonly used aquaponic configurations: Media Bed and NFT. By comparing water quality parameters and plant growth metrics across these systems, this research provides insight into the role of mussels in enhancing organic waste management and the overall sustainability of aquaponic production. The findings aim to contribute to both environmental conservation efforts and the development of innovative aquaculture practices.

Table 1. Previous Studies on Freshwater Mussels in Filtration and Aquaponic Systems.

Study (Year)	Mussel Species	System Type	Key Findings and Results
Demircan et al. (2022)	*Unio crassus*	Laboratory (aquaculture effluent, bacteria)	Reductions in *Staphylococcus* and *Aeromonas* loads within 48 h; depuration/antibiotic treatments improved outcomes.
Campos et al. (2022)	*Unio mancus*	Laboratory (*E. coli* challenge; clearance assay)	Suspended *E. coli* was rapidly cleared within the first 24 h; retention in soft tissues was observed for up to 4 days.
Tang et al. (2015)	*Hyriopsis cumingii*	Fish–mussel integrated culture (pond/IMTA-vari)	Fish–mussel integration (with feed supplementation) increased fish and pearl yields and improved nitrogen utilisation efficiency.
Ranjbar et al. (2021)	*Anodonta cygnea*	Closed-loop wastewater treatment reactor	Biological removal of pollutants in a closed-loop reactor; results indicated decreases in suspended solids and microbial load.
Geng et al. (2022)	*Mixed (mussel–microalgae–bacteria)*	Pilot-scale aquaculture wastewater treatment	Significant reductions in NH_3–N, TN, TP, COD and suspended solids using a mussel–microalgae–bacteria complex; improved water quality in pilot and field (river) applications.

Materials and Methods

Experimental Design

The experiment was conducted at the Aquaponics Research Unit of Istanbul University, Faculty of Aquatic Sciences. Two types of recirculating aquaponic systems were established: the Media Bed System and the NFT System. Both systems were designed to operate under similar environmental conditions and were run concurrently over 30 days.

Each system was replicated three times, with identical tank volumes (~150 L) and biological loads to allow for direct performance comparison (Figure 1). The systems shared a standard design in which water was continuously circulated between the fish tank and plant-growing units. In both systems, *Unio crassus* mussels were incorporated as a biological filtration component.

Media Bed System: Constructed using 130 × 40 × 33 cm plastic containers filled with ~100 L of expanded clay media (hydroton). Each container was fitted with a custom-made bell siphon (Ø50 mm outer shell, Ø20 mm inner air break pipe) to enable periodic flood-and-drain cycles. Water from the sump tank was pumped into the media bed and drained by gravity after reaching a set height.

NFT System: Comprised two 1-meter-long polyvinyl chloride (PVC) pipes (Ø110 mm) per unit. Each pipe featured five equally spaced openings (20 cm apart) to accommodate net pots. Submersible pumps delivered water from the sump tank to the top of each pipe. The water passed over the plant roots and returned to the tank by gravity, flowing past mussels located in mesh baskets at the return point to optimise contact with suspended solids.

Both systems utilised full-spectrum LED grow lights (100 W) to maintain consistent photoperiods of 16 hours of light and 8 hours of darkness. The ambient room temperature ranged from 22 °C to 25°C.

Biological Material

Two biological components were actively utilised in both aquaponic system types: plants and freshwater mussels. Nile tilapia (*Oreochromis niloticus*) were not directly involved in the experiment but served as the source of nutrient-rich effluent water.

Fish Effluent Source**:** Nile tilapia (*Oreochromis niloticus*) culture tanks supplied the daily effluent. Stocking density was 10 kg m^{-3} with a mean individual mass of 200 g. Fish were fed with a commercial trout feed, consisting of 45% crude protein and 12% crude fat at 2% BW day^{-1}, 2 times per day. On each experimental day, 15 L of clarified effluent was taken from the tilapia tanks and dosed to each replicate (≈10% of the ~150 L working volume). This standardised organic loading across replicates without manipulating the fish, allowing for a consistent comparison of design-level effects between systems.

Plants: Two leafy vegetable species—lettuce (*Lactuca sativa*) and collard greens (*Brassica oleracea* var. *acephala*)—were selected for their compatibility with aquaponic systems and rapid growth. Each replicate consisted of five plants of each species, arranged in an alternating order to minimise competition effects.

Plant performance was evaluated as fresh weight (FW) at harvest after 5 min surface-drying, and as relative growth rate (RGR):

$$RGR = (\ln W_2 - \ln W_1) / t \ (day^{-1}),$$

Where W_1 and W_2 are initial and final FW, and t is the cultivation period (days). Procedures followed standard small-scale aquaponic practice for leafy crops.

Mussels: Each replicate received 15 *U. crassus* (shell length 5.8 ± 0.4 cm; total biomass ~250 g), corresponding to ~1.7 g L^{-1} and 0.10 ind L^{-1}. The target density was selected to ensure measurable contact between suspended solids and suspension feeders while remaining within a conservative biomass-to-volume range for non-lethal husbandry. Individuals were quarantined for 14 days and housed in perforated containers placed near the return line to maximise exposure to suspended solids and maintain high oxygenation.

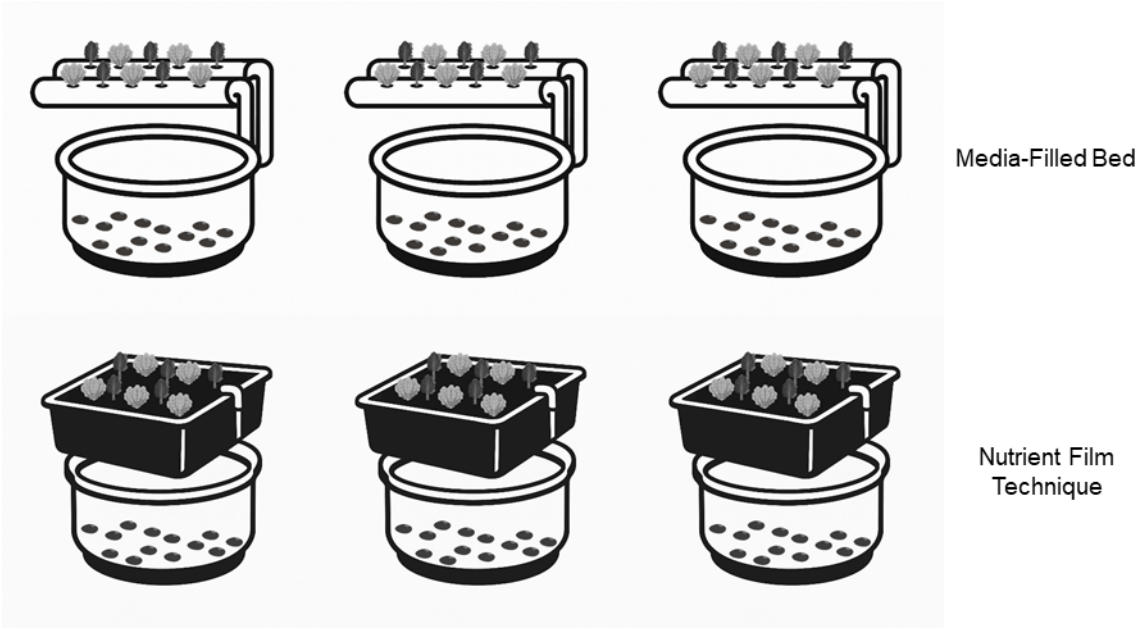

Figure 1. Experimental aquaponics system design

Figure 2. Lettuce and collard greens (left) and freshwater mussels (*Unio crassus*) (right).

Water Quality Analysis

Water samples were collected over 30 days. TSS, pH, and DO were measured using gravimetric and probe-based methods. The total suspended solids were calculated using:

$$TSS\ (mg\ L^{-1}) = (A - B) \times 1000 / V$$

Where:

A = final mass of crucible + residue,

B = mass of empty crucible,

V = volume of water sample (mL)

Statistical Analysis

Data were analysed using Student's t-test; significance was set at $p < 0.05$. Normality and homogeneity were checked by Shapiro–Wilk and Levene's tests, respectively.

Ethical Statement

This study did not involve any direct experimentation on live animals. Fish were not subjected to handling or invasive procedures, and only wastewater from existing aquaculture systems was utilised. Therefore, no ethical approval was required for the mussels, as per institutional and national guidelines. Mussel handling was non-lethal; individuals were returned to their source habitat.

Results and Discussion

Water Quality

Differences in water quality were observed between the two system types over the 30 days. Total Suspended Solids (TSS), dissolved oxygen (DO), pH, and ammonia (NH_3) were the primary indicators used to assess system performance (Table 1).

Localised visual clarity was noted around mussel clusters; however, no mussel-free control was included. Therefore, mussel-specific effects cannot be isolated from design-level effects (e.g., media trapping vs. NFT hydraulics). Visual comparisons are qualitative only and are not interpreted as quantitative filtration outcomes.

The media bed showed lower TSS (14.2 ± 2.1 mg L^{-1}) than NFT (22.7 ± 3.4 mg L^{-1}; $p < 0.01$). Dissolved oxygen was higher in NFT (5.8 ± 0.6 mg L^{-1}) than in the media bed (4.2 ± 0.9 mg L^{-1}). pH ranged between 6.8–7.4 in NFT and 6.7–7.3 in media beds. Ammonia concentrations were 0.18 ± 0.03 mg L^{-1} in NFT and 0.26 ± 0.04 mg L^{-1} in media beds.

Figure 3 illustrates the temporal profiles of TSS for both systems over 30 days.

Plant Growth Performance

Final plant fresh weight was higher in NFT than in media beds (by ~20–25%; Figure 4). Figure 4 presents the final plant fresh weight for both systems.

Table 1. Summary of water quality parameters in NFT and media bed systems over the 30-day trial.

Parameter	NFT System	Media Bed System
TSS (mg L⁻¹)	22.7 ± 3.4[a]	14.2 ± 2.1[b]
DO (mg L⁻¹)	5.8 ± 0.6[a]	4.2 ± 0.9[b]
pH	7.2 ± 1.3[a]	7.3 ± 1.2[a]
NH_3 (mg L⁻¹)	0.18 ± 0.03[a]	0.26 ± 0.04[b]

Values are mean ± SD. Different superscript letters within a row indicate significant differences between systems ($p < 0.05$; statistical test specified in Methods)

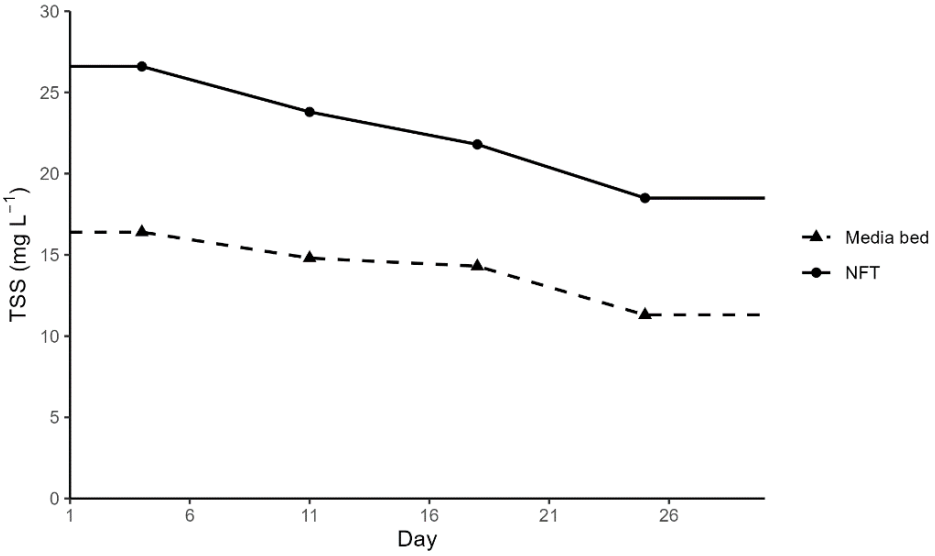

Figure 3. Temporal profiles of TSS in NFT and media bed systems over 30 days

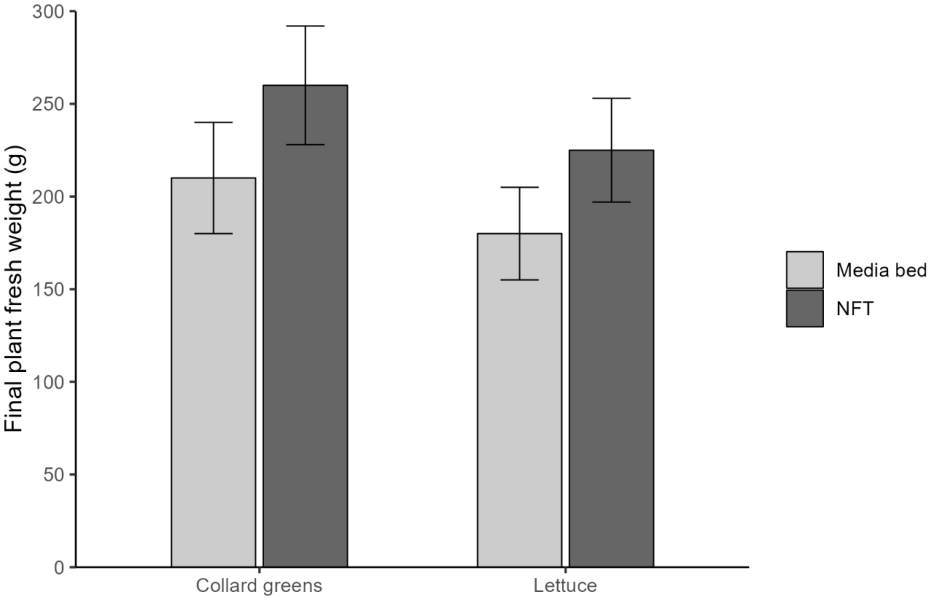

Figure 4. Final plant fresh weight in NFT and media bed systems (mean ± SD)

Mussel Filtration Activity

Localised zones of reduced turbidity were observed around mussel clusters, especially near water return areas. Quantitative filtration metrics were not collected; therefore, mussel-specific effects were not estimated.

The two designs differed in terms of suspended solids and dissolved oxygen. Potential mussel contributions require confirmation with mussel-free controls and quantitative turbidity/clearance measurements.

The present study compared two commonly used aquaponic configurations—media bed and nutrient film technique (NFT)—and documented apparent design-level differences in suspended solids, dissolved oxygen, and plant performance over a 30-day trial period. The media bed configuration exhibited lower total suspended solids (TSS; 14.2 ± 2.1 mg L^{-1}) than NFT (22.7 ± 3.4 mg L^{-1}; $p < 0.01$), which is consistent with the physical interception and settling that occur in porous substrates with longer hydraulic retention. Conversely, the NFT system maintained higher dissolved oxygen (DO; 5.8 ± 0.6 vs. 4.2 ± 0.9 mg L^{-1}), aligning with uninterrupted flow and intensive gas exchange along the film. Both systems held pH within operational ranges (NFT: 6.8–7.4; media bed: 6.7–7.3). The slightly higher ammonia levels in media beds (0.26 ± 0.04 vs. 0.18 ± 0.03 mg L^{-1}) likely reflect transient accumulation within the substrate before nitrification and capture, a known trade-off when solids retention is emphasised.

Regarding plants, the final fresh weight was ~20–25% higher in NFT than in media beds. This outcome is compatible with the well-established advantage of NFT for fast-growing leafy crops under adequate aeration and continuous nutrient delivery (e.g., Somerville et al., 2014; Goddek et al., 2019). Variation among media-bed units was also higher, which is plausible given the potential for micro-heterogeneity in flow paths (channelling) and moisture during flood–drain cycles. Collectively, these patterns underscore a practical compromise: media beds favour solids capture and clarification, whereas NFT favours oxygenation and crop growth. For applications prioritising water polishing and solids removal, a media component (or pre-filter) is advantageous; for maximising leafy biomass, NFT provides a performance edge, provided that upstream solids are effectively managed. Taken together, the media bed achieved lower TSS, whereas NFT maintained higher DO and supported greater plant biomass; these differences are attributable to system architecture rather than mussel effects, which were not quantified and require mussel-free controls.

A qualitative observation in both configurations was localised visual clarity around *Unio crassus* clusters, especially near water return points where suspended loads concentrate. Because the design lacked mussel-free controls and did not include measurements of turbidity, chlorophyll a, particle size distributions, or clearance rate assays, solids reduction cannot be attributed solely to mussels. The observation is therefore hypothesis-generating but consistent with the literature, which reports that suspension-feeding bivalves can polish water in recirculating and IMTA contexts through filtration and biodeposition (Ranjbar et al., 2021; Somerville et al., 2014; Goddek et al., 2019). Whether the net effect is beneficial depends on the hydraulic residence time, the availability of capture surfaces downstream, and maintenance that prevents resuspension.

Independent laboratory studies have shown that unionid mussels can rapidly reduce bacterial loads and suspended material under controlled conditions: in aquaculture effluent, *U. crassus* decreased *Staphylococcus* and *Aeromonas* within 48 h (Demircan et al., 2022), while *Unio mancus* cleared suspended *E. coli* in challenge assays with marked declines in the first 24 h (Campos et al., 2022). Likewise, a closed-loop reactor using *Anodonta cygnea* demonstrated biological removal of pollutants consistent with consumption of suspended solids and pathogen reduction (Ranjbar et al., 2021).

These results reflect a trade-off between particulate removal and oxygen/nutrient delivery, dictated by system design. Media beds outperform in solid filtration due to their high surface area and porous structure, while NFT systems favour faster plant growth through enhanced oxygenation and nutrient mobility (Tyson et al., 2011; Goddek et al., 2019). The integration of *U. crassus* provided additional ecological value in both systems, highlighting its role as a complementary filtration agent that does not impair plant productivity.

At a broader system scale, pairing filter-feeding bivalves with photosynthetic and microbial partners can enhance nutrient capture and water polishing. A pilot mussel–microalgae–bacteria "complex ecosystem" effectively reduced dissolved nutrients and suspended solids in aquaculture effluent (Geng et al., 2022). Similarly, pond-based fish–mussel integrations with *Hyriopsis cumingii* increased both yields and nitrogen-utilisation efficiency, underscoring how biotic assemblages and hydraulics jointly shape solids and nutrient dynamics (Tang et al., 2015).

Beyond system performance, the integration of *U. crassus* also offers ecological significance. As an endangered species (Lopes-Lima et al., 2024), its ex-situ inclusion in controlled aquaponic environments may support conservation efforts,

particularly in regions where natural habitats are compromised by pollution, habitat fragmentation, or declining host fish populations (Tomović et al., 2023). With appropriate water parameters and habitat design, aquaponic systems may even sustain the survival and potentially the reproduction of *U. crassus*.

While *Unio crassus* acts as a natural filter, it also produces waste and converts organic matter into biomass. These dual roles should be considered when evaluating their overall impact on nutrient dynamics. However, challenges remain. Like other unionid mussels, *U. crassus* produces glochidia larvae that require attachment to host fish for metamorphosis. In recirculating systems, unintentional interactions between glochidia and fish could stress cultured species or impair water quality if larval development fails. Future implementations may benefit from separating reproductive individuals or utilising sterile mussels. Moreover, the long-term viability of mussels under variable aquaponic conditions—such as fluctuating temperatures and nutrient loads—warrants further investigation.

Although previous literature highlights the bacterial filtration capacity of unionid mussels, this study did not conduct microbial analyses. Therefore, no direct conclusions can be drawn regarding bacterial removal by *Unio crassus* under the tested conditions. The role of nitrifying and heterotrophic bacteria, which are fundamental to nutrient conversion in aquaponics, was not monitored in this study. This omission limits a comprehensive understanding of biotic interactions within the system. Nevertheless, previous studies in aquaponic systems using koi carp and parsley have demonstrated that microbial compositions vary significantly between system compartments, with beneficial genera such as *Bacillus*, *Flavobacterium*, and *Pseudomonas* supporting system stability and inhibiting the growth of pathogenic strains. The integration of mussels could interact synergistically with such microbial communities, enhancing overall water quality.

Methodologically, the design-level contrasts observed here suggest tangible engineering refinements. First, pairing NFT with a dedicated solids-management stage (e.g., swirl separator, radial-flow settler, or a small media-polishing bed) could retain NFT's growth advantage while lowering TSS entering the plant channels. Second, distributing inflow across media beds (e.g., perforated manifolds) and verifying even flood–drain timing can reduce within-bed heterogeneity. Third, routine monitoring of DO not only in bulk water but also within root zones (NFT channels and media pores) would better resolve oxygen dynamics relevant to both nitrifiers and plant roots. Finally, adding quantitative optical metrics (NTU) and chlorophyll a alongside TSS will resolve "clarity" into size classes and trophic components (fine particulates vs. phytoplankton).

Limitations & Future Work

The main limitation is the absence of a mussel-free control, which prevents causal attribution of any observed clarity to *U. crassus*. We also did not measure turbidity (NTU), chlorophyll a, particle-size distributions, or mussel clearance rates, nor did we assess microbial load (indicator taxa), oxygen demand (BOD_5/COD), or root-zone DO. Future trials will therefore (i) implement parallel mussel-free loops; (ii) quantify optical and gravimetric metrics (NTU, TSS), phytoplankton proxies (chlorophyll-a), and particle spectra; (iii) measure mussel performance (clearance rate under controlled flows, biodeposit production); (iv) profile microbial communities and indicator bacteria across compartments; (v) track DO within root zones and media pores; and (vi) include a priori power analysis to size the study for detecting mussel effects against design-level variance. These steps will enable rigorous partitioning of contributions from system architecture versus mussel filtration, clarifying when mussel integration yields net benefits.

For stakeholders, two actionable messages emerge: (1) when solids reduction is the priority, incorporate a media or dedicated solids unit upstream of plants; (2) when maximising leafy biomass, NFT remains preferred but should be paired with effective solids control to avoid chronic channel fouling. Mussels may complement either pathway, but their deployment should be coupled to quantitative monitoring and clear maintenance protocols.

Conclusion

Within the scope of our measurements, the media bed design reduced suspended solids more effectively, whereas NFT maintained higher dissolved oxygen and supported greater plant biomass. The presence of *U. crassus* coincided with localised visual clarity; however, mussel-specific contributions cannot be inferred without mussel-free controls and quantitative turbidity/clearance data. Overall, these differences reflect system architecture rather than mussel effects, which were not quantified and require mussel-free controls. These results highlight design-dependent trade-offs and motivate future controlled evaluations of mussel integration in aquaponic loops.

Compliance with Ethical Standards

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

Ethics committee approval: Ethical committee approval was not required for this study as it utilised only aquaculture wastewater and invertebrate species (*Unio crassus*), with no direct experimentation on vertebrate animals.

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

Funding disclosure: This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University, project number FLO-2022-38607.

Acknowledgements: The authors thank the Limnology Lab at Istanbul University's Faculty of Aquatic Sciences for their assistance with water quality analysis.

Disclosure: This study is part of the undergraduate project of Batuhan Kapucuoğlu.

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AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 8(4), 253-264 (2025) • https://doi.org/10.3153/AR25025 *Research Article*

Determination of the minimum reproductive size of mussels *Mytilus galloprovincialis* (Lamarck, 1819) in the Eastern Mediterranean

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Cite this article as:
Kırtık, A. (2025). Determination of the minimum reproductive size of mussels *Mytilus galloprovincialis* (Lamarck, 1819) in the Eastern Mediterranean. *Aquatic Research*, 8(4), 253-264. https://doi.org/10.3153/AR25025

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Submitted: 12.06.2025
Revision requested: 22.09.2025
Last revision received: 29.09.2025
Accepted: 29.09.2025
Published online: 19.10.2025

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Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

The minimum shell length of sexual activity of the Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck 1819) was studied at three different stations in the Aegean Sea in November 2020. Individuals ranging between 7mm and 30mm in length were collected, and gonads were histologically observed in November, which is the initial sexually active month of mussels annually for this region. Water temperature was monitored in situ at the stations, and environmental parameters, chlorophyll-*a* and particulate matter were determined. Gonad activity was first observed in a male individual measuring 10 mm in length at Site I. First appearance of oogonia in females was observed at 14mm length in the shell at all Sites, while spawning was observed at 19mm. A statistically significant difference in the size at the onset of sexual maturity was found among stations ($p<0.05$).

Keywords: Mediterranean mussel, Reproduction size, Minimum gametogenesis size

Introduction

Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck 1819) is a very economically important species due to its practical culture methods and its worldwide distribution (Branch & Stephanni, 2004). Alongside their economic value, mussels have been used as indicator species in ecological studies for a long time (Banni et al., 2007; Viarengo et al., 2007; Dailianis, 2010; Okaniwa et al., 2010). Although it plays an economic and scientific role, the Mediterranean mussel is listed among the "World's worst 100 invasive species" (Lowe et al., 2000; Shinen et al., 2009).

The world's major mussel producers are France, Norway, Germany, the USA, Canada, the UK, the Netherlands, Denmark and Spain (Araújo et al., 2020). Global *Mytilus sp.* production was 1 925 902 tonnes in 2022 (FAO, 2024). In Turkey, production of *M. galloprovincialis* is increasing, and new mussel farms are being founded recently. The total mussel production in Türkiye was 8738 tonnes (TÜİK, 2024).

There are many studies on the reproductive cycle of the Mediterranean mussel (Bhaby et al., 2014; Eads et al., 2016; Villalba, 1995; Caceres-Martinez & Figueras, 1998; Okaniwa et al., 2010; Rouabhi et al., 2019; Smart et al., 2021). While the minimum reproductive size of some other bivalve species has been studied previously (Nakaoka, 1994; Franz, 1996; De Souza et al., 2019; Suchanec, 1981), limited knowledge exists about the minimum reproductive size of the Mediterranean mussel, especially in the Aegean Sea, where this study was conducted.

Identifying the minimum reproductive size is critical for sustainable fisheries management and setting regulatory harvest limits to protect mussel populations (Camacho–Mondragon et al, 2012). This study aims to determine the minimum reproductive size of mussels and the difference in this size across sites.

Materials and Methods

The Mediterranean mussels were collected from three different sites. Site I was a sea cage farm that produced sea bass and sea bream in Balıklıova, and the mussels were gathered from the buoys of the sea cages (38°27'0.23"N; 26°37'9.66"E). Site II was a shore with a rocky substrate of a natural mussel bed (38°25'25.94"N; 26°35'19.43"E) in Balıklıova. Site III was another natural mussel bed (38°24'47.40"N; 27° 3'7.14" E), which is the inner part of Izmir Bay, Türkiye (Fig. 1).

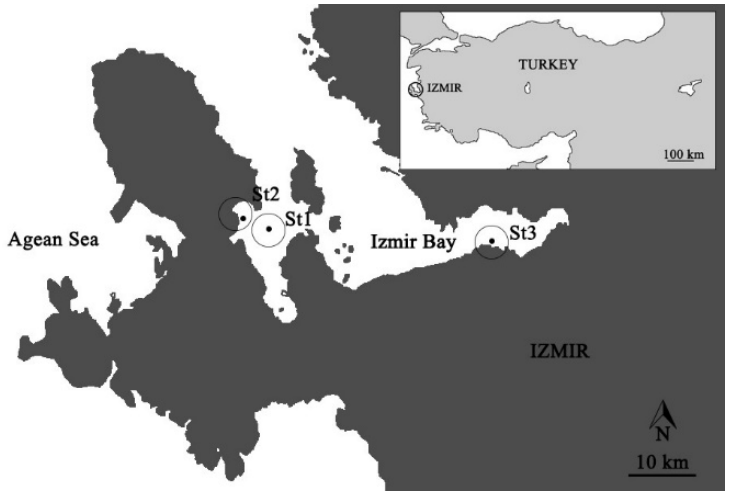


Figure 1. Location of stations at the study site

It was reported that adult mussels are sexually active in November in this region (Kırtık, 2014; Kurtay & Lök, 2023). In November 2020, when spawning peaked, 1000 mussels were collected from each site by free diving and transferred to the laboratory in ice containers. Shell lengths of mussels were measured with a digital calliper (Mitutoyo Absolute) and classified by size. Shell lengths which were between 7.00 and 7.99mm were named as "7", shell lengths which were between 8.00 and 8.99 were named as "8" and continued in this manner until between 30.00 and 30.99mm, so that 24 size groups were made up. Every size group is organised into 30 individuals. Mussels smaller than 7mm in length were not included in the study due to physical limitations regarding the dissection process.

Water samples were also collected to determine environmental parameters, including temperature, salinity, chlorophyll a, total particulate matter (TPM), particulate inorganic matter (PIM), and particulate organic matter (POM). Temperature was measured using a mercury-in-glass thermometer, and chlorophyll a, TPM, PIM and POM were determined by filtering through GF/C fibreglass filters according to the spectrophotometric method of Strickland and Parsons (1972).

A total of 2160 mussel gonads, 30 in each of 23 size groups between 7mm and 30mm, organised from mussels taken at every 3 stations, were dissected. Since the gonads of the most petite sizes were uncertain, all soft tissue was taken under a histological process.

Mussel gonads and whole soft tissue of small individuals were fixed in Davidson's solution for 48 h (Shaw & Battle, 1957). Gonadal tissues were dehydrated with alcohol and xy-

lene series, embedded in paraffin (Histowax, Leica), and sectioned to a thickness of 6 µm with a Thermo Shandon Finesse 325 microtome. Cross-sections of samples were stained with haematoxylin and 0.5% eosin (Howard et al.,1983). The reproductive stages were determined as resting, development, maturation, and spawning, according to Seed (1976).

The differences in reproductive sizes between sites were determined using the nonparametric Kruskal-Wallis test, and the Mann-Whitney U test was applied for the dual comparison between sites. The significance level used for the test was p = 0.05. Statistical analysis was performed using SPSS software.

Results and Discussion

In November, the highest temperature was 18.70 ±0.20 °C at the İnciraltı station, while the lowest was 16.53 ±0.67 °C at the natural mussel bed in Balıklıova. Chlorophyll-*a* values at Site I, Site II and Site III were 1.47 ±0.21, 1.03 ±0.31 and 1.77 ±0.16 µg/L, respectively. TPM, PIM, and POM values were much higher at Site III than at the other stations (Table 1).

The flesh of small mussels was dissected carefully from the shell and histologically examined with several sections to ensure the observation of the whole body in terms of not missing out possible gonad tissue (Figure 2). Sex could not have been determined in mussels that are sexually inactive due to the absence of gamete cells. Histological cross-section photographs of mussels 7mm and 9mm in size are shown in Figure 2A and 2B, respectively.

Spawning was observed in mussels 10mm in length, but formed follicles were very rare (Figure 3A). A developing gonad was observed in only one male individual, measuring 11mm in shell length (Figure 3B). The gonad structure, which consists of follicles containing oocyte samples at various stages of development, is seen in the female individual (Figure 3C). In the 28mm size group, the gonad tissue is covered with follicles in the mature male individual (Figure 3D). Although follicles are not fully developed in the mature female, mature egg sizes have reached their maximum (Figure 3E). In the 19 mm length group, the spawning stage was detected, although only a small number of individuals were observed (Figure 3F).

Table 1. Water conditions of the study sites

a	Aqua Farm Balıklıova	Natural Bed Balıklıova	Natural Bed Inciraltı
Temperature (ºC)	17.07 ±0.25	16.53 ±0.67	18.70 ±0.20
Chlorophyll-*a* (µg/L)	1.47 ±0.21	1.03 ±0.31	1.77 ±0.16
TPM (mg/L)	7.77 ±0.45	8.83 ±0.50	19.83 ±2.62
PIM (mg/L)	6.37 ±0.83	7.87 ±0.35	15.40 ±1.57
POM (mg/L)	1.40 ±0.44	0.97 ±0.38	4.43 ±1.19

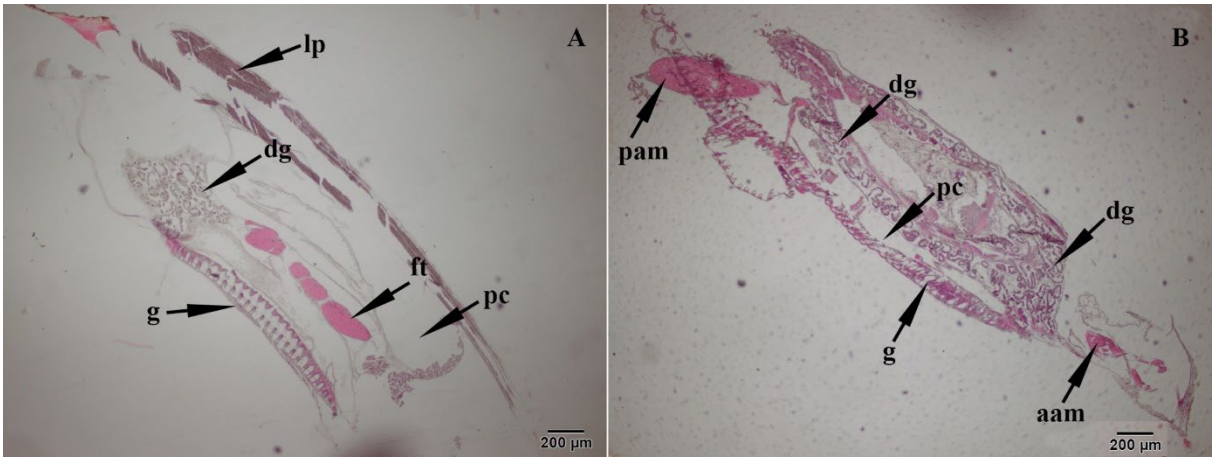
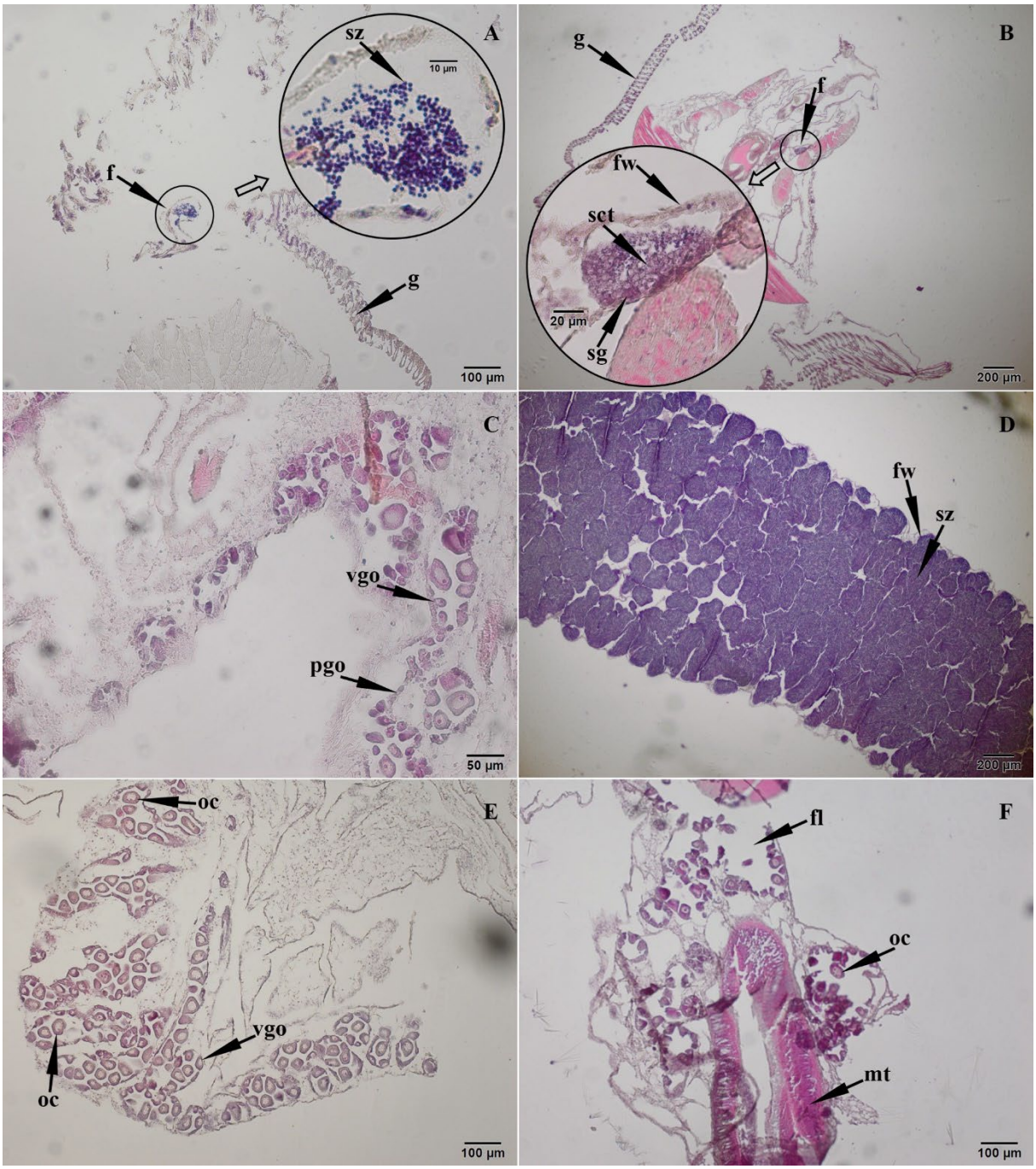


Figure 2. Inactive stage in 7 mm (A) and 10 mm (B) mussels (lp: Labial palp, dg: digestive gland, g: gill, ft: foot, pc: pallial cavity, pam: posterior adductor muscle, aam: anterior adductor muscle)



(Sz: Spermatozoa, g: gills, Sg: Spermatogonia, Sct: Spermatocyte, f: follicule, fw: follicular wall, g: gills, Pgo: Previtellogenic oocyte, Vgo: Vitellogenic oocyte, sz: spermatozoa, fw: follicular wall, oc: mature oocyte, vgo: vitellogenic oocyte, fl: follicular) lumen

Figure 3. Gonadal stages of *Mytilus galloprovincialis*: (A) Spawning in male, (B) Development in male, (C) Development in female, (D) Ripe male, (E) Spawning in female, (F) Spent in female

Site I

Sexual activity was first observed in 10 mm in length mussels. All mussels under this size were sexually inactive at Site I. Mussels with a shell length of 25mm or over were totally sexually active, and the highest spawning rate was observed in group "26", as 43% (Figure 4). The difference between the size groups in terms of gonad development stages was statistically significant ($p<0.05$).

Sex could not be determined under the 10mm group due to sexual inactivity. Only males were defined in 10-13 size groups. Of the 23 individuals, 3 males and 4 females were sexually inactive in the 14mm group. Sex was defined for 19mm and all oversized groups. The highest number of males was found as 21 in the 27mm group, and the highest number of females was 15 in the 29mm group (Figure 5). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 31% male, 20% female and 49% inactive in all size groups at Site I throughout the study (Figure 6).

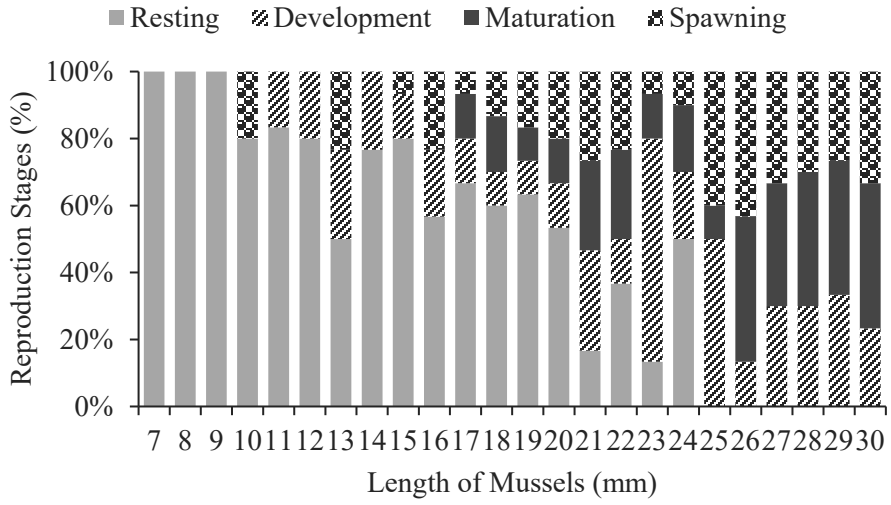


Figure 4. Reproductive stages according to size groups of mussels at Site I

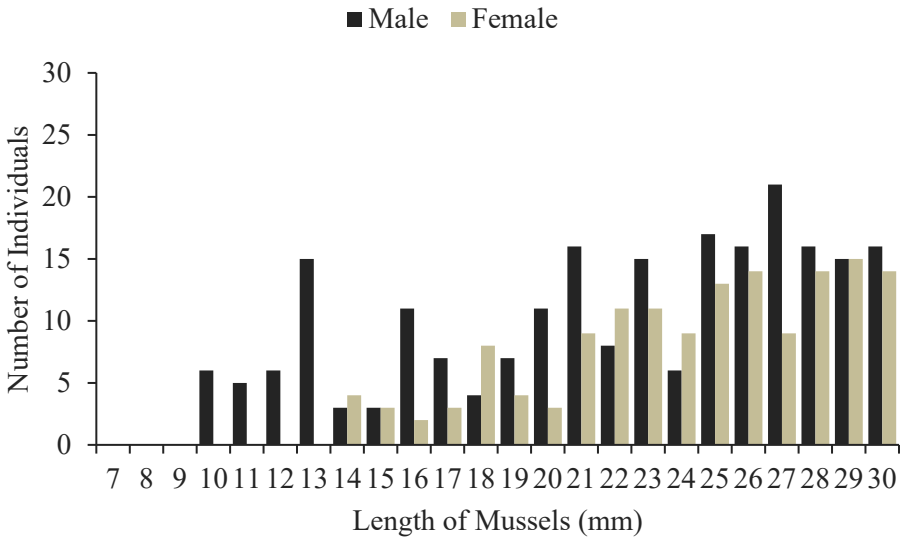


Figure 5. Number of males and females at Site I

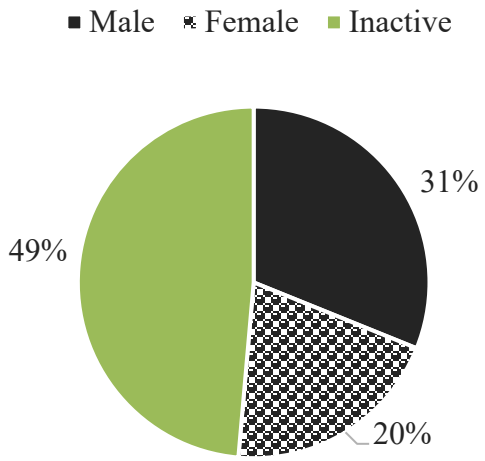


Figure 6. Sex ratio of mussels at Site I

Site II

Mussels in the 7-10mm size group were sexually inactive at Site II. Sexual activity was first observed in 11 mm-sized group mussels, and 23% of the group were in the development stage. All mussels which belong to groups 25mm and over were sexually active. It was observed that mussels in 28 and 29-mm sizes were spawning at Site II with a rate of 57% (Figure 7). The difference in sex between size groups was statistically significant ($p<0.05$).

Sex was not determined for the 11mm and under size groups due to sexual inactivity. All individuals in groups 11 to 13 were male, and both sexes were observed beginning at 14mm and above. The highest number of males was found as 19 in the 25mm group, and the highest number of females was 15 in the 28mm group (Figure 8). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 32% male, 25% female and 43% inactive in all size groups at Site II throughout the study (Figure 9).

Site III

Mussels in the 7-9mm size group were not sexually active at Site III. In contrast to the other two stations, mussels in the development (17%) and maturation (3%) stages were observed in the 10mm size group. All mussels from the 25 mm and over groups were sexually active. The highest spawning rate was 60% in the 30mm size group of mussels (Figure 10). The difference in sex between size groups was statistically significant ($p<0.05$).

Sex was not determined for the 10mm and under size groups due to sexual inactivity at Site III. Mussels belonging to groups 10-13 were only male. Both sexes were observed from 14mm onward, and oversized groups were observed. The highest number of males was 15 individuals in the 21mm and 25mm groups. At the same time, the highest number of females was 18 individuals in the 30mm size group (Figure 11). The difference in sex between size groups was statistically significant ($p<0.05$).

The sex rate of mussels was 35% male, 31% female and 34% inactive in all size groups at Site III throughout the study (Figure 12).

A total of 2160 mussels were observed across three sites, and no hermaphrodite individuals were found throughout the study.

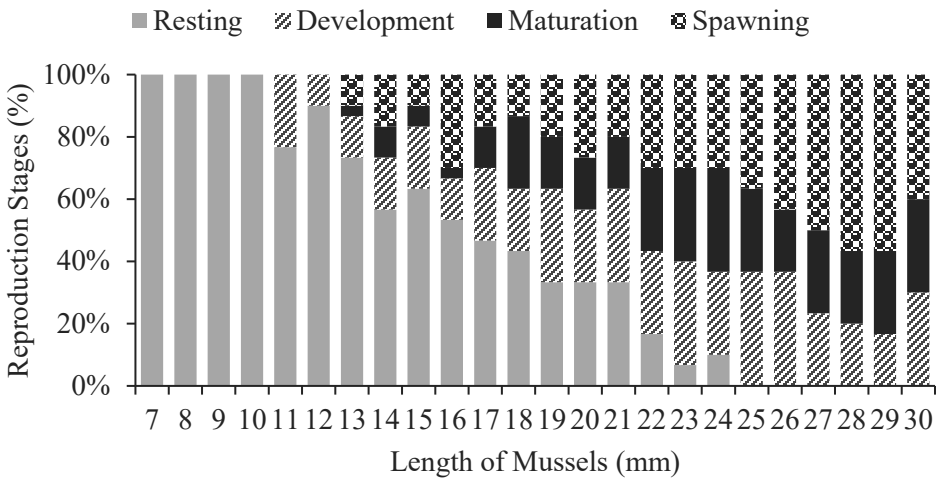


Figure 7. Reproductive stages according to size groups of mussels at Site II

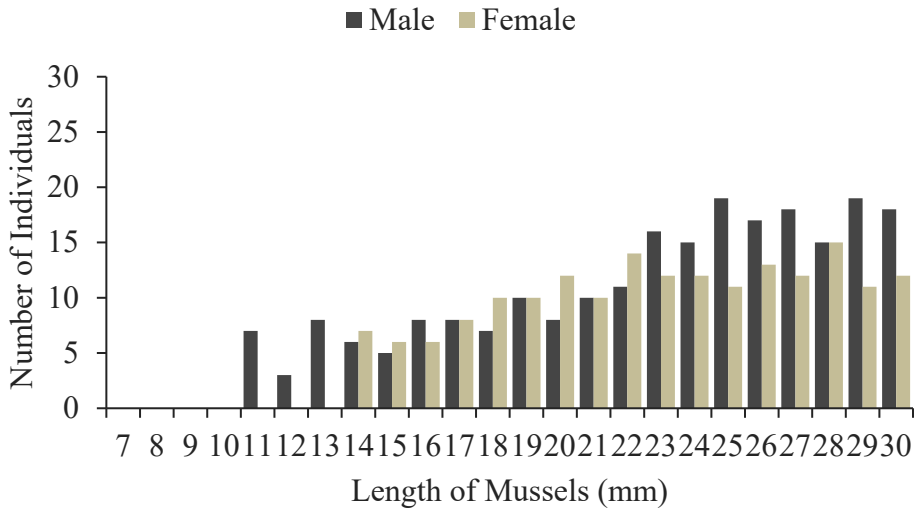


Figure 8. Number of males and females at Site II

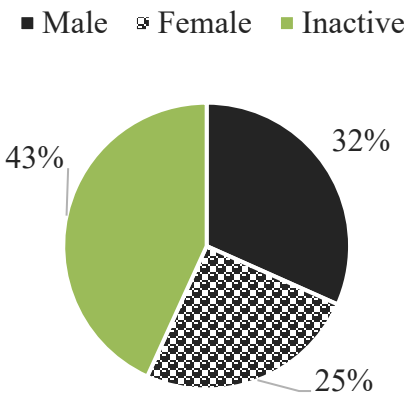


Figure 9. Sex ratio of the mussel at Site II

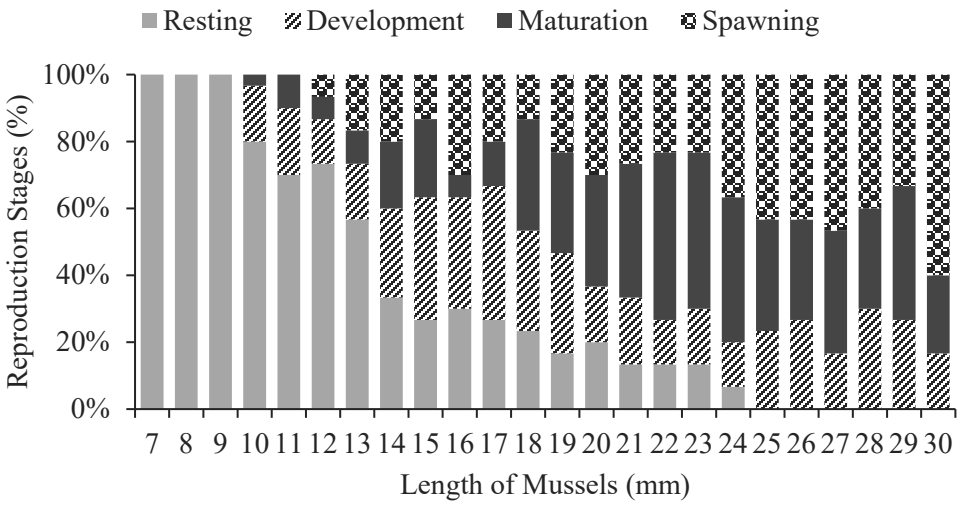


Figure 10. Reproductive stages according to size groups of mussels at Site III

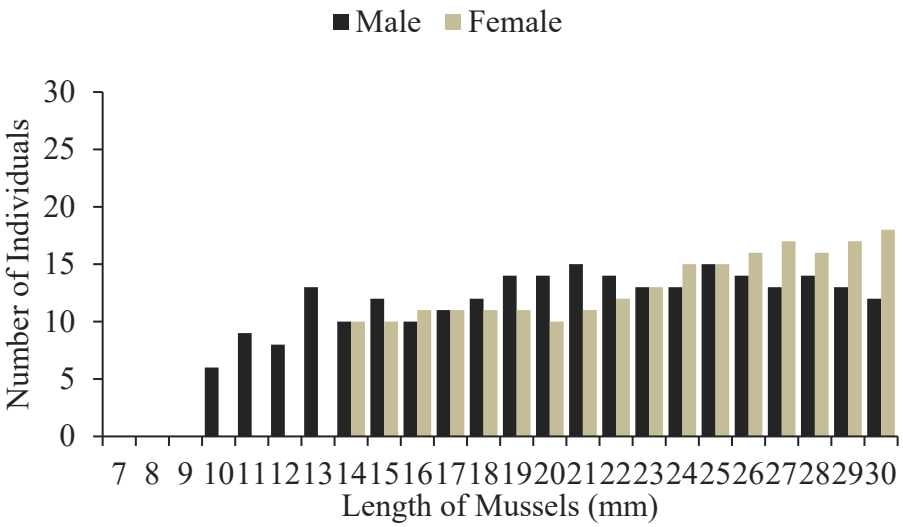


Figure 11. Number of males and females at Site III.

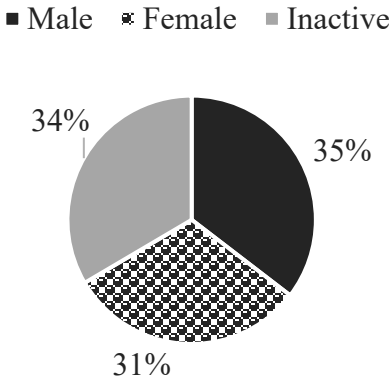


Figure 12. Sex ratio of the mussel at Site III

Spawning of mussel populations in the Southern Aegean Sea was reported as 3 times a year in October-November, January-February, and April (Kırtık, 2014). In Balıklıova, where the mussels were collected for this study, spawning occurs between October and May (Kurtay & Lök, 2023). Mussel samples in this study were collected in November to ensure the population was in the spawning period, as reported in previous studies. Okaniwa et al. (2010) reported that in Tokyo Bay, the gonads of *Mytilus galloprovincialis* developed when the length reached 20mm, and an extended spawning period was observed between November and March. *Mytilus edulis* is sexually active in the first year of their life span with small sizes (Seed and Brown, 1977). In North America, the first reproductive size of *M. edulis* and *M. californianus* was observed at 15-20 mm and 25-30 mm, respectively (Suchanec, 1981). Sunila (1981) reported that there was no sexual maturity in *M. edulis* until 5-10 mm in shell length. In 1996, Franz reported that the first sexual activity occurs in *Geukensia demissa* (Mytilidae), measuring 15-20 mm in length, in the New York Salt Marsh. First sexual maturity was observed in *Perna perna* mussels at 20.70 mm for males and 21.90 mm for females (De Souza et al., 2019). Minimum sexual maturity of mussels was observed in male individuals when the shell length was 10mm in Site I and Site III, while 11mm in Site II in this study.

Temperature and chlorophyll *a* are the most important environmental factors that trigger the reproduction (Yiğitkurt et al., 2020). Okaniwa et al. (2010) reported that the lowest chlorophyll a values were recorded in November-December, with a mean of <3 g/L. That water temperature was between 12 and 14 °C during the same period in Tokyo Bay. Similar results were observed in temperature and chlorophyll *a* in this study. Sea water temperatures ranged from 16.53 to 18.70 °C, while chlorophyll a was <2 µg/L in November. Compared to Okaniwa et al.'s (2010) results, lower chlorophyll a values did not affect reproduction in this study; in addition, seawater temperature was higher, and it was conceived that higher water temperatures can support mussels' reproduction in smaller sizes.

It is known that gametogenesis can occur in mussels even at 0 °C (Grey et al., 1997). In a previous study, the average annual water temperature was reported as 19.46 ±4.41 °C (min 14 °C in February; max 26.2 °C in August), and in November, water temperature was between 16 and 17 °C in Balıklıova, which includes Sites I and II (Kurtay & Lök, 2023). During a study conducted on the Inciraltı coast, including Site III, on bivalve reproduction, it was reported that water temperature ranged from 13.5 °C to 28 °C annually, with 19 °C in November (Kırtık, 2014). In a study on the reproductive cycle of *Mytilus galloprovincialis* in Italy, it was reported that sexual activity was not observed in the summer months when the temperature increased and gametogenesis began to be seen because of the end of the summer season and the temperature falling below 20°C (Da Ros et al., 1985). In this study, water temperature was measured at 17.07 ±0.25 °C and 16.53 ±0.67 °C at Sites I and II, respectively. Consistent with previous studies, reproductive activity was continuous at water temperatures below 20 °C.

Kurtay and Lök (2023) reported the lowest chlorophyll a values in Balıklıova (Site I and Site II in this study) as 0.2 µg/L in July and 1.91 µg/L in December, while the lowest TPM values in the same region were 6.69 mg/L in November and the highest in October reported as 15.45 mg/L. On the Balıklıova coast, the highest PIM value was 10.56 mg/L in October and the lowest 5.1 mg/L in June, while the highest POM value was 5.9 mg/L in October, and the lowest 0.73 mg/L in November and February (Kurtay & Lök, 2023). In the mussel breeding study conducted on the coasts of İncialtı, chlorophyll a values were reported as 1.31 g/L in December (the lowest), 41.21 g/L in June (the highest), and 1.52 µg/L in November (Kırtik, 2014). The amount of feed in the environment is considered a limiting factor in mussel gonad development (Grey et al., 1997). In his study, Kırtık (2014) reported that the year-round average TPM data for İnciraltı was 17.27 7.87 mg/L, while TPM was 21.84 mg/L (PIM=15.07 mg/L, POM=6.76 mg/L) in November. Annual water changes were examined alongside similar studies conducted in the same region and compared with the November data in this study. The results reported in the studies are consistent with the water data we obtained. The smallest size in which gametogenesis was observed in mussels obtained from İnciraltı Coasts, where water parameters are at higher values, was determined as 10 mm. In the Balıklıova farm region, both the development phase and the maturation phase were observed in mussels of this size. Higher POM and chlorophyll a levels may accelerate gametogenesis in smaller mussels, potentially triggering earlier reproductive maturity.

Hermaphroditism is very rare in the *Mytilus* spp. (Seed, 1976). Bhaby (2015) reported that 345 females, 400 males, and 2 hermaphrodites were detected among 750 individuals of *M. galloprovincialis* on the Moroccan Coast. Sunila (1981) reported that hermaphroditism was observed in only 2 of 1832 individuals of M. edulis in the Gulf of Finland. Supportive of the Seed 1976's determination, hermaphroditism was not found in any of 2160 individuals in *M. galloprovincialis* in this study.

Conclusion

Our results, unlike those of other studies, showed that gametogenesis began in mussels at much smaller sizes; however, there were slight differences between stations in the region. It has been determined that sexual maturity occurs faster in smaller mussels, especially in regions with higher nutritional value, and male mussels reach sexual maturity earlier than female individuals.

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 article.

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

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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AQUATIC RESEARCH

AQUATIC RESEARCH
E-ISSN 2618-6365

Aquatic Research 8(4), 265-271 (2025) • https://doi.org/10.3153/AR25026 *Research Article*

Investigation of oxidative stress, lipid peroxidation and DNA damage in the muscle tissue of young trout treated with arsenic

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Cite this article as:
Çilingir Yeltekin, A. (2025). Investigation of oxidative stress, lipid peroxidation and DNA damage in muscle tissue of young trout treated with arsenic. *Aquatic Research*, 8(4), 265-271. https://doi.org/10.3153/AR25026

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Submitted: 31.07.2025
Revision requested: 16.09.2025
Last revision received: 29.09.2025
Accepted: 02.10.2025
Published online: 19.10.2025

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ABSTRACT

Arsenic, a metalloid and metal compound commonly found in the earth's crust, dissolves in underground waters and can cause toxic effects by spreading through industrial processes or the environment. In this study, the changes in levels of antioxidant enzymes and several parameters (GSH-Px, CAT, MDA, SOD, and 8-OHdG) in the muscle tissue of juvenile trout (Oncorhynchus mykiss) after arsenic exposure were investigated, and the data obtained were analysed.

In our study, juvenile trout were divided into three groups, and arsenic was administered at saturations of 25, 50, and 75 mg/L. 96 hours after application, the muscle tissue from young trout was removed and homogenised. The 8-OHdG content in the muscle tissue was determined using an ELISA kit. The levels of SOD, CAT, GSH-Px and MDA were determined using spectrophotometric methods.

In young trout with arsenic accumulation in muscle tissue, DNA damage was observed, and the 8-OHdG content increased accordingly. There was deterioration in the cell membrane structure and lipid peroxidation. When the muscle tissue of young trout treated with arsenic was examined, a significant decrease in the concentrations of CAT, GSH-Px and SOD was observed. It is assumed that this is due to oxidative stress caused by the accumulation of free radicals in the muscle tissue of the fish. These changes occurred after arsenic was applied. The presence of heavy metals in large quantities in the habitats of aquatic organisms negatively affects their life and developmental stages, and their presence in the food chain and use as food by other species can cause significant toxicity.

Keywords: Arsenic, MDA, 8-OHdG, Antioxidants, Oxidative stress, Juvenile Rainbow Trout

Introduction

Arsenic, a metalloid and metal-based compound commonly found in the earth's crust, dissolves in underground waters and can cause toxic effects when it enters industrial processes or the environment (Hughes, 2002; Cilingir Yeltekin, 2018). Although it is used as a raw material in agriculture, pharmaceuticals and other industries, it has toxic effects on various organisms, including humans. Arsenic is found in water, especially after groundwater dissolves certain compounds and minerals (e.g., after arsenic has passed through soil and rock) (Baskan & Pala, 2009).

The toxicity of heavy metals to living organisms is generally associated with the formation of reactive oxygen species (ROS). Upon exposure to a toxicant, ROS levels increase, leading to oxidative stress. Oxidative stress alters the electron transport system and causes cellular damage in tissues and organs (Méndez-Armenta et al., 2011). Hydroxyl radicals and hydrogen peroxide are among the free oxygen radicals with the highest activity in ROS. To minimise the destructive effects of ROS arising from various influences, living organisms have developed an antioxidant self-defence system with two classes. These systems are enzymatic antioxidant systems containing enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT), as well as non-enzymatic antioxidants such as glutathione and thioredoxin (Özdek et al., 2020). CAT, SOD and GSH-Px are the most important antioxidant defence systems in living cells. Moreover, these enzymes are always fighting against free radicals and oxidative stress (Hematyar et al., 2019).

Low levels of ROS do not cause destruction, but high levels of ROS have adverse effects on intracellular components such as proteins, lipids and DNA. In particular, ROS can trigger lipid peroxidation (LPO) of polyunsaturated fatty acids, leading to cell membrane disruption and changes in membrane structure and permeability (Doğan et al., 2022). In addition to the excessive production of reactive oxygen species, lipid peroxidation is also observed in aquatic organisms exposed to heavy metals. An increase in malondialdehyde (MDA), a degradation product of polyunsaturated fatty acids, is an indicator of oxidative stress. One of the most important indicators of lipid peroxidation is MDA. As a result of these effects, free radicals cause lipid peroxidation and severe membrane damage through an autocatalytic effect (Atmaca & Aksoy, 2009).

The presence of intracellular reactive oxygen species affects more than twenty species. Among these damaged bases, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most studied base. An increase in the concentration of 8-hydroxy-2'-deoxyguanosine is an indicator of DNA damage (Özcan et al., 2015). It is known that intracellular oxidative stress disrupts base and sugar modifications on DNA through various mechanisms, induces single- and double-strand breaks, creates abasic sites and DNA-protein crosslinks, and causes other forms of damage. DNA damage, which occurs in the cell for various reasons, is one of the most common destructive events in the life of a cell. DNA damage in living organisms can lead to mutations, cancer, ageing and, as a result, cell death. In the course of its life, DNA is exposed to numerous changes caused by cellular metabolites (ROS) and exogenous substances. These changes in DNA can lead to cell death in unicellular organisms. They can also lead to wear and tear and ageing in multicellular organisms (Sancar et al., 2004).

Studies show that the amount of arsenic in water is increasing daily. This rising arsenic level poses a threat to all living things (Berg et al., 2001; Fernández et al., 2012). This rising arsenic level threatens all living things. The doses for our study were determined based on the arsenic concentrations found in these studies. The aim of this study is therefore to investigate the effects of increasing arsenic concentrations in water on the metabolism of living organisms.

Materials and Methods

Fish

Juvenile trout (*Oncorhynchus mykiss*) were purchased from trout farms in the Van-Çatak district. 60 juvenile trout (80-100 g) from this facility were distributed among 4 fibreglass aquaria (300 L), each containing the same number of fish. The fish were maintained for one week to acclimatise to the environment. The young trout were fed twice a day, morning and evening, with commercial food. The fish were kept in tanks at 18.0±1°C, which were aerated with an air stone.

The fish in one of the tanks served as the control group, and the fish in the other three tanks served as the groups treated with the As concentration. Arsenic-containing $NaAsO_2$ (Sigma-Aldrich, purity 90-95%, CAS number: 7784-46-5, Germany) was added to the aquaria to be treated with arsenic at concentrations of 25, 50, and 75 mg/L, respectively, and the concentrations were maintained for 96 hours. Determination of the lethal dose according to the work of Buhl and Hamilton (Yao et al., 2023). 96 hours after arsenic application, the fish were anaesthetised with MS222 (0.1 g/L) and their muscle tissue was detached. The detached muscle tissue was homogenised and frozen at -83 °C for analysis.

Preparation of Tissue Homogenate

The fish muscle tissue is weighed, a homogenization buffer containing EDTA and KH2PO4 is added, and the mixture is homogenised using a homogeniser. The resulting mixture was centrifuged, transferred to Eppendorf tubes, and used for the analyses.

Determination of the Concentrations of the Mentioned Antioxidant Enzymes and Apoptosis Markers

The SOD and GSH-Px content in homogenised muscle tissue of young trout was determined using a UV kit, and the CAT and MDA content was determined using spectrophotometric methods. The Randox-Ransel enzyme kit (SD 125, LOT 343177, United Kingdom BT294QY, RS 505, LOT 430430, United Kingdom BT294QY) was used to detect SOD and GSH-Px, and the absorbance values were measured using the spectrophotometer. Xanthine and xanthine oxidase were used together to determine the SOD enzyme activity, and the solution took on a red appearance, forming the superoxide radical 2-(-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) at the end of the reaction. The apparent absorbance value of the resulting red solution was measured on the spectrophotometer at 505 nm. To determine the activity of the enzyme GSH-Px, the reagents were pipetted into the cuvette, and the absorbance of the samples was measured at 340 nm on a spectrophotometer using the Randox-Ransel enzyme kit (Yeltekin, 2024).

The 8-OHdG content was measured using an ELISA kit specifically manufactured for fish (BT LAB, Cat. No. E0084FI, LOT 202403002, China). The solutions and standard solutions required for the analysis were added to each well of the ELISA kit plates, and the numerical data obtained were read and calculated in a microplate reader at 450 nm (Yeltekin, 2022).

Statistical Analyses

The SPSS v25 package was used to precisely interpret the results. The significance levels were determined at the .05 level. Duncan's multiple-comparison test was applied to data that appeared to be significantly skewed.

Results and Discussion

The study of the data obtained shows that the levels of antioxidant enzymes in the muscle tissue of young rainbow trout treated with a specific arsenic concentration change significantly. Statistically, a significant decrease in the levels of SOD, CAT and GSH-Px was observed in the muscle tissue of juvenile trout. There was a statistically significant increase in MDA and 8-OHdG levels due to oxidative stress in the muscle tissue of the arsenic-treated fish (Figure 1-2).

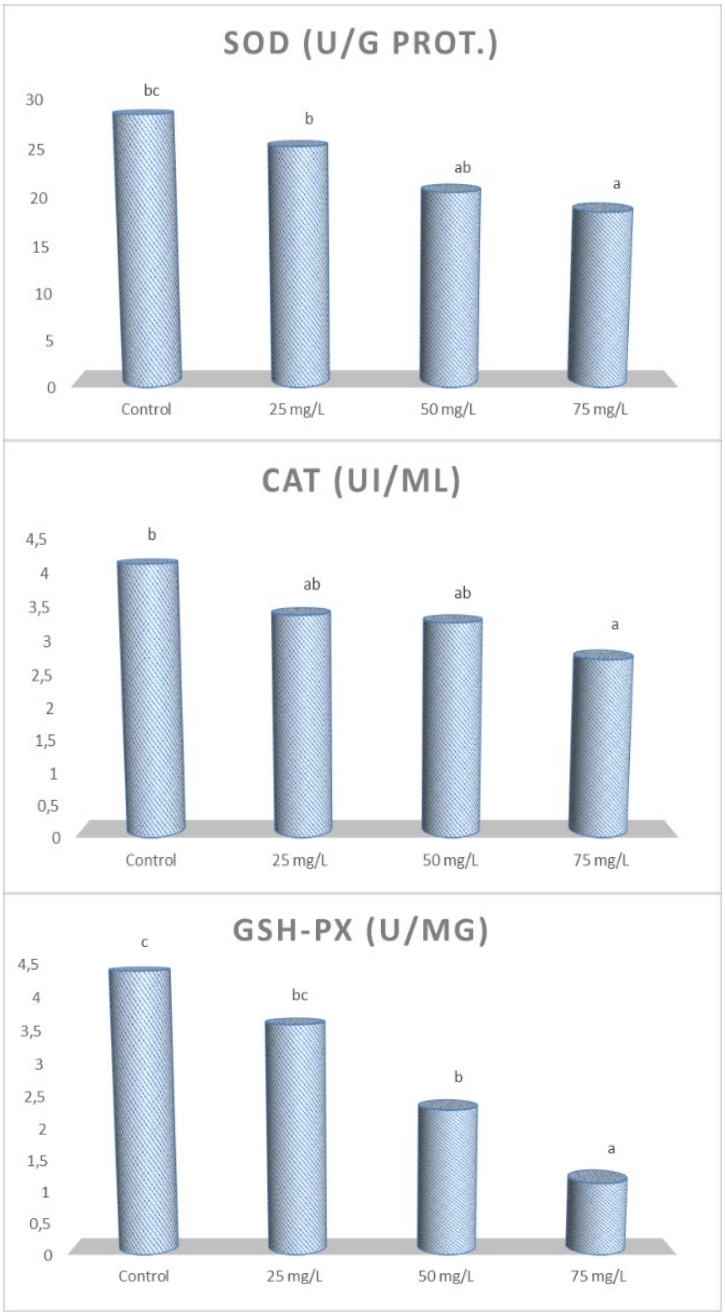

Figure 1. Changes in CAT, SOD and GSH-Px activity levels in rainbow trout muscle tissue. The letters a, b, c, and d indicate a significant difference between the groups ($p<0.05$)

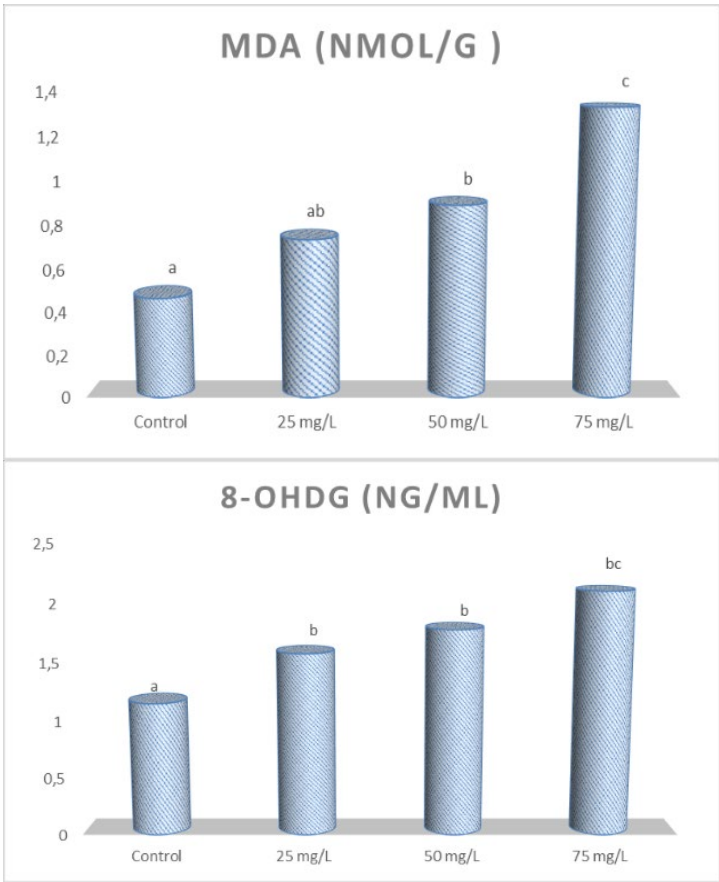

Figure 2. Changes in MDA and 8-OHdG levels in rainbow trout muscle tissue. The letters a, b, c, and d indicate a significant difference between the groups ($p<0.05$)

In our study, significant changes in the levels of antioxidant enzymes and several parameters (MDA, CAT, SOD, GSH-Px, and 8-OHdG) were examined in the muscle tissue of arsenic-treated juvenile trout (Oncorhynchus mykiss), and the results were verified. The data obtained after the study were analysed.

It is known that reactive oxygen species are produced in aquatic organisms following heavy metal exposure and that oxidative stress occurs within cells as a result. It has been found that the effectiveness of some antioxidant enzymes increases or decreases, depending on whether they eliminate or reduce ROS-induced oxidative stress in the cell (Lee et al., 2023). An arsenic study was conducted on carp (Cyprinus carpio). The study found that these fish had significantly increased levels of oxidative stress, which disturbs the charge balance in the electron transport system and causes disorders (Lu et al., 2024; Saç, Yeltekin, 2023). The results of the present study support this finding.

Li et al. In *Channa argus* fish exposed to lead (Pb), the SOD, CAT and GSH-Px levels in the liver, kidney and gill tissues of the fish were examined as a result of lead exposure. Following exposure, significant decreases in SOD, CAT, and GSH-Px levels were observed in the tissues. Intracellular oxidative stress increased with lead accumulation in tissues (Li et al., 2021). In our arsenic study, a decrease in SOD, CAT and GSH-Px concentrations was observed in the muscle tissue of the young trout. This decrease parallels the results of the study mentioned above.

In a study conducted by Abdal-Gawad et al. on Nile tilapia, the oxidative status of the fish was examined after exposure to copper (Cu) and zinc (Zn). After the study, a decrease in the levels of SOD, CAT, GST and GSH-Px was observed in the liver tissue of the fish exposed to the metal (Abdel-Gawad et al., 2020). The changes in the parameters in our study were found to be statistically significant compared with those in this study. In addition, an arsenic study conducted in zebrafish showed that arsenic exposure had significant adverse effects on fish development. Arsenic toxicity during development was even found to affect the sex of the fish (Rachamalla et al., 2024).

Wagh et al. (2023). In zebrafish, the fish were exposed to specific concentrations of lead (Pb), nickel (Ni) and cadmium (Cd). After the application, the gill and liver tissues of the fish were examined, and significant decreases in SOD, CAT, and GSH-Px levels and significant increases in MDA levels were observed. The changes in trout muscle tissue parameters in our study are comparable to those reported in this study.

In a study by Wang et al., liver tissue from Nile tilapia exposed to copper (Cu) and zinc (Zn) was examined after application. Examination of the tissue revealed significant decreases in SOD, CAT, and GSH-Px levels and a marked increase in MDA levels (Wang et al., 2020). The results of this study are similar to the results of the study in which we administered arsenic. In a study conducted by Iyorah et al., kidney and gill tissues of African catfish living in the Warri River and exposed to heavy metal pollution (Pb, Cd, Mn, Ni, Cu) were examined, and MDA levels were measured. It was found that the tissues of fish exposed to heavy metal pollution showed a significantly greater increase than those of the control group (Iyorah et al., 2023). In our application, a similar increase in MDA levels was observed in trout muscle tissue.

In a 2023 study on rainbow trout, oxidative stress and lipid peroxidation were investigated in fish exposed to heavy metals, and MDA levels increased significantly in gonadal samples from trout exposed to heavy metals compared with control groups (Bhat et al., 2023). In our study, the increase in

MDA levels was statistically significant in the muscle tissue of juvenile trout.

In one study, four fish species (argus fish, striped eel, giant catfish, and mullet) were sampled from Pattani Bay in Thailand, which is known to be contaminated with heavy metals, and their muscle and liver tissue were analysed. After examination, it was found that the MDA levels in these tissues were significantly higher than those in the control group (Aggarwal, 2004). In our study, in which we administered arsenic, the MDA levels in the fish's muscle tissues were parallel to the results of this study. In another study involving about 1600 fish, it was found that arsenic concentration increased steadily and had a severe adverse effect on the organisms (Lau et al., 2025).

In a 2023 study with goldfish, the fish were exposed to various concentrations of lithium (Li), and the muscle and gill tissue were examined after exposure. After the study, it was found that MDA and 8-OHdG levels increased in the tissues (El-Tekreti & Yeltekin, 2023). In another arsenic study, oxidative stress was found to increase at very high levels (Saç & Yeltekin, 2023). In our study on arsenic application, a similar increase in muscle tissue was observed after application. It showed results similar to those of the above study. In a study conducted on zebrafish, specific concentrations of antimony (Sb) were administered to the fish every two days and the livers of the zebrafish were examined after application. After the examination, it was found that the 8-OHdG content in the zebrafish liver tissue increased. In our study, 8-OHdG levels increased similarly in the muscle tissue of the fish. This increase was found to be statistically significant.

Conclusion

If we look at the results of our study on arsenic application: It has been observed that due to the presence of a heavy metal such as arsenic in high concentrations in the regions where aquatic organisms live, and especially as a result of long-term exposure of aquatic organisms to this heavy metal, excessive arsenic accumulates in the tissues of the organism and oxidative stress occurs in the cells of the organism due to the action of reactive oxygen species formed as a result of arsenic accumulation. Free radicals, which are the leading cause of oxidative stress in the cells of muscle tissue of young trout, accumulate in large quantities in the tissues of the fish, and in order to reduce or eliminate oxidative stress in the cells, the intracellular levels of some antioxidant enzymes, cytokines and antioxidant markers increase, leading to lipid peroxidation. This has been hypothesised to lead to DNA damage in tissues.

The presence of heavy metals in large quantities in the habitats of aquatic organisms negatively affects their life and developmental stages, and their accumulation in the food chain and use as food by other species can cause significant toxicity.

In order to eliminate the situation caused by the presence of a heavy metal such as arsenic in aquatic organisms and prevent heavy metals from entering the food chain, all fish farms must regularly check the water quality and water health and take precautions. and the amount of heavy metals in the water must be eliminated in a controlled manner or harmful heavy metals in the water must be eliminated.

This study can serve as a valuable resource for other scientific studies on the toxicity of heavy metals, such as arsenic, in aquatic organisms and the environment.

Compliance with Ethical Standards

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

Ethics committee approval: The study was approved in accordance with the decision of the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee dated 29/04/2021, numbered 2021/04-19.

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

Funding disclosure: This study was supported by YYU Scientific Research Projects Presidency as project number FYL-2021-9693.

Acknowledgements: -

Disclosure: -

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AQUATIC RESEARCH
E-ISSN 2618-6365

Aquatic Research 8(4), 272-285 (2025) • https://doi.org/10.3153/AR25027 *Research Article*

Rotifers´ assemblage and seasonal distribution in two shallow lagoons in Türkiye

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Cite this article as:
Korucu, K.V., Tugaytimur, T., Emir Akbulut, E. C. (2025). Rotifers' assemblage and seasonal distribution in two shallow lagoons in Türkiye. *Aquatic Research*, 8(4), 272–285. https://doi.org/10.3153/AR25027

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Submitted: 06.08.2025
Revision requested: 02.10.2025
Last revision received: 10.10.2025
Accepted: 10.10.2025
Published online: 19.10.2025

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ABSTRACT

In this study, temporal variations in rotifer abundance, biomass, and size structure were determined and compared across samples collected between 2014 and 2016 from two shallow lagoons in different climate zones. Paradeniz Lagoon is a high-salinity ecosystem (around 31‰), located in the Mediterranean Sea Region, on the south coast of Turkey, and formed within the delta of the Göksu River. Uzungöl Lagoon is a low-salinity ecosystem (≤ 1‰) located in the Kızılırmak Delta, in the north of Turkey. Key environmental parameters, particularly temperature, salinity, conductivity, dissolved oxygen, and chlorophyll *a*, were determined in interactions with rotifer assemblages. Rotifera were the dominant group in the zooplankton of slightly saline Uzungöl Lagoon; mainly, *Keratella cochlearis*, *K. quadrata*, *Polyarthra vulgaris*, *Filinia longiseta*, *Notholca acuminata*, and *Brachionus calyciflorus* accounted for more than 70% of the rotifer community. In the saline Paradeniz Lagoon, rotifers *Synchaeta pectinata* and *Hexarthra fennica* were constant species. However, their population density was very low, whilst copepods made up more than 90% of the total zooplankton. Moreover, an increase in nauplius lengths correlated with maximum salinity in both lagoons. Results of this research indicated that climate-dependent salinisation of shallow lagoons is an important factor in ecological explanations of zooplankton biodiversity, density, and biomass.

Keywords: Zooplankton, Biomass, Diversity, Chemicals, Uzungöl and Paradeniz Lagoon

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Introduction

Rotifers are among the most important groups of zooplankton and play an important role in nutrient cycling and energy flow in aquatic systems (Herzig, 1987; Wallace, 2002; Emir, 1994). Seasonal zooplankton dynamics and population structure are highly susceptible to changes in environmental variables, especially in shallow lakes. Several previous studies have indicated that nutrients, primary production, temperature, abundance of predators, competitors, and potential food resources are important factors influencing the structure of the zooplankton abundance as well as the population dynamics and the productivity patterns (Galkovska, 1987; Herzig, 1987; Devetter, 1998; Caglierani et al., 2009; Donisete et al., 2022). Among the zooplankton, the studies on the population dynamics of rotifers, dissolved oxygen concentration, water conductivity and salinity were the most important factors driving differences in species diversity and abundance (Egborge, 1994; Khaleqsefat & Malekzadeh-Viayeh, 2013; Yuan et al., 2020); dissolved oxygen and temperature concentration (Armengol et al., 1993; Mikschi, 1989); pH (Grajner, 2001) and turbidity (Duggan et al., 2002); trophic level (Duggan et al., 2001; Yoshida et al., 2003). In addition, predation pressure decreased the maximum population density and maximum population growth rate of rotifers (Sarma et al., 2011)

The differences observed in the saline and freshwater environments, abundance and biomass of zooplankton in the lagoons were caused not only by salinity and temperature conditions, but also by dissolved oxygen concentrations, the trophic state of the studied water body and the existing biocenotic relationships (Paturej & Kruk, 2011; Paturej et al., 2017)

As transition zones between marine and terrestrial environments, lagoons are important areas where biodiversity is high and biogeochemical cycling occurs. (Kjerfve, 1994). The high productivity of coastal lagoons makes them habitats for many fish and invertebrates, and most lagoons support important fisheries or aquaculture (Ruzafa et al., 2024).

Although various parameters affect zooplankton species diversity and density in lagoons, high salinity is among the most important limiting factors, as in temperate lakes. The significant suppressive effect of salinity on zooplankton distribution, especially in small-bodied zooplankton groups, has been studied by various researchers. Danni et al. (2020) observed that zooplankton species number, richness, evenness, Shannon index, and Simpson's index values decreased with increasing salinity. The abundances of total zooplankton, rotifers, and cladocerans were negatively correlated with salinity, while there was little correlation between copepod abundance and salinity (Akbulut & Tavşanoğlu, 2015). Saline wetlands were colonised by more salt-tolerant species such as *Brachionus plicatilis*, *Trichocerca* sp., and calanoid copepods. The results indicate that increasing salinity may reduce freshwater zooplankton richness, shifting the community toward more salt-tolerant species (Toruan, 2012). Even relatively small increases in salinity levels can drive such systems to a state of depleted biodiversity and abundance, altering ecosystem functioning (Schallenberg et al., 2003). Grajner and Cudak (2014) observed that increased salinity decreased rotifer species richness and diversity. Min-Chul et al. (2022), based on their laboratory work, found that among the abiotic factors, salinity and temperature induced adverse effects on reproduction in the tropical rotifer species.

There are some studies on zooplanktonic organisms in lakes, streams and rivers in the Kızılırmak Basin, like on the Cladocera and Copepoda taxonomy in Bafra Balık Lake (Gündüz, 1991a, 1991b); on the Rotifera taxonomy and ecology (Emir, 1989,1990; Akbulut et al., 2008), Zooplankton fauna in Çernek Lake (Bekleyen, 2008), Rotifers from Kızılırmak River and its branches were observed (Günsel &Akbulut, 2012). Also, recent studies are available on the zooplankton community and physicochemical properties of Uzungöl Lagoon (Özdemir et al., 2021), on zooplankton population dynamics and biomass distribution in Gıcı Lake (Gül, 2021), and on zooplankton population dynamics in Tatlı Lake in the Kızılırmak Delta (Tugaytimur, 2023). Although rotifers predominate in most aquatic environments, such as lagoons, few studies have examined zooplankton, including rotifers, in saline waters to compare them with those in temperate lakes (Akbulut & Tavşanoğlu, 2015, 2018)**.** The purpose of our study was to determine the effects of saline intrusions and temperature on zooplankton biomass and community structure in two lakes located in different climate zones.

Materials and Methods

Study Sites

Uzungöl Lagoon is an eutrophic lake with typical brackish water characteristics, located in the Kızılırmak Delta, Black Sea Region of Turkey (41°34'N 36°05'E, Figure 1). Although the closest point to the sea is 1 km away, a channel opens to the sea in line with anthropogenic interventions. This lake, located in the eastern part of the basin, lies parallel to the sea and covers 219 ha. Its depth varies between 1 meter and 1.5 meters. Although it is common in the southern part of

the lake, emergent macrophytes, especially *Phragmites australis*, were observed (Akbulut & Bayramıalemdarı, 2015).

Figure 1. Satellite images of study sites with geographical location and coordinates

Paradeniz Lagoon is a saline lake located on the southern coast of Turkey and is one of the four lakes in the Göksu Delta that pour into the Mediterranean Sea (36° 24'N, 33° 45'E, Fig. 1). Paradeniz is the second-largest lake in the basin, with a surface area of 492 ha. There is a sand bar between the sea, and it has a saltier water chemistry than other lakes in the basin due to its direct connection to the sea. It has a depth of 1 meter to 0,5 meters. It is possible to say that anthropogenic interference is high, though it is linked to neighbouring Akgöl due to fishing activities.

Sampling

Samples were collected at Uzungöl Lagoon, located in the Kızılırmak Delta (Samsun, Turkey), and Paradeniz Lagoon, located in the Göksu Delta (Mersin, Turkey), at four stations in each lake between August 2014 and May 2016. Zooplankton samples were collected horizontally using a standard plankton net (44 µm mesh size), and Secchi depth was measured with a 20 cm disc. Samples were preserved in 4% Lugol solution and examined using a Leica DM5000B trinocular microscope. Physicochemical parameters, including salinity, pH, dissolved oxygen, and temperature, were measured using a Consort C933 Multi-Parameter Analyser. Physical and chemical data were obtained from project report no. 014 D04 818 001-598, which supported the study.

Species were identified using the keys of Koste (1978a, 1978b), Kutikova (1970), Nogrady (1980), Segers (1995, 2007, 2008), De Semet (1996, 1998), Dumont (2006), De Smet and Pourriot (1997), and species were checked according to Ustaoğlu et al. (2012) and Ustaoğlu (2015).

Data Analysis

The organisms observed in the samples taken from Uzungöl and Paradeniz Lagoons were counted in 5 replicates on a 1 mL capacity Seewick-Rafter counting slide, after homogenization by shaking the sample tube. It was prepared following the methods described by Downing and Rigler (1984), Edmondson (1971), and Telesh (1986). Considering the density of the samples, plankton counts were recorded by examining a minimum of 0.25 ml and a maximum of 1 ml of water samples, and the dilution rate was determined for each diluted sample.

Biomass measurements of zooplankton samples were made using a trinocular light microscope with a Leica-branded differential interference contrast (DIC) attachment, and a computer program called LAS 4.1 to measure Rotifera species. Moreover, nauplii, the larvae of Copepoda, were also measured. The weights of organisms whose biometric measurements were made were calculated using equations from the literature; biovolume was calculated from biometric data of Rotifera members, and biomass was determined using geometric equations (Kolisko, 1977).

Statistical evaluations of zooplankton communities; for population evaluation, relative abundance, and frequency (Tischler, 1949), species richness (S), Shannon-Weiner diversity (H') and Pielou evenness (J) indices were used. Moreover, to test seasonal differences in zooplankton community parameters, One-Way Analysis of Variance (ANOVA) was used; statistical analyses were performed using Past 4.03 Statistical Software.

Results and Discussion

Physicochemical Data

The physicochemical data for the sample collection dates from Lake Uzungöl and Lake Paradeniz are given in Table 1. The highest water temperature was measured in August 2014, with 27.6 C° recorded in Uzungöl and 32.4 C° in Paradeniz. However, the lowest water temperatures were measured in November 2015: 10 °C in Uzungöl and 13,5 °C in Paradeniz. In the physicochemical examinations conducted in Uzungöl,

the pH ranged from 8.89 to 9.57, indicating an alkaline water state. Salinity ranged from 0.55‰ to 1.21‰; the lowest was recorded in March 2015, and the highest in May 2016. Dissolved Oxygen (DO) was measured in the range of 6.3 mg/L to 10.9 mg/L and recorded above the eutrophication limit according to the Water Pollution Control Regulation, except for August 2014 (T.C Orman ve Su işleri Bakanlığı 2012, 2014). Electrical Conductivity (EC) was observed in November 2015, with a maximum of 2240.5 µS cm-1, while a minimum of 1078.5 µS cm^{-1} was recorded in March 2015. Chemical data show that anions and cations reached maximum levels in November 2015 at 27.12 mg/L and 28.58 mg/L; moreover, they are at minimum levels in March 2015 at 12.68 mg/L and 13.37 mg/L.

In the physicochemical examinations conducted in Paradeniz, pH levels ranged from 6.03 to 9.515, indicating an alkaline water state, except in August 2014. Salinity varied between 11.67‰ and 31.4‰; the lowest was recorded in March 2015, and the highest in August 2014. Dissolved Oxygen (DO) was measured at 1.15-9.15 mg/L; these values are much lower than those in Uzungöl. Electrical Conductivity (EC) ranged from 20834.5 to 48484 µS cm-1, with the highest value recorded in August 2014 and the lowest in March 2015. Secchi disk measurements showed that turbidity did not differ significantly between the dates, with a standard deviation of 13.36 and a mean of 81.56 cm. Chemical data show that the anion and cation values ranged from 27.12 to 28.58 in November 2015, with the minimum values recorded in March 2015 at 12.68 and 13.37 in Uzungöl.

In terms of the highest Chl-a values, the two lakes differ, while the lowest values coincide with the same date. While Chl-a values were at their maximum (26.91 µg/L) in Uzungöl in August 2014, they were at their maximum (5.01 µg/L) in Paradeniz in March 2015. On the other hand, the lowest values were measured as 3.963 µg/L for Uzungöl and 0.125 µg/L for Paradeniz in May 2016.

Biotic Data

In this study, 43 species and 1 genus of Rotifera were identified. While 41 of the identified species were recorded in Uzungöl and 8 in Paradeniz, 5 species were found in both lakes: *Brachionus calyciflorus*, *Keratella cochlearis*, *Keratella tropica*, *Lecane bulla*, and *Synchaeta pectinata*. However, unlike Uzungöl, *Hexarthra fennica*, *Synchaeta grandis* and *Synchaeta* sp. species were identified in Paradeniz (Table 2).

A comparative abundance and frequency table of Rotifera species in lakes is shown in Table 3. Based on the frequency classification presented by Tischler, 15 incidental species, nine accessory species, and 17 fixed species were recorded in Uzungöl (Tischler, 1949). Three recorded species *Brachionus angularis*, *Brachionus calyciflorus*, and *Trichocerca marina* were evaluated in the definitely fixed species category. Among the species detected in Paradeniz, B*rachionus bidentata*, *Hexarthra fennica*, *Keratella tropica*, L*ecane bulla*, *Synchaeta grandis* and *Synchaeta pectinata* are considered as incidental species. At the same time, *Keratella cochlearis* is an accessory species, and *Synchaeta* sp. has been identified as a definite fixed species. However, it was determined that 2 species, *Brachionus bidentata* and *Lecane bulla*, were incidental species for both lakes.

Density values of zooplankton in samples taken from Uzungöl and Paradeniz lagoons are given in Table 4. The population density difference between the two lakes is remarkable. While the highest individual count in Uzungöl was 43353.84 ind.m^{-3} for *Hexarthra mira* in August 2014, the lowest was 23.87 ind.m^{-3} for *Mytilina ventralis*, *Trichocerca rattus*, and *Trichothria pocillum* in March 2016. In terms of the total number of individuals, the highest value recorded in Uzungöl was 120416.73 ind.m^{-3} in August 2014, while the lowest value was 62189.846 ind. m^{-3} in March 2015. The highest number of individuals measured in Paradeniz was recorded in May 2016 as *Synchaeta* sp. with 6923.24 ind.m^{-3}, while the lowest number of individuals recorded was in November 2015 as *Brachionus calyciflorus* and *Lecane bulla* and in May 2016 as *Hexarthra fennica* with 15.915 ind.m^{-3}

Also, the highest total number of individuals was recorded at 8307.90 ind.m^{-3} in May 2016, while the lowest was 15.91 ind.m^{-3} in March 2015.

The Shannon-Wiener diversity index and Pielou Evenness index were calculated from the density values for the two lakes per cubic meter, as given in Table 5. Calculated values are shown in Table 5. In line with Uzungöl data, species diversity decreased significantly in March 2015 and then increased; the highest value was recorded in May 2016. Statistical analysis shows that the Shannon-Wiener Diversity Index differs significantly among lakes (One-Way ANOVA; $F=19.69$, $p < 0.05$) and sampling dates (One-Way ANOVA; $F=3.857$, $p < 0.05$). Based on Pielou's Evenness Index calculations, the date with the lowest species homogeneity was March 2015. Statistical analysis shows that there is no significant difference in Pielou Evenness index across sampling dates (One-Way ANOVA; $F=0.2779$, $p>0.05$); however, statistically significant differences were observed between Pielou Evenness index and lakes (One-Way ANOVA; $F=59.58$, $p<0.01$) (Table 5, Figure 2).

Table 6 presents the species identified in the study areas and their corresponding biomass values. Among the species common in Uzungöl and Paradeniz, only *Keratella cochlearis*

seems to provide comparable data (Figure 3). It was determined that *Keratella cochlearis* individuals whose biomass was measured showed no statistical difference across measurement dates and lakes (F=0.8655, p>0.05).

Table 1. Average values of physicochemical parameters in Lake Uzungöl and Paradeniz between August 2014 and May 2016

	Uzungöl				Paradeniz			
	19.08.2014	3.03.2015	19.11.2015	13.05.2016	31.08.2014	15.03.2015	24.11.2015	7.05.2016
DO (mg L^{-1})	6.3 (±0)	10.99 (±0.447)	9.8825(±2.299)	8.13 (±0.955)	1.15 (±0.08)	9.1475 (±0.38)	7.89 (±0.11)	5.2575 (±0.26)
DO %	80.7 (±0)	97.775 (±4.147)	94.475 (±12.092)	92.625 (±10.946)	18.8 (±1.1)	95.375 (5.19)	84.575 (±0.82)	69.6 (±3.79)
EC (µS cm^{-1})	2055 (±0)	1078.5 (±283.98)	2240.5 (±1132.21)	2187.5 (±534.461)	48484 (±887.6)	20834.5 (±5999.03)	27200.75 (±2931.9)	39760.75 (±2005.92)
Salinity ‰)	1 (±0)	0.55 (±0.15)	1.175 (±0.63)	1.2125 (±0.314)	31.4 (±0.54)	11.675 (±2.9)	17.9 (±1.16)	27.6 (±1.15)
Temperature (C°)	27.6 (±0)	10.1 (±0.255)	10.075 (±0.415)	21.45 (±0.173)	32.4 (±0.47)	14.225 (±0.606)	13.55 (±0.29)	21.235 (±0.83)
pH	9.25 (±0)	8.89 (±0.308)	9.57 (±0.17)	9.0075 (±0.342)	6.03 (±0.25)	8.7425 (±0.024)	9.515 (±0.69)	8.3375 (±0.016)
Secchi Disk (cm)	48 (±0)	45 (±5)	76.25(±23.81)	85 (±26.926)	97.5 (±18.9)	68.75 (±29.66)	72.5 (±17.85)	87.5 (±25.86)
Depth (cm)	48 (±0)	95 (±22.941)	92.5 (±34.187)	130 (±25.495)	120 (±25.5)	107.5 (±17.85)	91.25 (±32.09)	97.5 (±34.91)
Chl-a (µg/L)	26.91 (±0)	22.07 (±6.5)	3.978 (±1.469)	3.963 (±3.011)	2.17 (±0.518)	5.01 (±3.14)	1.155 (±0.411)	0.125 (±0.069)
Cl (mg/L)	473.91 (±0)	242.57 (±100.38)	690.451 (±427.594)	610.07 (±198.754)	19053.17 (±515.58)	7657.2 (±1916.495)	14724.47 (±1395.193)	18294.575 (±954.034)
NO2 (mg/L)	0	0.0314 (±0.033)	0.1008 (±0.175)	0.366 (±0.157)	0	0	0	1.58 (±0.996)
NO3 (mg/L)	1.988 (±0)	1.279 (±0.692)	0.2955 (±0.261)	0.832 (±0.827)	0	1.5 (±1.552)	0	0
PO4 (mg/L)	0	0	0	0	0	0	0	0
SO4 (mg/L)	280.543 (±0)	88.899 (±26.03)	167.196 (±52.416)	135.68 (±28.531)	2228.1 (±61.62)	892.62 (±219.276)	1722.601 (±165.147)	2115.9675 (±112.302)
CO3 (mg/L)	0	16.5 (±2.598)	24.519 (±11.079)	19.853 (±14.91)	33.66 (±2.53)	30 (±0	28.85 (±0	29.4117 (±4.16)
HCO3 (mg/L)	148.78 (±0)	207.4 (±11.206)	200.89 (±25.014)	239.215 (±39.894)	139.85 (±5.15)	198.25 (±16.425)	156.9 (±7.62)	167.451 (±14.65)
Na (mg/L)	306.68 (±0)	157.665 (±53.994)	422.428 (±241.635)	371.231 (±111.60)	9196.1 (±267.15)	4040.55 (±1020.87)	8102.63 (±717.466)	10119.996 (±489.611)
NH4 (mg/L)	1.28 (±0)	0.197 (±0.197)	1.239(±1.488)	0	37.15 (±14.47)	0	18.834 (±20.404)	0
K (mg/L)	12.864 (±0)	8.438 (±1.934)	16.736 (±8.131)	13.24 (±3.321)	391.49 (±16.33)	157.77 (±38.812)	280.02 (±28.98)	337.25275 (±15.482)
Mg (mg/L)	71.881 (±0)	36.998 (±9.533)	79.879 (±34.594)	76.42 (±16.398)	1816.43 (±95.94)	725.21 (±180.746)	1133.636 (±75.296)	1595.25225 (±71.561)
Ca (mg/L)	53.224 (±0)	64.963 (±2.196)	62.703 (±19.788)	70.55 (±11.382)	1261.11 (±328.43)	439.3 (±106.001)	663.979 (±33.49)	675.62395 (±15.752)
Anion (mg/L)	21.70 (±0)	12.684 (±3.558)	27.12 (±13.45)	24.667 (±6.75)	587.94 (±15.83)	239.18 (±58.463)	455.353 (±42.75)	564.52485 (±29.158)
Cation (mg/L)	22.31 (±0)	13.372 (±3.210)	28.58 (±14.497)	26.298 (±6.803)	624.58 (±5.49)	261.43 (±65.21)	487.171 (±40.38)	613.9234 (±28.13)

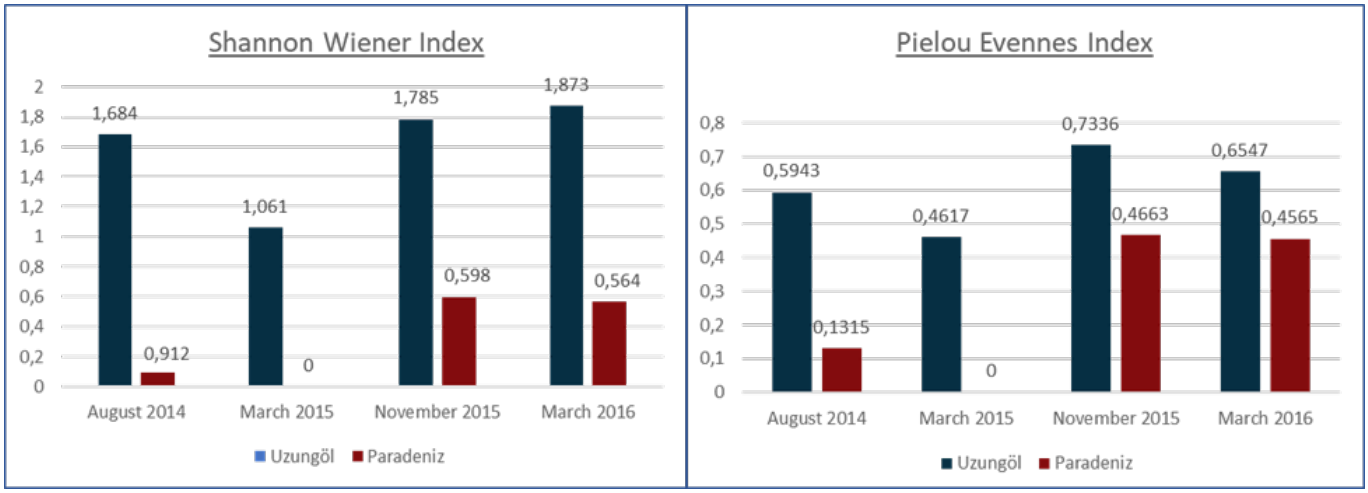

Figure 2. Bar graph of Shannon Wiener Index and Pielou Evenness Index data of Uzungöl and Paradeniz from August 2014 to May 2016.

Table 2. Seasonal Distribution of the Rotifers in Uzungöl and Paradeniz Lagoons

	Uzungöl				Paradeniz			
	Aug. 2014	Mar. 2015	Nov. 2015	May 2016	Aug. 2014	Mar. 2015	Nov. 2015	May 2016
Anuraeopsis fissa				*				
Asplanchna priodonta	*		*	*				
Brachionus angularis	*	*	*	*				
Brachionus bidentata	*							
Brachionus calyciflorus	*	*	*	*			*	
Brachionus diversicornis	*		*					
Brachionus plicatilis		*						
Brachionus quadridentatus	*		*	*				
Brachionus urceolaris		*		*				
Cephalodella catellina			*	*				
Cephalodella delicata			*	*				
Cephalodella gibba		*	*	*				
Colurella colurus		*	*	*				
Filinia longiseta	*		*	*				
Filinia terminalis		*						
Hexarthra fennica								*
Hexarthra intermediata	*			*				
Keratella cochlearis	*			*			*	*
Keratella quadrata		*	*	*				
Keratella tropica	*		*	*			*	*
Lecane bulla	*						*	*
Lecane closterocerca	*		*	*				
Lecane hamata	*							
Lecane luna	*		*	*				
Lecane imbricata		*						
Lecane lunaris	*							
Lecane ohioensis	*		*					
Lepadella ovalis	*		*					
Mytilina mucronata				*				
Mytilina ventralis				*				
Notholca acuminata		*						
Monommata sp.			*					
Polyarthra dolichoptera				*				
Polyarthra vulgaris		*	*	*				
Synchaeta grandis					*			
Synchaeta oblonga		*	*	*				
Synchaeta pectinata		*	*	*				*
Synchaeta sp.					*	*	*	*
Testudinella patina		*						
Trichocerca gibba	*	*	*	*				
Trichocerca marina				*				
Trichocerca rattus		*	*					
Trichothria pocillum		*	*					
Trichothria tetractis			*	*				

Table 3. Abundance and frequency of rotifers by sampling areas and sampling dates (%)

	Frequency (%)									
	August 2014		March 2015		November 2015		May 2016			
	Uzungöl	Paradeniz	Uzungöl	Paradeniz	Uzungöl	Paradeniz	Uzungöl	Paradeniz	Uzungöl	Paradeniz
Anuraeopsis fissa	0	0	0	0	0	0	1.543	0	**25**	**0**
Asplanchna priodonta	2.85	0	0	0	1.271	0	0.364	0	**75**	**0**
Brachionus angularis	2.69	0	0.2687	0	1.617	0	23.520	0	**100**	**0**
Brachionus bidentata	0.24	0	0	0	0	0	0	0	**25**	**25**
Brachionus calyciflorus	4.92	0	0.729	0	0.404	1.785	0.728	0	**100**	**0**
Brachionus diversicornis	0.079	0	0	0	0.1443	0	0	0	**50**	**0**
Brachionus plicatilis	0	0	0.0383	0	0	0	0	0	**25**	**0**
Brachionus quadridentatus	0.238	0	0	0	0.1444	0	0.2572	0	**75**	**0**
Brachionus urceolaris	0	0	0.0767	0	0	0	0.0428	0	**50**	**0**
Cephalodella catellina	0	0	0	0	10.568	0	3.473	0	**50**	**0**
Cephalodella delicata	0	0	0	0	0.3753	0	0.557	0	**50**	**0**
Cephalodella gibba	0	0	1.343	0	1.443	0	2.294	0	**75**	**0**
Colurella colurus	0	0	0.3454	0	1.530	0	1.843	0	**75**	**0**
Filinia longiseta	35.29	0	0	0	5.948	0	13.765	0	**75**	**0**
Filinia terminalis	0	0	23.0710	0	0	0	0	0	**25**	**0**
Hexarthra fennica	0	0	0	0	0	0	0	0.1915	**0**	**25**
Hexarthra mira	36.00	0	0	0	0	0	1.672	0	**50**	**0**
Keratella cochlearis	0.079	0	0	0	3.176	50	8.490	2.681	**75**	**50**
Keratella quadrata	0	0	22.226	0	8.431	0	1.372	0	**75**	0
Keratella tropica	0.952	0	0	0	20.589	7.142	0.321	0	**75**	**25**
Lecane bulla	7.61	0	0	0	0	1.785	0	0	**25**	**25**
Lecane closterocerca	2.854	0	0	0	3.522	0	0.750	0	**75**	**0**
Lecane hamata	0.158	0	0	0	0	0	0	0	**25**	**0**
Lecane imbricata	0	0	0	0	0	0	0.042	0	**25**	**0**
Lecane luna	0.555	0	0	0	2.3678	0	0.107	0	**75**	**0**
Lecane lunaris	0	0	0	0	0.144	0	0	0	**25**	**0**
Lecane ohioensis	0.396	0	0	0	0.721	0	0	0	**50**	**0**
Lepadella ovalis	0.158	0	0	0	1.270	0	0	0	**50**	**0**
Monommata sp.	0	0	0	0	0	0	0.064	0	**25**	**0**
Mytilina mucronata	0	0	0	0	0	0	0.364	0	**25**	**0**
Mytilina ventralis	0	0	0	0	0	0	0.0214	0	**25**	**0**
Notholca acuminata	0	0	8.867	0	0	0	0	0	**25**	**0**
Polyarthra dolichoptera	0	0	0	0	0	0	0.064	0	**25**	**0**
Polyarthra vulgaris	0	0	38.925	0	6.901	0	17.645	0	**75**	**0**
Synchaeta grandis	0	96.417	0	0	0	0	0	13.409	**0**	**25**
Synchaeta oblonga	0	0	0.422	0	3.696	0	8.983	0	**75**	**0**
Synchaeta pectinata	0	0	0.2687	0	23.390	0	10.463	0.383	**75**	**25**
Synchaeta sp.	0	3.582	0	100	0	39.285	0	83.333	**0**	**100**
Testudinella patina	0	0	0.076	0	0	0	0.0857	0	**50**	**0**
Trichocerca marina	4.916	0	1.190	0	1.126	0	0.364	0	**100**	**0**
Trichocerca rattus	0	0	0.383	0	0.490	0	0.0214	0	**75**	**0**
Trichocerca stylata	0	0	0	0	0	0	0.686	0	**25**	**0**
Trichothria pocillum	0	0	1.765	0	0.173	0	0.021	0	**75**	**0**
Trichothria tetractis	0	0	0	0	0.548	0	0.064	0	**50**	**0**
SUM	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**		

Table 4. Amount of individuals in cubic meters (ind.m^{-3}) by sampling areas and sampling dates

	Uzungöl				Paradeniz			
Dates / **Species** *(ind.m^{-3})*	August 2014	March 2015	Novem-ber 2015	May 2016	August 2014	March 2015	November 2015	May 2016
Anuraeopsis fissa	0	0	0	1718.87	0	0	0	0
Asplanchna priodonta	3437.74	0	1050.42	405.85	0	0	0	0
Brachionus angularis	3246.76	167.11	1336.90	26188.96	0	0	0	0
Brachionus bidentata	286.47	0	0	0	0	0	0	0
Brachionus calyciflorus	5920.56	453.59	334.225	811.69	0	0	15.92	0
Brachionus diversicornis	95.49	0	119.36	0	0	0	0	0
Brachionus plicatilis	0	23.87	0	0	0	0	0	0
Brachionus quadridentatus	286.47	0	119.36	286.47	0	0	0	0
Brachionus urceolaris	0	47.75	0	47.746	0	0	0	0
Cephalodella catellina	0	0	8737.61	3867.47	0	0	0	0
Cephalodella delicata	0	0	310.35	620.70	0	0	0	0
Cephalodella gibba	0	835.56	1193.66	2554.44	0	0	0	0
Colurella colurus	0	214.86	1265.28	2053.11	0	0	0	0
Filinia longiseta	42494.40	0	4917.89	15326.63	0	0	0	0
Filinia terminalis	0	14347.83	0	0	0	0	0	0
Hexarthra fennica	0	0	0	0	0	0	0	15.92
Hexarthra mira	43353.84	0	0	1862.11	0	0	0	0
Keratella cochlearis	95.49	0	2626.05	9453.81	0	0	445.64	222.82
Keratella quadrata	0	13822.62	6970.99	1527.88	0	0	0	0
Keratella tropica	1145.92	0	17021.64	358.09	0	0	63.66	0
Lecane bulla	9167.33	0	0	0	0	0	15.915	0
Lecane closterocerca	3437.75	0	2912.54	835.56	0	0	0	0
Lecane hamata	190.98	0	0	0	0	0	0	0
Lecane imbricata	0	0	0	47.746	0	0	0	0
Lecane luna	668.45	0	1957.61	119.36	0	0	0	0
Lecane lunaris	0	0	119.36	0	0	0	0	0
Lecane ohioensis	477.46	0	596.83	0	0	0	0	0
Lepadella ovalis	190.99	0	1050.42	0	0	0	0	0
Monommata sp.	0	0	0	71.61	0	0	0	0
Mytilina mucronata	0	0	0	405.85	0	0	0	0
Mytilina ventralis	0	0	0	23.87	0	0	0	0
Notholca acuminata	0	5514.73	0	0	0	0	0	0
Polyarthra dolichoptera	0	0	0	71.62	0	0	0	0
Polyarthra vulgaris	0	24207.48	5705.71	19647.69	0	0	0	0
Synchaeta grandis	0	0	0	0	4631.41	0	0	1114.08
Synchaeta oblonga	0	262.61	3055.77	10002.89	0	0	0	0
Synchaeta pectinata	0	167.11	19337.34	11650.15	0	0	0	31.83
Synchaeta sp.	0	0	0	0	190.98	15.91	350.14	6923.25
Testudinella patina	0	47.74	0	95.49	0	0	0	0
Trichocerca marina	5920.57	740.07	931.057	405.84	0	0	0	0
Trichocerca rattus	0	238.73	405.85	23.87	0	0	0	0
Trichocerca stylata	0	0	0	763.94	0	0	0	0
Trichothria pocillum	0	1098.17	143.24	23.87	0	0	0	0
Trichothria tetractis	0	0	453.59	71.62	0	0	0	0

Table 6. Biomass values of rotifers in Uzungöl and Paradeniz between August 2014 and May 2016

	Uzungöl				Paradeniz			
	August 14	March 15	November 15	May 16	August 14	March 15	November 15	May 16
	x̄ µg ± SD	**x̄ µg ± SD**	**x̄ µg ± SD**	**x̄ µg ± SD**	**x̄ µg ± SD**	**x̄ µg ± SD**	**x̄ µg ± SD**	**x̄ µg ± SD**
Asplanchna priodonta	0	0	0.1775 ± 0.101	0	0	0	0	0
Brachionus angularis	0.133 ± 0.031	0	0.359 ± 0.037	0.1615 ± 0.036	0	0	0	0
Brachionus calyciflorus	1.115 ± 0.357	1.351 ± 0.225	1.505 ± 0.099	0.950 ± 0.101	0	0	0	0
Brachionus diversicornis	0	0	0	0.3062 ± 0.082	0	0	0	0
Brachionus plicatilis	0	0	0	0.0412 ± 0.004	0	0	0	0
Brachionus quadridentatus	0	0	0	0.3446 ± 0.13	0	0	0	0
Filinia longiseta	0.2501451 ± 0.116	0	0. 2635 ± 0.056	0.2042 ± 0.048	0	0	0	0
Filinia terminalis	0	0.3888 ± 0.173	0	0	0	0	0	0
Hexarthra mira	0.2023399 ± 0.091	0	0	0.1768 ± 0.06	0	0	0	0
Keratella cochlearis	0	0	0. 0.0184 ± 0	0.0271 ± 0.005	0	0	0.04716 ± 0.006	0.06529 ± 0.008
Keratella quadrata	0	0.6436 ± 0.112	0.3142 ± 0.778	0.3818 ± 0.076	0	0	0	0
Keratella tropica	0.22143255 ± 0.038	0	0.226 ± 0.017	0.225 ± 0.008	0	0	0	0
Notholca acuminata	0	0.3908 ± 0.086	0	0	0	0	0	0
Polyarthra vulgaris	0.277014 ± 0.091	0.351 ± 0.113	0.2905 ± 0.0916	0.2971 ± 0.078	0	0	0	0
Synchaeta grandis	0	0	0	0	0.3697 ± 0.118	0	0	0.3625 ± 0.143
Synchaeta oblonga	0	0	0.1772 ± 0.055	0.1788 ± 0.02	0	0	0	0
Synchaeta pectinata	0	0	0.5455 ± 0.135	0	0	0	0	0
Synchaeta sp.	0	0	0	0	0	0	0	0.1001 ± 0.296
Trichocerca gibba	0.4800944 ± 0.164	0	0.00198 ± 0.035	0.0007 ± 0.055	0	0	0	0
	x̄ µm ± SD	**x̄ µm ± SD**	**x̄ µm ± SD**	**x̄ µm ± SD**	**x̄ µm ± SD**	**x̄ µm ± SD**	**x̄ µm ± SD**	**x̄ µm ± SD**
Nauplii	177.319 ±41.198	153.616 ± 25.706	175.086 ± 31.340	173.303 ± 27.936	120.481 ± 21.503	98.876 ± 6.384	97.054 ± 5.894	107.971 ± 11.065

Table 5. Shannon Wiener and Pielou Evenness Index of Uzungöl and Paradeniz between August 2014 and May 2016

	Uzungöl		Paradeniz	
	Shannon Wiener Diversity Index	Pielou Evenness Index	Shannon Wiener Diversity Index	Pielou Evenness Index
August	1.6837	0.5942	0.0911	0.1315
March	1.0611	0.4617	0	0
November	1.7854	0.7336	0.5975	0.4663
May	1.8726	0.6547	0.5642	0.4564
(Min-Max)	(0.2509-2.4019)	(0.1289-0.831)	(0- -1.0735)	(0- -0.7253)
Mean	1.5816	1.5777	-0.3132	-0.2635
(± SD)	0.5181	0.1613	0.3466	0.2605

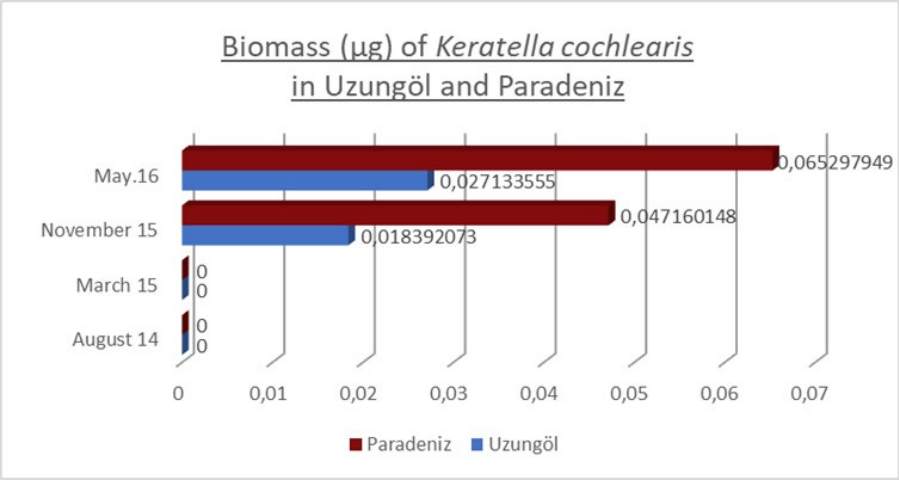

Figure 3. Biomass values of *Keratella cochlearis* in Uzungöl and Paradeniz between August 2014 to May 2016

The possible effects of physical and chemical parameters on zooplankton species diversity, density, and distribution have been studied by many researchers (Berzins & Pejler, 1987; Shaw & Kelson, 1992; Williams et al., 1990). It is known that many environmental factors, such as salinity (Williams, 1998), temperature, and EC, are effective. Zooplanktonic organisms have a wide distribution and are found in almost all aquatic systems (Galkovskaja, 1987). The most diverse rotifer assemblages occur in soft, slightly acidic, oligo- to mesotrophic waters. These are particularly vulnerable to eutrophication and salinisation (Segers, 2008).

In areas where eutrophication and salinity increase, species diversity and population density also decrease. During our study, salinity was changed to 0.55 in March and 1.21 in May in Uzungöl, and to 11.67 in March and 31.04 in August in the Paradeniz Lagoon. Species diversity and distribution also varied significantly with salinity. In the Paradeniz lagoon, where salinity and EC were high, species diversity and population density were low, and these species are highly salt-tolerant, such as the genera *Synchaeta* and *Hexarthra*.

Egborge (1994) observed that species richness would decrease with increasing conductivity from 2800 to 50000 µS cm. When the EC values determined in both areas were analysed, it was observed that they varied between 1078.5-2240.5 µS cm^{-1} in Uzungöl and 20834.5-48484 µS cm^{-1} in Paradeniz Lagoon, and that the number and density of species showed significant differences in both areas.

During the study, 41 species were recorded in Uzungöl and 8 in Paradeniz. Among the sampling dates, species diversity and density were higher in November and May in Uzungöl than on other dates. Although there was no significant difference in physicochemical parameters between the sampling dates, except salinity and EC, significant differences were observed in chlorophyll a. Generally, rotifer population growth increases with increasing temperature and food concentration

(Kauler & Enesco, 2011). In November and May, when zooplankton density was high, chlorophyll a was 3.97 - 3.96 µg/L due to feeding pressure; accordingly, in March and August, when zooplankton density was lower, chlorophyll a was much higher at 26.9 - 22 µg/L.

In conclusion, the major environmental factor affecting rotifer communities is salinity. An increase in salinity resulted in a decrease in species richness and diversity (Bielańska & Cudak, 2014). The highest densities were recorded in freshwater and hyposaline Uzungöl, whereas the lowest were in high saline Paradeniz lagoon, and species richness also differed significantly between the two sampling areas. Statistical analysis also showed that the Shannon-Wiener Diversity Index and Pielou Evenness index differ significantly among the lakes (One-Way ANOVA; F F= 59.58; $p < 0.01$).

Conclusion

In both areas, biomass calculations were made by utilising the length-weight relationship. For each species for which biomass was measured, the lengths of about 20 individuals were measured, average values were taken, and the weights of each species in micrograms were calculated. Analysis of biomass from the lagoons reveals that nauplii have a significant mass in both areas. No significant difference in rotifer species biomass was found in Uzungöl compared with previous studies conducted in eutrophic lakes (Kolisko, 1977; Downing & Rigler, 1984; Akbulut, E., 1998). Depending on the plankton density, zooplankton biomass is also high in November and May. However, nauplii biomass was high in all seasons sampled.

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 article.

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

Funding disclosure: This research was funded by Hacettepe University (BAP-014 D04 818 001-598; FHD 2015-8292).

Acknowledgements: The authors thank İbrahim Arslan and Vahid Bayramıalemdarı for their assistance with the field experiments.

Disclosure: Abstract/summary of this MS has been published in the XVI Rotifer Conference Proceedings in Zagreb, 2022

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AQUATIC RESEARCH
E-ISSN 2618-6365
Aquatic Research 8(4), 286-294 (2025) • https://doi.org/10.3153/AR25028 *Research Article*

Investigation of the effect of a commercial probiotic-based product containing enzymes on rainbow trout (*Oncorhynchus mykiss*)

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Cite this article as:
Akaylı, T. (2025). Investigation of the effect of a commercial probiotic-based product containing enzymes on rainbow trout (*Oncorhynchus mykiss*). *Aquatic Research*, 8(4), 286-294. https://doi.org/10.3153/AR25028

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Submitted: 03.09.2025
Revision requested: 09.10.2025
Last revision received: 15.10.2025
Accepted: 19.10.2025
Published online: 21.10.2025

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Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

In this study, the aim was to test the effect of a candidate biotechnological product developed by a commercial company and containing different probiotic bacteria and enzymes on juvenile rainbow trout (*O. mykiss*) and water quality. The product's protective effect against fungal infections in fish eggs and the pathogen *Aeromonas hydrophila* was also investigated, as were its effects on the fish's intestinal tissue. Fifty fish were placed in aquariums in two experimental setups (A and B). Different doses of the product were added to the feed in Group A and water for the fish in Group B. Four different bath doses (d1-d4) of the product were applied to fish in Group B. At the end of the study, fish in Group A demonstrated better health, growth, feed conversion, and survival rates, as well as water quality, compared to the other groups. The product was found to be effective in improving the fish's intestinal health, reducing fungal infections in their eggs, and preventing infection by *A. hydrophila*. The data obtained from this study aims to contribute to the development of our country's aquaculture sector.

Keywords: Rainbow Trout, *O. mykiss*, Probiotic, Egg, *Aeromonas hydrophila*

Introduction

Rainbow trout (*O. mykiss*) farming has developed rapidly in Turkey in recent years and has become an important sector contributing to the national economy (Çakmak et al., 2024; TÜİK, 2024). Turkey is the largest trout producer in Europe, with an annual production of 75,567 tons (FAO, 2024). Bacterial diseases resulting from many adverse conditions such as excessive stocking density, inadequate water quality, stress, and inadequate nutrition, along with the development of antibiotic resistance in pathogens and feed costs, pose a risk to the sustainable development of the sector and the health of cultured fish (Lieke et al., 2020; Simon et al., 2021; Deekshit et al., 2022). Nowadays, preventive measures including new biotechnological applications (such as prebiotics, probiotics and enzymes) have become increasingly important in order to control fish diseases and to strengthen the non-specific defence mechanism of fish for protection against diseases (Süzer et al., 2008; Merrifield et al., 2010; Lieke et al., 2020; Simon et al., 2021; Deekshit et al., 2022; Amenyogbe et al., 2024).

Probiotics are live bacterial and yeast cultures, or biological products contained within these cultures, that are beneficial to the digestive system and health of fish (Balcazar et al., 2006; Vine et al., 2006; Amenyogbe et al., 2024). These products increase fish immunity against diseases, inhibit pathogenic bacteria, promote growth, and help regulate water quality (Balcazar et al., 2006; Vine et al., 2006; Kristiansen et al., 2011; Amenyogbe et al., 2024). Probiotic products are applied by adding them to the fish growing water, by adding them to the feed, and by adding them to both the water and feed. Researchers have reported that there are different steps in selecting the probiotics (alone or mixed) to be used in fish and shellfish and determining the optimal doses, but first, the pathogenicity test of the product should be performed under in vivo conditions (Balcazar et al., 2006; Vine et al., 2006; Feckaninova et al., 2017).

Studies on the use of probiotics against fish pathogens (such as bacteria and fungi) are gaining increasing importance. Studies have shown that Lactobacillus species, which have a probiotic effect, can produce substances that inhibit the growth of pathogens that cause Aeromonas infections in freshwater and marine fish, and are therefore important for fish health (Feckaninova et al., 2017; Redhwan et al., 2024). Studies are also underway to identify probiotic bacteria effective in preventing saprolegniasis, a fungal infection seen in cultured fish that primarily affects fish eggs (González-Palacios et al., 2018; Fregeneda-Grandes et al., 2023). The bacterial and fungal cultures found in probiotic mixtures generally have a positive impact on fish health by producing various minerals and trace elements, vitamins, and digestive enzymes such as amylase, lipase, and protease. For this reason, in recent years, commercial products containing enzymes obtained from these microorganisms have been widely used successfully in fish feed production (Assan et al., 2022; Amenyogbe et al., 2024; Asha et al., 2024).

This study will investigate the effects of an enzyme-containing probiotic product (codenamed Enzyfish), produced in the laboratories of a commercial biotechnology company, on in vivo cultured rainbow trout fry. In addition to determining the effective dose of this product, the product's effects on growth, feed conversion, disease resistance, and water quality of the cultured trout fry will be comprehensively investigated. The product's antifungal activity on eggs and its protective effect against the pathogen *Aeromonas hydrophila* were also investigated.

Materials and Methods

Experimental Fish and Eggs

The fry used in this study were purchased from a private trout farm while they were still in the fertilised egg stage. After 15-20 days, the observed fish eggs developed into larvae and began their first feeding stage. In our study, 1,200 fish samples, weighing an average of 0.14 g, were used. Additionally, approximately 400 egg-stage samples were used in our study on fungal infections.

Application of the Commercial Probiotic

The study aimed to determine the effective dosage of an enzyme-based product (Enzyfish), produced by a commercial company and containing various probiotic bacteria. Company officials stated that the product contains enzymes derived from various probiotic bacteria. Because it is a new commercial product, no further information about its ingredients has been provided. Therefore, the relevant studies were conducted on young trout during their initial feeding phase over 45 days. The product was applied to the fish feed and water at different rates in line with the company's recommendations.

Commercially available powdered trout feed was used to feed the young fish. Following the manufacturer's recommendations, 1 mL of the product liquid was taken per 1 kg of fish feed and diluted in 10 mL of sterile saline solution (0.8%). This solution was then sprayed onto the powdered feed using a spray bottle, and the product-enriched feeds were prepared.

In the process of adding the product to the water as a bath, the main stock was first prepared, and 100 mL of the product was diluted 36 times in water. Different dilutions were then made from this stock, and four different doses (d1-d4) were applied to the aquariums (Table 1). Juvenile fish in the first feeding phase were kept in these aquariums for 14 days using the long-term bathing method. No product was added to the water of the test fish during the remaining 30-day trial period.

Experimental Design

To determine the effect of the product on fish eggs, three experimental groups (y1, y2, y3) and one control group (y4) were prepared, each containing 100 eggs. Fish eggs were incubated in egg development baskets. The temperature of the water in the experimental groups was measured as 17 °C, pH: 7.2, and the oxygen amount was measured as 5-6 (mg/L).

Three different doses of the product (5, 10, and 15 µL/L) were applied to the eggs via 20 minute, 1 hour, and 24 hour baths. The number of dead white eggs and the rate of fungal growth on the eggs were examined during this approximately 5 day study. Eggs were not subjected to experimental infection.

The experimental setups in this study were divided into two groups: A and B. Fifty fish were placed in each aquarium in each experimental group, and the study was conducted in three replicates. The product was added to the food of all fish in aquariums in group A (A1-A5). While four different application doses (d1-d4) of the product were added to the aquarium water by the bath method for the fish in group B (B1-B5) aquariums, no product was added to the feed.

Similarly, four different doses (d1-d4) of the product were added to the aquarium water using the long-term bath method, except for aquarium A5 (Table 1). Four different application doses (d1-d4) of the product were added to the aquarium water of fish in Group B (B1-B5) aquariums using the bath method. In contrast, no product was added to the feed. During the 45-day study, the aquariums were cleaned daily, and the ambient temperature, oxygen level, pH, ammonia, and nitrate levels were measured.

Experimental Challenge

An experimental infection setup was established to determine the effectiveness of a commercial product against *Aeromonas hydrophila*, a pathogenic fish pathogen. Four experimental groups (h1-h4) were formed, each containing approximately 35 fish, and the experimental infection study was conducted over 1 month. The fish in aquariums h1, h2 and h3 were given a 0.01 mL volume of bacterial solution with a density of 1x 10^6 mL^{-1} by injection into the muscle. The individuals in the control tank (h4) were also treated with the bacteria in the same manner, but the disease was not treated. After the infection occurred, fish in group h1, which had previously been fed the product, were fed only normal food. Fish in group h2 received the product added to their feed, and fish in group h2 received a 1 hour bath with the product. At the end of the study, the effectiveness of the product in treating the disease was investigated by comparing the percentage of fish that died with the percentage of surviving individuals.

Table 1. Addition of the product to aquarium water in the bathing method

Dose	Aquarium volume	Product quantity	Application period
d1	30 L	5.550 mL	14 days
d2	30 L	55.5 mL	14 days
d3	30 L	55,5 mL	14 days
d4	30 L	275 mL	14 days

Growth Performance Measurement

Growth indicators such as weight gain (WG), specific growth rate (SGR), survival rate and feed conversion ratio (FCR) were calculated for all experimental fish in groups A and B at the beginning and end of the feeding period according to the equations specified by Korkut et al. (2007).

$$\text{Weight gain (WG)} = \frac{\text{Mean final weight gm} - \text{Mean initial weight gm}}{\text{t(days)}}$$

$$\text{Specific growth rate (SGR)} = \frac{\log W2 - \log w1 \ \text{x}\ 100}{T2 - T1}$$

$$\text{Feed conversion ratio FCR} = \frac{\text{Total amount of feed consumed (gm)}}{\text{Wet weight gain (gm)}}$$

$$\text{Survival rate} = \frac{\text{Number of fish harvested x 100}}{\text{Number of fish stocked}}$$

Bacteriological Inoculations of Water

Water samples were taken twice a week from all aquariums containing fish, and these samples were inoculated onto PCA (Plate Count Agar) medium, which was used for total bacterial counts. Petri dishes were incubated at 22°C for 24-48 hours. At the end of this period, the number of bacterial colonies (cfu) grown on the petri dish was recorded, and changes in the bacterial load in the water throughout the study were recorded (Huss, 1988).

Histological Examination

In this study, tissue samples were taken from intestinal sections of fish larvae fed regular trout feed (N) and feed supplemented with the product for histological analysis. The tissue samples were fixed in a 10% formaldehyde solution and subjected to routine tissue processing and procedures used in our Faculty's Histology Laboratory. Preparations were stained with H&E. These sections were examined under a light microscope. Changes in the structure of the villi in the intestinal tissue taken from 5 fish in each group were investigated. The mean values of the number and length of villi in the intestinal tissue were calculated (Kristiansen et al., 2011; El-Kady et al., 2022; Marco et al., 2023; Asha et al., 2024).

Results and Discussion

Preventing Fungal Infection

Application of various bath doses of a probiotic containing enzymes to eggs showed that, except for d1, d2, d3, and d4 doses, reduced fungal infection in eggs. Considering the mortality rate in eggs, it was determined that the best results were obtained with the d3 dose applied every other day or for a minute every three days. Similar to the findings of other researchers, the addition of probiotic-containing products to water during bathing has been shown to have a protective effect against fungal infections in fish eggs (González-Palacios et al., 2018; Fregeneda-Grandes et al., 2023; Amenyogbe et al., 2024).

Improved Growth Performance

After the trial was completed, the development of the fish we used as material was examined, and it was noted that the fish in Group A1 developed much faster than those in Group B, with increased transverse muscle thickness in the fish's body and a distinct colouration in the fins (Figure 1).

Figure 1. Appearance of the fish at the end of the experiment

In our research on the determination of the effective dose in the fish in the trial groups of the commercial product, the weight increase of the fish in group A, the specific growth rate (SGR), the feeding (FCR) and the survival rates were found to be better than those of the other group (Table 2).

As a result of our study, It has been observed that the chances of the fish in group A are still high and that individuals are more willing and healthier against eating. More successful results have been obtained on the growth of the fish with the addition of both water and food to the fish in group A. The data we obtain show that probiotics contribute to scientific data showing that they can be used commercially to accelerate growth, increase feed efficiency and improve fish production safety by using antibiotics in fish and shellfish breeding (Vine, 2006; El-Kady et al., 2022; Asha et al., 2024).

Water Quality and Effect on Bacterial Load

At the end of the trials, some of the values related to water quality in the aquariums remained constant (such as temperature, oxygen and pH), while some of them had changed. In the aquariums of the A and B groups, variability in ammonia and phosphate levels in water was observed, while the nitrate level was always measured low. However, when the total bacterial load in the water was examined, it was observed that there was a significant decrease at the end of the start and trial in all aquariums. In group B5, which was not added to the aquarium juice, the bacterial load in water increased at the end of the experiment. With this, it is noteworthy that algae in A4, A5 and B4 occurred in aquariums (Table 2).

When the effect of the product on water quality values in the A and B groups is examined, there are no major differences, as stated by the researchers, while measured in optimal values for fish (Sunithha & Krishna, 2016; al-Kady et al., 2022; Redhwan et al., 2024). In addition, when the bacterial load in the water in the essay aquariums was examined, the values in group B were higher than those in group A. The fact that the product added to water and eating is effective in reducing the bacterial load of water is consistent with the results of the previous studies (Sunithha & Krishna, 2016; Gamoori et al., 2022; Redhwan et al., 2024).

Development of Gut Tissue

The experimental findings of this study demonstrated that the probiotic bacteria present in our product contributed to the development of intestinal tissue in trout fry, as previously reported (Bagheri et al., 2008; Kristiansen et al., 2011; Asha et al., 2024).

Table 2. Findings of the experiments in groups A and B

	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Feed probiotic addition	X	X	X	X	X	-	-	-	-	-
Bathroom application	D1	D2	D3	D4	-	D1	D2	D3	D4	-
Survival rate (%)	94	86	85	86	88	86	85	84	83	79
FCR	0.8	1,04	1.2	1	0.9	1.13	1.33	1.2	1	1.4
Water quality values										
Water temperature (°C)	17-18	17.1-7.8	17.2-7.8	17.4	17-17.5	17.1	16.9-17.9	17-18	17-18	16.8-17
Oxygen in water (mg/L)	4.9-6.24	5.35-.22	4.8-6.44	5.06-5.91	5.34-6.24	5.72-6	4.9-6.2	4.77-6.11	5.4-6.1	4.72-5.7
pH	7.2-7.6	7.4	7.4	7.4-7.6	7.2-7.6	7.4	7.4	7.4-7.6	7.2-7.6	7.2-7.6
Ammonia (mg/L)	0.5-1	1-2.5	2-5	2.5-7	0.1-0.5	0.5-5	1-5	1-5	0,5-5	5-7
Phosphate (mg/L)	1-5	1-5	2-5	2-7	1-5	1-5	1-5	1-5	2-7	1-5
Nitrate (mg/L)	0	0	0	0	0	0	0	0	0	0
Salinity	0	0	1	3	0	0	0	1	3	0
Algae in water	-	-	-	+	+	-	-	-	+	-
Bacterial load in water (cfu/mL^{-1})	$2x10^6$ — $1x10^3$	$1.9x10^6$ — $2.1x10^4$	$1.2x10^6$ — $2x10^4$	$1.1x10^6$ — $1.6x10^4$	$2.8x10^6$ — $2x10^4$	$2.6x10^6$ — $2x10^4$	$1x10^6$ — $3,1x10^4$	$1.7x10^6$ — $5x10^4$	$3.7x10^6$ — $4 x10^4$	$3x10^6$ — $2.6x10^6$

Differences have been observed in the appearance of tissues obtained as a result of histological studies on the gut tissue of the trout cubs fed by regular feed (N) and commercial product added (F) (Figure 2). Intestinal samples from 5 fish in each group were examined. Approximately 18 villus were seen in the intestinal cross -section of the fish in the N group. At the same time, their length towards the lumen was approximately 100 µm, and it was measured as 100 µm in the thickness of the outer round muscle layer of the gut tissue. It is noteworthy that the villus numbers of the fish in the commercial product (F) have increased to 25, the villus length was generally more than 100 µm, and the outer muscle layer became thicker than the other group.

Researchers reported that different feed additives (enzymes, prebiotics, probiotics) contribute to the healthy development of fish larvae, causing changes in the villi in the intestines due to the increase in absorption ability (Kristiansen et al., 2022; El-Kady et al., 2024). In the histological study we have made, the increase in the number and length of the villi of the fish fed with the product enriched with the product is beneficial in the digestion of the enzymes in the product.

Increasing Fish Health

As a result of the experimental infection study, the disease occurred 2 days after the *A. hydrophila* bacteria were given to fish by injection method, and fish deaths started accordingly. Experimental groups and data obtained within the scope of the 30-day study are shown in Table 3. At the end of the study, the product had a preventive and therapeutic effect against experimental infection with *A. hydrophila*. As in the h1 group, the most effective application in the treatment of the disease was obtained as a result of the bath applications performed in fish fed before the disease (min. 15 days ago) and in the stage after the start of the disease. *A. hydrophila* is a bacterium that lives in freshwater fish and is found in the intestinal flora of the fish. The stress of the fish due to adverse environmental conditions causes the disease. Similar to other studies, in our experimental infection study, it has been shown that the product is effective in the prevention and treatment of bacterial diseases in celestial trout (Fečkaninová et al., 2017; Simon et al., 2021).

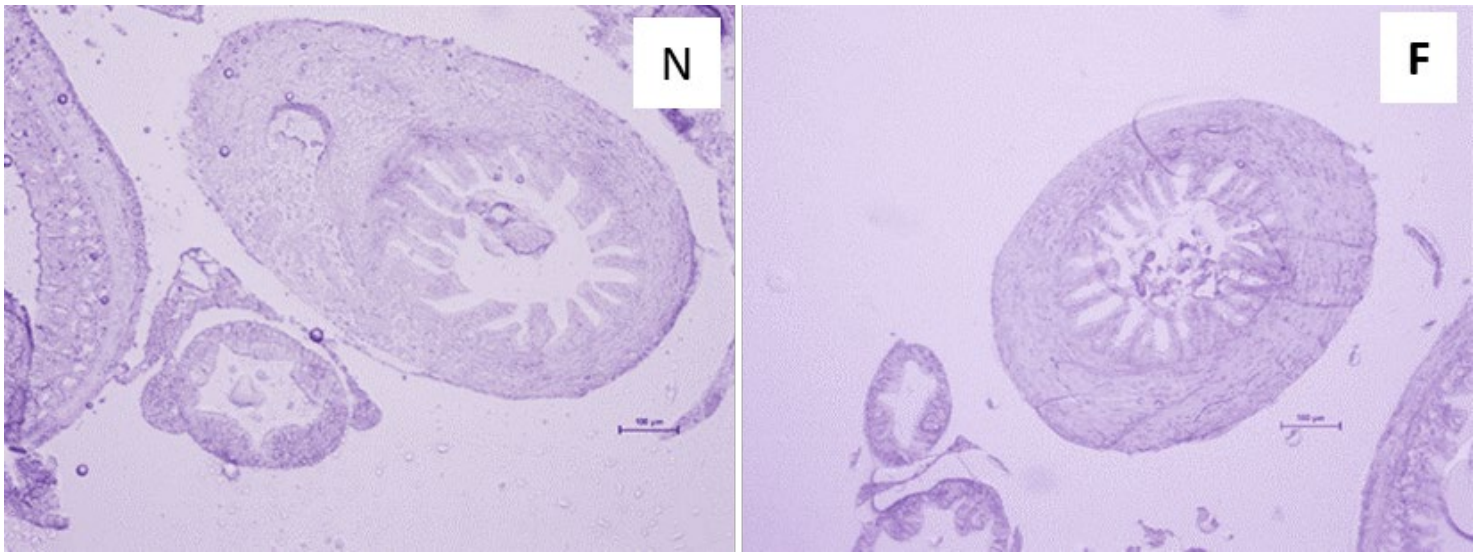

Figure 2. Appearance of intestinal sections of fish fed regular feed (N) and commercial product (F) (H&E)

Table 3. Findings on infected experimental groups with *A. hydrophila*

	h1	h2	h3	h4
Number of fish	35	35	35	35
The probiotic added before the disease feeding with feed	+	-	-	-
Eating probiotics after the development of the disease	-	+	-	-
Nutrition with normal feed after disease	+	-	+	+
Short-term bathroom application (2 times)	-	-	+	-
Number of dead fish	2	5	3	30
Ratio of survivors (%)	95	86	92	14

Conclusion

Probiotics are preferred, environmentally friendly products for increasing fish welfare and feed efficiency in sustainable aquaculture cultivation, and to protect fish health. It shows that commercial probiotics developed by adding enzymes, similar to today's biotechnological developments, are much more effective in increasing the health of fish, the immune system and production performance (Assan et al., 2022; Amenyogbe et al., 2024; Asha et al., 2024).

As a result of this study, the effects of adding the candidate biotechnological product (Enzyfish) considered for commercialisation or applying a bathing method to the water were tested on the growth and health of the fish. With the results obtained from this study, it was aimed to contribute to the development of the cultural fisheries sector in our country, as well as to shed light on the domestic studies to be carried out in the following years.

Compliance with Ethical Standards

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

Ethics committee approval: The local ethics committee report was received at İstanbul University, Animal Experiment Local Ethic Committee Report at 2015/76 report number and 05.011.2015 report date.

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

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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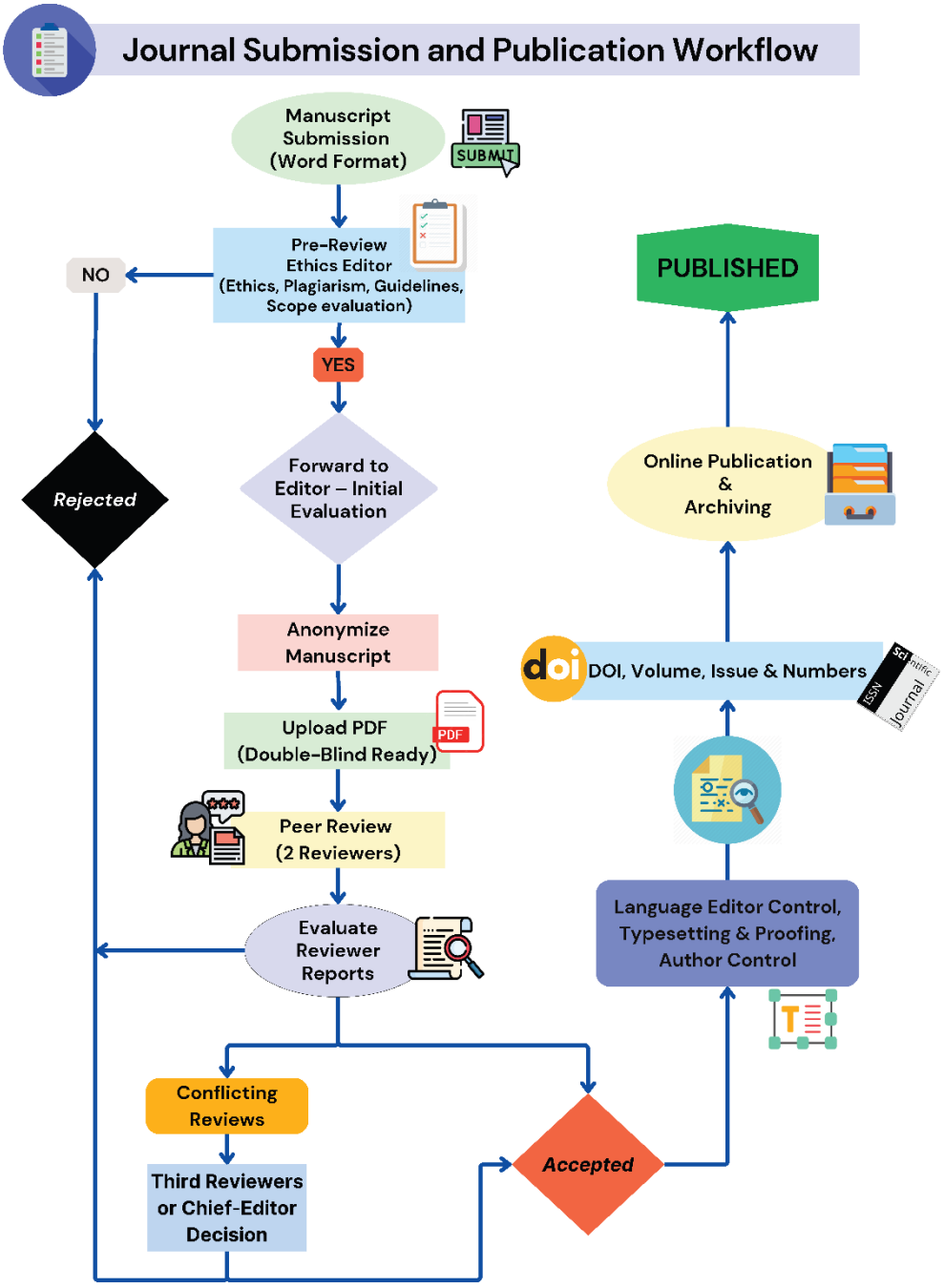

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Publications must be original, and appropriate attribution must be made when quoting other sources. In our journal, plagiarism is considered a serious crime. For this reason, all articles submitted to the "Aquatic Research" journal must undergo a preliminary 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**)

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.

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.