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RESEARCH ARTICLE

Evaluating the Ecological Integrity of a Tropical Lake Using Community Degradation Index

Samuel K.K. Amponsah*

University of Energy and Natural Resources

<https://orcid.org/0000-0001-5559-3139>

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Abstract: This study evaluated the ecological integrity of Stratum VII (Yeji sector) of Lake Volta, Ghana, using fish-based indicators, specifically diversity indices and the Community Degradation Index (CDI). Fish samples were collected from four fishing communities, namely; Fante Akura, Brekente, Tonka, and Vutideke between January and December, 2024. A total of 41 species belonging to 13 families were recorded, with *Chrysichthys walkeri* (39.46%), *Chrysichthys nigrodigitatus* (13.76%), and *Schilbe mystus* (13.12%) as the most dominant species, reflecting the resilience of opportunistic taxa. Diversity analysis showed healthy ecological environment at Stratum VII, with Shannon–Wiener index values ranging from 1.98 (Fante Akura) to 3.27 (Vutideke). The CDI values ranged from 1.10 (Vutideke) to 5.29 (Fante Akura), suggesting that despite existing anthropogenic stress, Stratum VII has not yet reached critical degradation level. Generally, CDI provided a more robust assessment by accounting for trophic interactions and species resilience, offering deeper insights into ecosystem degradation. Therefore, this study emphasizes the importance of integrating functional indices with conventional diversity measures in monitoring frameworks to inform sustainable management of Lake Volta and similar inland freshwater ecosystems in Ghana.

Anahtar kelimeler:

Volta gölü
Gana
Shannon–Wiener endeksi
Tür kompozisyonu
Tatlısu balıkları

Topluluk Bozulma İndeksi Kullanarak Tropikal Bir Gölün Ekolojik Bütünlüğünün Değerlendirilmesi

Öz: Bu çalışmada, Volta Gölü'nün Stratum VII (Yeji sektörü) bölgesinin ekolojik bütünlüğü, özellikle çeşitlilik indeksleri ve Topluluk Bozulma İndeksi (CDI) gibi balık-temelli göstergeler kullanarak değerlendirilmiştir. Balık örnekleri, Ocak–Aralık 2024 tarihleri arasında dört bölgeden, Fante Akura, Brekente, Tonka ve Vutideke toplanmıştır. Toplamda 13 familyaya ait 41 tür kaydedilmiş olup, fırsatçı taksonların dayanıklılığını yansıtabilecek şekilde *Chrysichthys walkeri* (%39,46), *Chrysichthys nigrodigitatus* (%13,76) ve *Schilbe mystus* (%13,12) türleri en baskın türler olarak belirlenmiştir. Çeşitlilik analizleri, stratum VII'de sağlıklı bir ekolojik ortam olduğunu göstermiş olup, Shannon–Wiener indeksi değerlerinin Fante Akura'da 1.98 Vutideke'de 3.27 arasında değiştiğini göstermiştir. Vutideke'de 1,10 ile Fante Akura'da 5,29 arasında değişen CDI değerleri halihazırda antropojenik stresi işaret etmekle beraber, stratum VII kritik bozulma eşiğine henüz ulaşmamıştır. CDI, trofik etkileşimleri ve türlerin dayanıklılığını da hesaba katarak daha kapsamlı bir değerlendirme sunarak ekosistem bozulmasına dair daha derinlemesine içgörüler sağlamıştır. Bu nedenle bu çalışma, Gana'daki Volta Gölü ve benzer içsu ekosistemlerinin sürdürülebilir yönetimini desteklemek amacıyla, izleme çalışmaları kapsamında işlevsel indekslerin geleneksel çeşitlilik ölçütleriyle birlikte kullanılmasının önemini vurgulamaktadır.

Introduction

The impacts of human activities on water bodies require appropriate monitoring tools to facilitate detection and characterization of the causes and sources of chemical, physical and biological impairment of the aquatic habitats. Among these tools, aquatic biota is identified as potential bioindicators to detect pollutant loads in water (Muyibi et al., 2008). Bioindicators are organisms or biological responses that reveal the health of an ecosystem. In aquatic environments, fish, macroinvertebrates, algae, and aquatic plants are commonly used to detect pollution, habitat degradation, and hydrological alterations (Olsen et al., 2021; Badu, 2012). Their presence, absence, or abundance

reflects cumulative environmental conditions over time, making them more integrative than snapshot chemical tests (Holt and Miller, 2010). Aquatic ecosystems are intricate and vital environments characterized by the interaction of living organisms with non-living components like water chemistry and temperature (Gopal, 2020). These systems are crucial not only for biodiversity conservation but also for sustaining vital ecological services. Despite their importance, they are increasingly degraded by human-induced factors such as chemical pollution, physical habitat alteration, overfishing, and changing climate

*Corresponding author: samuelamponsah09@gmail.com

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patterns and also mining activities (Tickner et al., 2020; Pinna et al., 2023).

Amidst this degradation, fish are widely recognized as effective ecological indicators, offering valuable insights into the health of aquatic ecosystems (Deere, 2021; Pinna et al., 2023). Alterations in fish diversity and abundance, often reflect underlying stressors such as pollution or hydrological disturbances (Okwuosa et al., 2019). To assess these roles quantitatively, several fish-based indices including Index of Biotic Integrity (IBI), Fish Assemblage Integrity Index (FAII), Community Degradation Index (CDI), among others have been developed (e.g. Karr, 1999). These metrics aid in evaluating the ecological state of freshwater systems.

In Ghana, diversity indices including species richness, evenness and Shannon Weiner indices have been widely applied in assessing the ecological health of ecosystem (Nero, 2021). However, indices that incorporates the trophic interaction of fishes in assessing the ecological integrity of aquatic systems in Ghana are highly nonexistent. Despite the known importance of aquatic biodiversity, ecological monitoring in Ghana largely relies on basic diversity measures, often omitting trophic-based and functional metrics such as the CDI. This leads to inadequate understanding of ecological processes and insufficient tools to guide sustainable management. This study contributed to ecosystem assessment of Lake Volta in Ghana by applying a suite of fish-based indicators, including diversity metrics and the CDI, to offer a more holistic evaluation of ecosystem health. The findings from the study informed data-driven conservation and management strategies tailored to the unique dynamics of

Stratum VII of Lake Volta. To obtain an insight into estimating the ecological health of the lake for increased production and management purposes, CDI was used to estimate the level of degradation of the lake, while Shannon Weiner index was also used to estimate the richness and evenness of the lake. In light of the above, this study aimed to assess the ecological integrity of Stratum VII (Yeji Sector) of Lake Volta, Ghana using fish-based indices to ensure sustainable management of the freshwater ecosystems of the lake.

Material and Methods

Study Area

Lake Volta is the largest man-made lake in Ghana, stratified into eight strata for effective management (Ghansah et al., 2016). From the eight strata, the study focused on Stratum VII which is centered around Yeji. At Yeji, four landing communities within the Stratum VII of the Lake Volta, which lies between longitude 0°10' and 1°05'W and latitude 8°8' and 8°20'N, and extends 60 km south and 50 km north of Yeji, were selected. These communities were Tonka, Vutideke, Brekente and Fante Akura which are all landing sites within the Stratum VII of Lake Volta (Figure 1). Yeji is the capital of Pru District in the Brong-Ahafo region with a population of 28,515 (GSS, 2014). Selection of these sampling inland fishing communities was based on two-stage stratified sampling criteria, including geographical isolation and the level of fishing activities based on the number of active fishing boats.

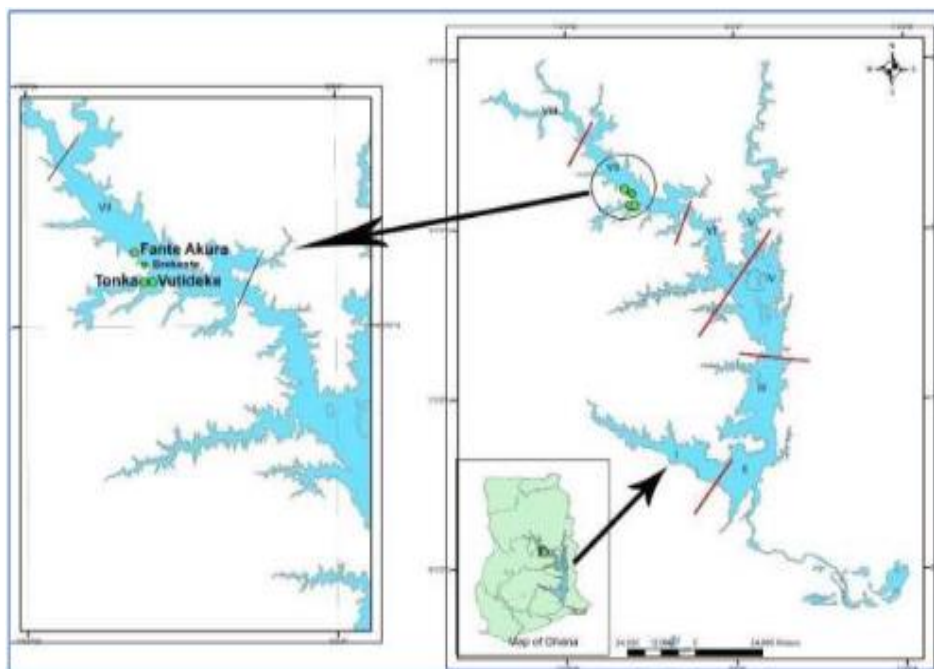


Figure 1. Map of study areas within Stratum VII of the Lake Volta

Data collection

Fish samples were obtained from the commercial catches of random small-scale fishermen between January and December, 2024. The main fishing gears used at the sampling locations were set nets, traps as well as hook and line. Fish samples obtained from these fishermen were sorted in-situ and identified to the species level using Dankwa et al. (1999) identification keys.

Methods

Relative abundance

This indicates how rare or common a species is relative to other species in a defined location. This was expressed in percentage, using the expression below (Oliveira et al., 2003):

$$\frac{\text{Number of individuals of species}}{\text{Total number of individuals}} \times 100$$

Diversity indices

Shannon–Wiener Index (H') which considers both the number of species and the distribution of individuals among species was calculated following the procedure by Shannon & Wiener, (1963). Shannon Wiener index ranges from 1.5 to 3.5 for healthy ecological environment (Magurran, 2004).

Development of the Community Degradation Index

Comparison among communities

In order to establish the magnitude of degradation at a given site, the "observed" and "potential" species composition must be compared. An accepted ecological method for making comparisons is the use of a similarity index. While many similarity indices have been developed and used by ecologists, one simple and commonly used index, developed by Jaccard, has been in use for over 50 years (Ellenberg and Mueller-Dombois, 1974). It is expressed as

$$J = \frac{C}{T}$$

where 'C' is the number of species common to two assemblages and T is the total number of different species contained within the two.

The value of 'J' will range between 0 and 1, where 1 represent identical species lists, and 0 indicates no species in common. In this application, the observed species assemblage will always be a subset of the potential assemblage. If the number of species in the observed assemblage is defined as "O", and the number in the potential list is P, then $O = C$ and $P = T$, and equation (1) becomes

$$J = \frac{O}{P}$$

This provides a measure of similarity between the observed and potential assemblages.

Dissimilarity calculation

Jaccard's index provides a measure of similarity between the above assemblages. What is really required, however, is a measure of dissimilarity. This is readily accomplished by subtracting the above result from unity, a procedure commonly employed in community ordination techniques. Thus, dissimilarity (D) is given as:

$$D = 1 - J$$

Consequently, a factor must be incorporated in the degradation measure (D) which relates the potential richness at a site (P), to the maximum potential richness of any site within the area of study (Pmax). In practice, the potential richness (P) of each biological segment is determined, and Pmax is the highest individual value of P available from all the biological segments within the area of study. The most straightforward manner of incorporating this richness factor into the index is $P / P_{\max}$. However, this simple relationship does not correctly express the appropriate relationship between community structure and species richness. From a theoretical basis, information content of a community is instead directly related to the logarithm of species richness. Thus, information content is directly related to the logarithm of richness, and the factor can better be represented as

$$\frac{\log P}{\log P_{\max}}$$

The relationship then becomes: $D = (1 - J) \{\log P / \log P_{\max}\}$

While the degradation measure (D), defined in (5) above, always lies in the range $0 < D < 1$, we have in practice defined the community degradation index (CDI) as $10 \times D$. The CDI thus ranges from 0 (no degradation) to 10 (totally degraded site).

Data analysis

Descriptive statistics was used to provide the mean, minimum and maximum information of the composition of the species and diversity indices from the sampling locations. Kruskal-Wallis test was used in identifying significant difference in species composition at a confidence level of 95 % ($p < 0.05$). Paleontological statistical tool (PAST) version 5.0 was used to determine the Shannon-Wiener index and also, perform the Kruskal-Wallis test.

Results

Species composition

Overall, forty-one (41) species belonging to sixteen (16) families were recorded in all four sampling locations (Table 1). *Chrysichthys walkeri* was the most abundant species, making up 5289 out of 13402 individuals, representing 39.46%. It was followed by *Chrysichthys nigrodigitatus* (13.76%) and *Schilbe mystus* (13.12%). All the three top species were semi pelagic omnivorous fish belonging to the families, Claroteidae for the top two and Schilbeidae for the third highest which was also the most

abundant families in all the landing sites. *Petrocephalus bane*, *Citharinus citharus*, and *Synodontis arnoulti* were the least abundant species.

Table 1 shows the composition of species and families from the sampling locations. At Fante Akura, 11 species were recorded with *Chrysichthys walkeri* as the dominant species and *Labeo coubie* as the least represented species. At Brekente, 26 species were recorded with *Chrysichthys walkeri* as the dominant species and *Synodontis arnoulti* as

the least represented species. At Vutideke, 30 species were recorded with *Schilbe mystus* as the dominant species and *Citharinus citharus* as the least represented species. At Tonka, 28 species were recorded with *Chrysichthys walkeri* as the dominant species and *Synodontis clarias* as the least represented species. Kruskal- Wallis test showed significant difference in the abundance of species obtained from the four sampling locations ($H = 15.15$, $p\text{-value} = 0.001$).

Table 1. Species composition from the sampling locations

Species	Family	Brekente	Fante Akura	Tonka	Vutideke	Total	Relative abundance (%)
<i>Chrysichthys walkeri</i>	Claroteidae	2627	1138	1271	253	5289	39.46
<i>Chrysichthys nigrodigitatus</i>	Claroteidae	864	279	605	96	1844	13.76
<i>Schilbe mystus</i>	Schilbeidae	751	143	565	299	1758	13.12
<i>Coptodon zillii</i>	Cichlidae	552	288	621	90	1551	11.57
<i>Sarotherodon galilaeus</i>	Cichlidae	262	0	172	45	479	3.57
<i>Oreochromis niloticus</i>	Cichlidae	122	0	184	23	329	2.45
<i>Synodontis sorex</i>	Mochokidae	289	0	4	11	304	2.27
<i>Hemichromis bimaculatus</i>	Cichlidae	0	0	283	2	285	2.13
<i>Chrysichthys auratus</i>	Claroteidae	107	131	20	5	263	1.96
<i>Synodontis ocellifer</i>	Mochokidae	254	0	0	8	262	1.95
<i>Hemichromis fasciatus</i>	Cichlidae	4	0	153	9	166	1.24
<i>Labeo coubie</i>	Cyprinidae	45	4	52	21	122	0.91
<i>Distichodus rostratus</i>	Distichodontidae	44	0	44	2	90	0.67
<i>Synodontis eupterus</i>	Mochokidae	13	0	65	2	80	0.60
<i>Brycinus leuciscus</i>	Alestidae	0	0	74	2	76	0.57
<i>Synodontis clarias</i>	Mochokidae	42	7	1	10	60	0.45
<i>Alestes baremoze</i>	Alestidae	18	11	8	17	54	0.40
<i>Synodontis schall</i>	Mochokidae	25	12	0	8	45	0.34
<i>Bagrus bajad</i>	Bagridae	2	12	8	14	36	0.27
<i>Distichodus engycephalus</i>	Distichodontidae	0	0	24	12	36	0.27
<i>Brycinus nurse</i>	Alestidae	0	0	18	15	33	0.25
<i>Barbus callipterus</i>	Cyprinidae	7	0	22	0	29	0.22
<i>Brycinus macrolepidotus</i>	Alestidae	0	0	27	2	29	0.22
<i>Marcusenius senegalensis</i>	Mormyridae	25	0		4	29	0.22
<i>Clarias gariepinus</i>	Clariidae	4	0	16	2	22	0.16
<i>Bagrus docmak</i>	Bagridae	17	0	4	0	21	0.16
<i>Labeo senegalensis</i>	Cyprinidae	8	0	0	12	20	0.15
<i>Mormyrus macrophthalmus</i>	Mormyridae	0	0	0	16	16	0.12
<i>Mormyrops anguilloides</i>	Mormyridae	12	0	0	2	14	0.10

Table 1 Continued

<i>Penollula leonensis</i>	Clupeidae	0	0	12	0	12	0.09
<i>Hyperopisus bebe</i>	Mormyridae	6	0	0	0	6	0.04
<i>Synodontis batensoda</i>	Mochokidae	0	0	0	6	6	0.04
<i>Synodontis gambiensis</i>	Mochokidae	6	0	0	0	6	0.04
<i>Synodontis violaceus</i>	Mochokidae	0	0	0	6	6	0.04
<i>Marcusenius psittacus</i>	Mormyridae	0	0	5	0	5	0.04
<i>Hemichromis niloticus</i>	Cichlidae	0	0	4	0	4	0.03
<i>Polypterus senegalus</i>	Polypteridae	0	0	4	0	4	0.03
<i>Synodontis macrophthalmus</i>	Mochokidae	0	4	0	0	4	0.03
<i>Petrocephalus bane</i>	Mormyridae	0	0	3	0	3	0.02
<i>Citharinus citarus</i>	Citharinidae	0	0	0	2	2	0.01
<i>Synodontis arnoulti</i>	Mochokidae	2	0	0	0	2	0.01
Total		26	11	28	30	13402	100

Shannon-Weiner index

Shannon-Weiner index for the sampling locations is shown in Figure 2. The Shannon-Weiner index ranged from 1.98 ± 0.13 in Fante Akura to 3.27 ± 0.12 in Vutideke, with Brekente (2.79 ± 0.05) and Tonka (3.21 ± 0.05) being intermediaries. Overall, the mean Shannon-Weiner index recorded during the study period was 3.047 ± 0.034 .

Community degradation index

The Community Degradation index for the four sampling locations is shown in Table 2. The CDI ranged from 1.10 in Vutideke to 5.29 in Fante Akura, with Brekente (1.99) and Tonka (1.54) being intermediaries.

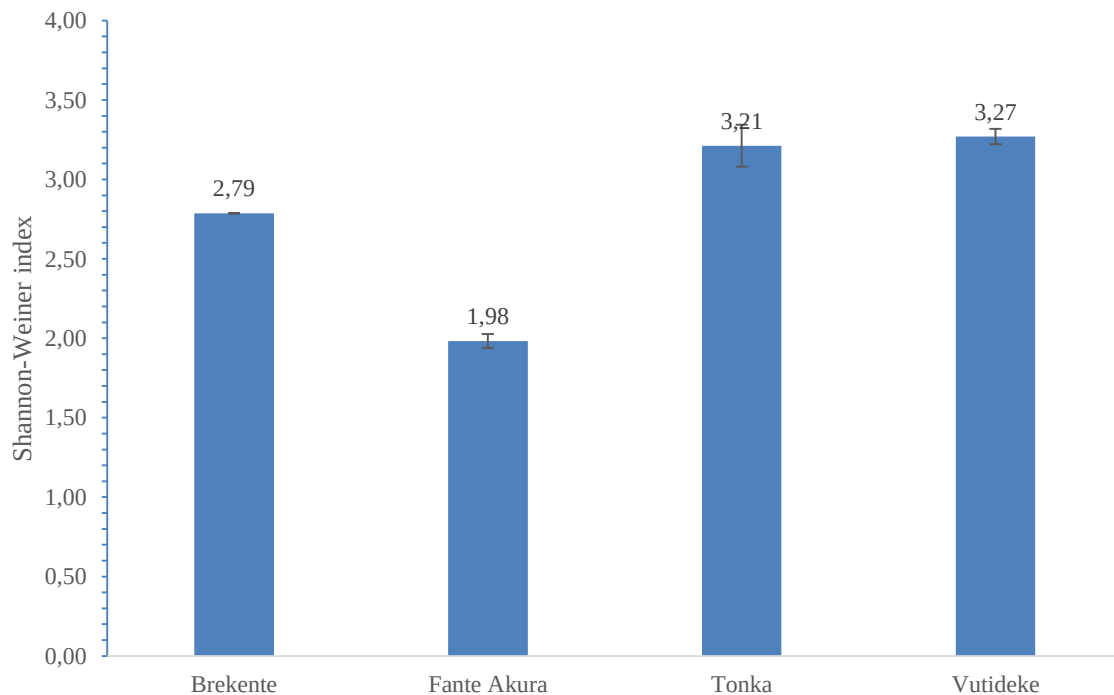


Figure 2. Mean Shannon-Weiner index from the four sampling locations

Table 2: Estimated adjusted degradation and community degradation index for Fante Akura, Brekente, Tonka and Vutideke sampling sites

Location	Adjusted Degradation index	Community Degradation Index
Fante Akura	0.53	5.29
Brekente	0.20	1.99
Tonka	0.15	1.54
Vutideke	0.11	1.10

Discussion

Species Composition

A total of 41 fish species were recorded across the four study sites in Stratum VII of Lake Volta, dominated by *Chrysichthys walkeri*, *Chrysichthys nigrodigitatus* and *Schilbe mystus*. The persistence of *Chrysichthys* spp. as dominant taxa was consistent with long-term studies on Lake Volta. For instance, Braimah (2003) reported that catfishes (*Chrysichthys* spp.) and tilapias (*Oreochromis* and *Sarotherodon* spp.) together constituted more than 70% of inland fishery landings. Similarly, Mensah et al. (2019) observed that artisanal catches in Yeji and surrounding fishing communities were dominated by tilapias and catfishes, confirming their ecological and economic importance in the Volta basin. The dominance of *Chrysichthys* spp. may be attributed to their omnivorous feeding strategies and ecological plasticity. Amaeze et al. (2012) identified *Chrysichthys nigrodigitatus* as resilient and commercially important species due to its tolerance to hypoxic and turbid environments. This adaptability likely explains its dominance in Stratum VII, particularly in habitats impacted by high fishing pressure and nutrient runoff from adjacent farmlands. In contrast, species such as *Bagrus docmak*, *Labeo coubie*, and *Marcusenius senegalensis* were rare (<1% of total catch). Agyapong (2021) noted that these large-bodied benthic feeders and slow-reproducing species in Lake Volta are under high fishing pressure due to their high market value, leading to population decline. This observation was in line with findings by Wilson (2024), who linked the scarcity of such species to selective exploitation and habitat alteration caused by shoreline development and siltation from catchment activities. Ecologically, the presence of 41 species indicates moderate richness while the dominance of resilient taxa suggests incipient homogenization of fish communities.

Diversity Indices

Shannon-Wiener diversity index is a widely used diversity index that centers on species abundance and evenness in a community. It is also an indicator of pollution, without significant variation in its values (Farukuzzaman et al., 2023). Shannon-Wiener values from Tonka and Vutideke were higher than estimates from Berekente and Fante Akura which may suggest higher species diversity at Vutideke and Tonka (Islam et al.

2023). The higher species diversity could be linked to less fishing activities as a result of recurring floods during rainy seasons. Comparatively, Shannon-Wiener index recorded from the study was relatively higher than the values reported by Mensah et al. (2019) from Stratum III of Lake Volta. The observed variation may be due to changes in biophysical conditions, and habitats adaptations (Poff and Allan, 1995, Grenouillet et al., 2002). Ecologically, Magurran (2004) stated that for healthy environment, the range of Shannon-Weiner index spanned from 1.5 to 3.5. This suggest that the aquatic environment of Stratum VII may be ecologically sound (Pathak and Naresh, 2021). According to Wilhm and Dorris (1966), Shannon-Wiener index greater than 3.0 indicates clean water, a value between 1.0 – 3.0 suggests moderate pollution and a value below 1.0 indicates heavy pollution. This implies that, inland waters around Tonka and Vutideke are clean waters while Berekente and Fante Akura are characterised by moderately polluted waters. The presence of moderate pollution at Fante Akura and Berekente could be due to high population as well as intense fishing and boating activities (Islam et al., 2013). The moderate pollution at these fishing communities may degenerate to heavy pollution over time if proper measures are not instituted. Therefore, routine monitoring of water quality parameters of Stratum VII is needed which will allow for prompt interventions and management measures to protect commercially important species and dependent livelihoods.

Community Degradation Index (CDI)

Nero (2021) cautioned that diversity-based metrics alone underestimate ecosystem degradation in freshwater systems, and recommended complementary indices that incorporate trophic and functional dynamics such as the Community Degradation Index (CDI). To date, CDI has rarely been applied in Ghanaian inland waters. Most studies, such as Mensah et al. (2019) and Nero (2021), have relied on diversity-based metrics. However, recent studies in Ghana have underscored the importance of trophic-based assessments. For instance, Amponsah (2024) applied fish assemblage responses to assess ecological change during the Tema port expansion. Similarly, the present study, by applying CDI, therefore provides a more holistic assessment of Lake Volta's ecological integrity. The low CDI values recorded from the study may indicate healthy ecosystem at Vutideke, Tonka and Berekente.

However, the ecological degradation of inland waters at Fante Akura evinced by moderate CDI may indicate a semi degraded area. The semi degraded locations may be due to intense anthropogenic activities such as pollution from water transport, use of illegal fishing activities and high fishing pressure (Badu et al., 2019; de-Graaf and Ofori-Danson, 1997). The estimated CDI values may imply that while some sites in Stratum VII remain relatively intact, others show some degree of ecological degradation. Comparatively, the CDI values in Stratum VII were lower than values estimated by Ramm (1990), who reported CDI values up to 8.2 in severely degraded estuaries in South Africa. This suggests that stratum VII of Lake Volta, while under stress, has not yet reached critical degradation levels.

Conclusion

This study assessed the ecological integrity of Stratum VII of Lake Volta using fish-based indices, namely species composition, diversity metrics, and the Community Degradation Index (CDI). A total of 41 fish species were recorded, dominated by resilient taxa such as *Chrysichthys walkeri*, *Chrysichthys nigrodigitatus*, and *Schilbe mystus*. Shannon–Wiener values ranged from 1.98 to 3.27, implying relatively healthy ecological environment of Stratum VII. CDI values spanned from 1.10 to 5.29, suggesting semi degradation at Fante Akura. As part of recommendations, further studies should incorporate the Community Degradation Index (CDI) in ecosystem assessment to provide a more comprehensive assessment of ecosystem integrity of Lake Volta, Ghana. This will ensure sustainable management of the fisheries resources along the Lake Volta, Ghana.

Conflicts of Interest

The author declares no conflicts of interest.

Ethics Approval

Ethics committee approval is not necessary for this study.

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RESEARCH ARTICLE

Comparative Study of the Fixed Lift Net Fisheries in Wiritasi and Sarang Tiung, Indonesia

Ahmadi Ahmadi^{1*}, Muhammad Nasir², Rajat Anhari Saputra³

¹Department of Capture Fishery, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarbaru, Indonesia

²Department of Capture Fishery, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarbaru, Indonesia

³Department of Capture Fishery, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarbaru, Indonesia

<https://orcid.org/0000-0001-9691-9107>

<https://orcid.org/0009-0003-6707-3538>

<https://orcid.org/0009-0003-1833-165X>

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Abstract: This study compares the structural and operational characteristics of the fixed lift net fisheries in two fishing villages: Wiritasi (Tanah Bumbu) and Sarang Tiung (Kotabaru) in South Kalimantan, Indonesia. Field observations and qualitative interviews were conducted to document gear construction and fishing operations, while quantitative catch data were collected to analyze catch composition. The results revealed notable differences in material selection, CPUE, and catch composition between two sites. Wiritasi used lighter materials like bamboo and aniong wood for ease and efficiency, while Sarang Tiung's gear incorporated mangrove timber for durability in deeper waters. Wiritasi exhibited a higher mean CPUE (12.83 kg/haul) than Sarang Tiung (8.46 kg/haul), with the difference being statistically significant based on an independent samples t-test ($p < 0.05$). Catch composition also differed between sites and was dominated by small pelagic species, particularly *Sardinella fimbriata*, *Selaroides leptolepis*, and *Stolephorus* sp. Collectively, these findings are consistent with a possible trade-off between energy consumption and catch optimization, underscoring the relevance of cost-effective lighting strategies in small-scale lift-net fisheries. The use of LED lamps in Sarang Tiung highlights economic benefits and environmental considerations, while Wiritasi's mercury lamps demonstrate the need for sustainable transition. The key recommendations for local fishermen are also discussed.

Anahtar kelimeler:

Bagan tancap
Uygun maliyetli aydınlatma
Balık tutma verimliliği
Ekipman performansı
Sürdürülebilirlik

Wiritasi ve Sarang Tiung (Endonezya)'da Sabit Kaldırma Ağı Balıkçılığının Karşılaştırmalı Çalışması

Öz: Bu çalışma, Endonezya'nın Güney Kalimantan bölgesinde yer alan iki balıkçı köyünde—Wiritasi (Tanah Bumbu) ve Sarang Tiung (Kotabaru)—sabit kaldırma ağı (fixed lift net) balıkçılığının yapısal ve operasyonel özelliklerini karşılaştırmaktadır. Ağ yapısı ve balıkçılık faaliyetlerini belgelemek amacıyla arazi gözlemleri ve nitel görüşmeler gerçekleştirilmiş; av kompozisyonunu analiz etmek için nicel av verileri toplanmıştır. Bulgular, iki saha arasında kullanılan malzemeler, birim çaba başına av (CPUE) ve av verimliliği açısından belirgin farklılıklar olduğunu ortaya koymuştur. Wiritasi'de bambu ve aniong ağacı gibi daha hafif malzemeler kullanım kolaylığı ve verimlilik sağlarken, Sarang Tiung'da daha derin sulara dayanıklılığı artırmak amacıyla mangrov kerestesi tercih edilmiştir. Wiritasi'de ortalama CPUE (12,83 kg/çekim), Sarang Tiung'a (8,46 kg/çekim) kıyasla daha yüksek bulunmuş ve bu fark bağımsız örneklem t-testi sonucuna göre istatistiksel olarak anlamlıdır ($p < 0,05$). Av kompozisyonu da sahalar arasında farklılık göstermiş olup, başlıca küçük pelajik türler olan *Sardinella fimbriata*, *Selaroides leptolepis* ve *Stolephorus* sp. baskın türler olarak belirlenmiştir. Bu bulgular birlikte değerlendirildiğinde, enerji tüketimi ile av optimizasyonu arasında olası bir denge (trade-off) bulunduğu işaret etmekte ve küçük ölçekli kaldırma ağı balıkçılığında maliyet-etkin aydınlatma stratejilerinin önemini vurgulamaktadır. Sarang Tiung'da LED lambaların kullanımı ekonomik avantajlar ve çevresel hususları öne çıkarırken, Wiritasi'de kullanılan cıva lambaları sürdürülebilir bir geçiş ihtiyacını ortaya koymaktadır. Ayrıca yerel balıkçılar için temel yönetim ve uygulama önerileri de tartışılmaktadır.

Introduction

The fixed lift nets, locally known as *bagean tancap*, are an essential fishing method widely used along Indonesia's coastal waters. These passive fishing gears rely on light attraction to aggregate pelagic fish near the surface during night time operations (Puspito et al., 2015; Fatmawati et al., 2020). Originating from South Sulawesi, the technology spread throughout Indonesia following cultural and trade exchanges among fishing communities

(Sudirman et al., 2019). The lift net technique provides an efficient yet low-cost fishing approach suitable for artisanal fishers, contributing significantly to local livelihoods and food security (Krisnafi et al., 2020).

Despite widespread application, the technical design and efficiency of stationary lift nets vary across regions due to local adaptations in construction materials, water depth, and target species (Silitonga et al., 2014). Previous

*Corresponding author: ahmadi@ulm.ac.id

studies have shown that frame strength, light intensity, and mesh size strongly influence catch composition and total yield (Sudirman et al., 2020; Alamsah et al., 2021). However, comparative analyses across regions remain limited, particularly for South Kalimantan, where variations in environmental and socio-economic contexts may lead to differing fishing outcomes.

The fixed lift net fishery is a vital component of Indonesia's small-scale fishing sector, which supports regional food availability (FAO, 2024). As coastal fisheries face growing pressures from overfishing, habitat degradation, and climate change, sustainable fishing technologies have become increasingly important (Mardiyani et al., 2020). The fixed lift nets rely on minimal fuel consumption compared to mobile fishing gears such as trawls or purse seines, positioning them as an eco-friendly option (Riyanto et al., 2017). Moreover, their stationary nature enables consistent fish aggregation, allowing for manageable exploitation and long-term planning by local fishers (Muna et al., 2023).

Technological innovation in light fishing, particularly through the use of LED lamps, has significantly transformed stationary lift net operations across Indonesia. LED systems reduce operational costs, increase light intensity efficiency, and lower emissions compared to traditional kerosene lamps or compact fluorescent lamps (Fauziyah et al., 2021; Musbir et al., 2021). However, the ecological effects of light-based attraction, such as species selectivity and behavioural changes among pelagic fish, remain an area of active research (Susanto et al., 2017; Nakiyende et al., 2025). In broader fisheries research, understanding how light configurations and environmental variables influence catch performance is, therefore, crucial for optimizing fishing efficiency without compromising ecosystem health (Solomon and Ahmed, 2016).

From a socio-economic perspective, stationary lift nets play a strategic role in supporting rural coastal economies. Many fishing households depend on the lift net operations as their primary source of income, employing family members and local workers in fishing, net maintenance, and post-harvest processing (Nasution et al., 2023). In regions such as Tanah Bumbu and Kotabaru, the development of fixed lift nets has contributed not only to employment generation but also to community cohesion and intergenerational knowledge transfer.

Nevertheless, differences in access to capital, materials, and supporting infrastructure often result in technological disparities among fishers, affecting catch rate and long-term viability (Krisnafi et al., 2020). For example, limited access to durable construction materials, reliable power sources, or energy-efficient lighting systems may constrain some fishers from adopting more efficient fishing technologies, thereby reducing catch stability, operational efficiency, and long-term sustainability of lift-net fisheries.

Environmental conditions also shape lift-net operations and performance. Variations in sea depth, turbidity, salinity, and plankton concentration can significantly influence the abundance and behaviour of target fish

species (Fatmawati et al., 2020). The waters of Tanah Bumbu, characterized by shallower coastal areas with sandy-muddy substrates, provide different ecological dynamics compared to Kotabaru, which has deeper and more oceanic characteristics. These site-specific differences justify a comparative approach to assess how environmental and operational factors affect fishing success and sustainability.

This study aims to comparatively assess the fixed lift net fisheries in Wiritasi and Sarang Tiung fishing villages by examining gear structure, operational characteristics, and catch composition. The findings are expected to inform the development of appropriate fishing technology and management practices tailored to local ecological conditions. By identifying the key determinants of lift-net performance, this study contributes to broader efforts in advancing sustainable small-scale fisheries in Indonesia's coastal regions.

Material and Methods

Study Sites

This study was conducted between September and October 2024 in two coastal fishing villages: Wiritasi and Sarang Tiung, South Kalimantan, Indonesia (Figure 1). Both locations are representative of their active small-scale lift net fisheries, which primarily target small pelagic fish species. Wiritasi is situated along the southeastern coast of Tanah Bumbu Regency (03°42' S, 115°55' E) with shallow coastal waters (3-10 m depth). The seabed consists mainly of muddy-sandy substrates, likely influenced by river outflows, where small pelagic species such as sardine and yellowstripe scads are commonly observed.

In contrast, Sarang Tiung, located on the northern side of Kotabaru Regency (03°37' S, 115°55' E), features relatively deeper coastal waters (10-20 m) and clearer visibility due to lower sediment input. The fishing grounds are more open and directly influenced by oceanic currents from the Makassar Strait, which supports a wider variety of pelagic species such as anchovy, sardine, and cephalopods. The local fishers generally construct larger and sturdier lift nets to withstand stronger currents and deeper mooring conditions compared to those used in Wiritasi.

Both sites are located within a tropical monsoon climate zone, with seawater temperature and salinity conditions generally characterized by warm waters (approximately 27–31 °C) and salinity levels typically ranging from 30 to 34 ppt, based on regional climatological and oceanographic information. The northeast monsoon typically enhances fishing activity from May to November, providing calm sea conditions suitable for stationary operations. The study was conducted in local fishing communities where lift-net operations are widely practiced and typically supported by family labor and long-established fishing knowledge.

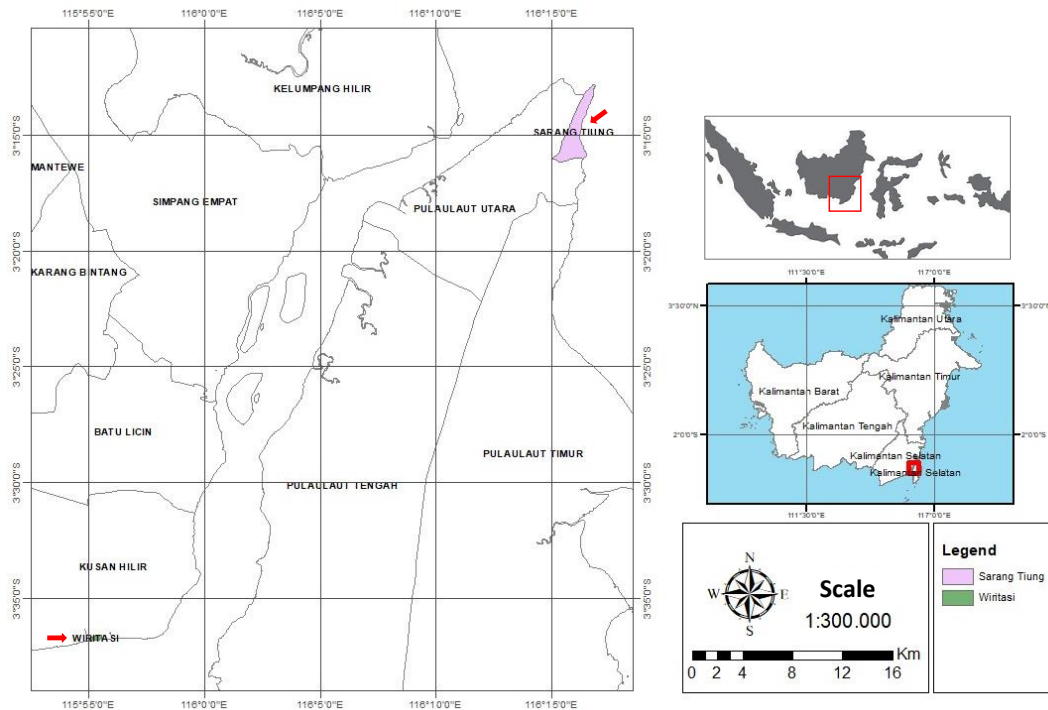


Figure 1. Map of Wiritasi and Sarang Tiung villages, where the fixed lift nets were studied

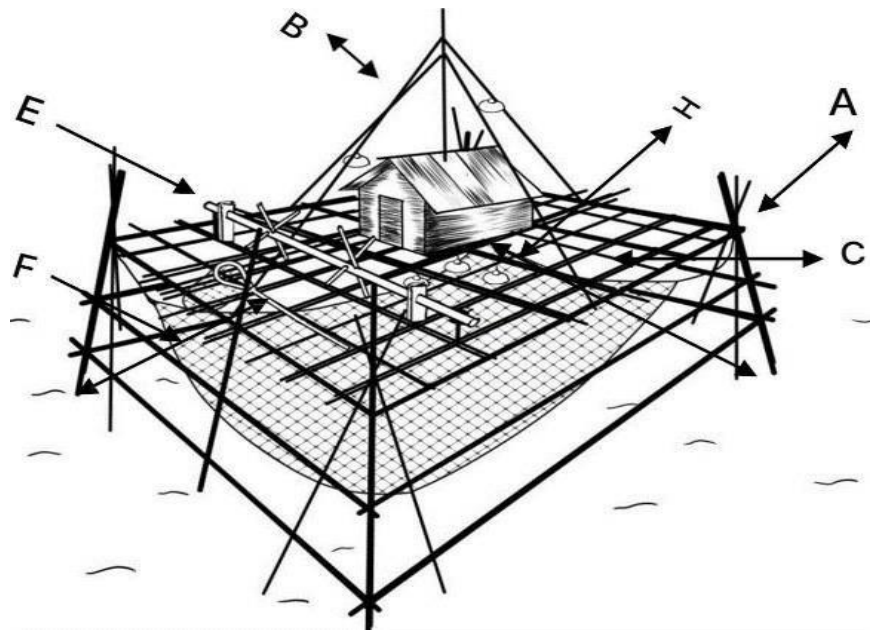


Figure 2. Schematic (not to scale) diagram of the fixed lift-net construction representative of the gear configuration used at both study sites. A. Main pole, B. Bugis pole, C. Deck platform, D. Lift-net house, E. Hauling roller, F. Net, G. Sinkers, H. Lamp

Notes: Bugis pole refers to a locally used secondary support pole to stabilize the net frame and hauling mechanism. Diameter (mm) refers to the characteristic diameter of the primary structural element for each component (e.g., pole diameter for wooden and bamboo

structures, rope diameter for roller rope, and twine diameter for netting). Number (n) indicates the total amount of each component. Each sinker weighs approximately 10 kg.

Table 1. Gear specification of the fixed lift net in Wiritasi, Tanah Bumbu Regency

Components	Materials	Length (m)	Width (m)	Diameter (mm)	Number (n)
Main pole	Aniung wood	10	-	120-200	30
Bugis pole	Aniung wood	5	-	80-150	6
Lift-net house	Wooden beam	2.5	2	-	1
Deck platform	Bamboo	15	15	60-90	1
Hauling roller	Iron Wood	10	-	145	1
Roller pole	Aniung wood	4	-	85	2
Roller rope	Polyethylene	30	-	6	12
Sinker	Paving Stone	-	-	-	13
Net	Polyethylene	15	15	4	1

Table 2. Gear specification of the fixed lift net in Sarang Tiung, Kotabaru Regency

Components	Materials	Length (m)	Width (m)	Diameter (mm)	Number (n)
Main poles	Mangrove wood	10-15	-	300-400	24
Bugis poles	Mangrove wood	6	-	150-200	5
Lift-net house	Wooden beam	3	2	-	1
Deck platform	Bamboo	15	15	60-90	1
Hauling roller	Mangrove wood	10	-	150	1
Roller pole	Mangrove wood	4	-	100	2
Roller rope	Polyethylene	40	-	6	8
Sinker	Paving Stone	-	-	-	14
Net	Polyethylene	15	15	4	1

Data Collection

The study employed a mixed-method approach combining direct observation, interviews, and participatory monitoring across six fishing operations at each site. Quantitative data were obtained from measurements of gear dimensions, operational characteristics, and catch composition, while qualitative information was derived from interviews and participatory observations to document fishing practices, operational procedures, and local knowledge.

Field observations recorded the technical specifications of the fixed lift net, including dimensions, materials, net type, and lighting. Wiritasi's lift nets utilize lighter materials like aniung wood and bamboo for shallow water. In contrast, the Sarang Tiung lift net used mangrove timber to enhance structural stability under deeper-water conditions and stronger currents. The core fishing sequence (preparation, light deployment, fish aggregation, hauling, and handling) was generally consistent across

sites, typically commencing around 18:00 and concluding at dawn. This standardized overnight operating period ensured comparable fishing effort among trials and was therefore, used as the basis for CPUE calculation. Both sites have similar net dimensions (15×15 m) but differ in hauling mechanisms, with Wiritasi using ironwood roller and Sarang Tiung employing mangrove wood roller, each adapted to specific environmental conditions.

In terms of lighting system (Figure 3), Wiritasi employed 15 white mercury lamps (38–100 W), while Sarang Tiung utilized 18 white LED lamps (30–100 W), each powered by portable generators (Yakusa 23850 W and Krisbow 1200 W, respectively). The reported wattage ranges reflect differences among individual lamps within each system, whereas the number, type, and configuration of lamps were kept consistent across all fishing operations at each site to ensure comparability of catch performance. The lamps were lowered through a hole in the floor of the lift net house (1×1 m) and positioned at a height of 2–3 m

above sea level. Wiritasi operated 7 miles offshore, necessitating a 1.5-hour trip with a larger 4.5 GT vessel,

whereas Sarang Tiung's closer 5-mile nearshore location required only a 1-hour trip using a smaller 1.3 GT boat.



Figure 3. Lighting system performance and portable generators used in study sites

Catch assessment were conducted for each haul over the six fishing operations. The net was hauled two times per night during each fishing trip (total of 12 hauls per site). The catches were removed from the net using a scoop-net (a 5-mm mesh), sorted and identified by species using the guide by White et al. (2013). Each specimen was then counted, weighed to the nearest 0.1 kg using a calibrated digital scale. The recorded data were standardized and aggregated to derive total and mean catch per trip (kg), providing a quantitative basis for comparative analysis between fishing operations.

Semi-structured interviews were conducted with lift-net fishers at each site to obtain socio-economic and technical information, including fishing experience, material selection, maintenance practices, operational challenges, and perceptions of fish stock changes. The results were cross-checked with field observations.

Statistical Analysis

An independent samples *t*-test was applied to compare catch number and mean CPUE between the two fishing gears, with statistical significance evaluated at the 95% confidence level ($\alpha = 0.05$). The analysis was based on six fishing operations per site, comprising a total of 12 hauls per site (24 hauls in total). Each fishing operation was defined as a single analytical unit, corresponding to a separate night of fishing activity. The *t*-test was considered appropriate given the independence of observations and the comparable variance structure between groups. Prior to hypothesis testing, data normality was examined using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Microsoft Excel was used for data processing and graphical visualization, whereas SPSS (version 18.0; IBM Corp., Armonk, NY, USA) was used for inferential statistical analyses, including independent samples *t*-test. The statistical outcomes were subsequently integrated with the quantitative results to

enable a comprehensive comparison of lift-net fisheries between the two study sites.

Results

Catch composition

The total catch composition from six fishing trips across Wiritasi and Sarang Tiung study sites showed significant site-specific differences in species dominance (Table 3). Wiritasi's total catch reached 154 kg, primarily dominated by *Sardinella fimbriata* (64.29%), and secondarily by *Selaroides leptolepis* (35.71%). Based on direct length measurements, *S. fimbriata* specimens (50–110 mm) were relatively smaller than *S. leptolepis* (80–140 mm). Meanwhile, Sarang Tiung produced 101.5 kg, featuring a more varied composition of *S. fimbriata* (47.29%), *Stolephorus* sp. (37.44%), and *Loligo* sp. (15.27%). The total catch differed between sites, with Wiritasi exceeding Sarang Tiung by 52%. Comparatively, the total catch at Wiritasi was 1.5 times higher than that at Sarang Tiung, and this difference was statistically significant based on an independent samples *t*-test comparing total catch between sites ($p < 0.05$).

CPUE

Wiritasi exhibited a higher CPUE than Sarang Tiung (Table 4). Under a comparable total fishing effort of 12 hauls per site, mean CPUE at Wiritasi was 12.83 kg/haul, compared with 8.46 kg/haul at Sarang Tiung, and this difference was statistically significant based on an independent samples *t*-test ($p < 0.05$). Higher CPUE at Wiritasi was largely attributable to increased catches of *S. fimbriata* and *S. leptolepis*.

Over the six fishing operations, Wiritasi exhibited a wider range of daily catch (0–60 kg), with a mean of 25.67 ± 23.80 kg/trip, whereas Sarang Tiung showed a narrower range (0–24 kg) and a lower mean catch of 16.92 ± 8.73 kg/trip. Each daily fishing trip was treated as

an independent observational unit in the analysis, as catches were obtained from separate nights with no overlap in fishing effort. The highest single-day catch at Wiritasi (60 kg) was primarily composed of *Sardinella*

fimbriata (16.50 ± 16.05 kg/trip) and *Selaroides leptolepis* (9.17 ± 12.81 kg/trip). Zero-catch days coincided with periods of strong currents at both sites (Figure 4).

Table 3. Total catch composition over six fishing trips across Wiritasi and Sarang Tiung

Species	Wiritasi		Sarang Tiung		Total Catch	
	(kg)	(%)	(kg)	(%)	(kg)	(%)
<i>Selaroides leptolepis</i>	55	35.71	0	0	55	21.53
<i>Stolephorus</i> sp.	0	0	38	37.44	38	14.87
<i>Sardinella fimbriata</i>	99	64.29	48	47.29	147	57.53
<i>Loligo</i> sp.	0	0	15.5	15.27	15.5	6.07
Total	154	100	101.5	100	255.5	100

Table 4. Comparative CPUE for the fixed lift nets in Wiritasi and Sarang Tiung

Study Sites	Total Catch (kg)	Total Effort (haul)	CPUE (kg/haul)
Wiritasi	154	12	12.83
Sarang Tiung	101.5	12	8.46

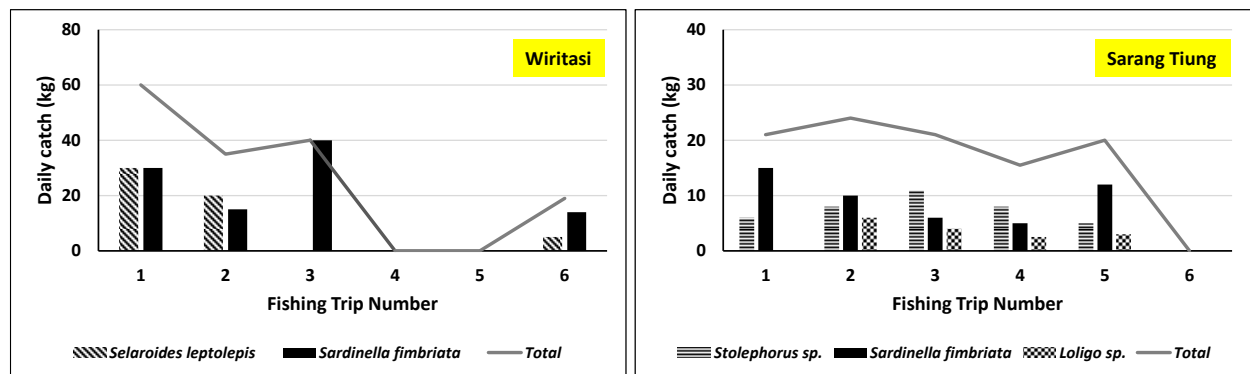


Figure 4. Daily catch trends of the fixed lift nets in Wiritasi and Sarang Tiung. Grey lines represent total daily catch summed across all species

Discussion

The differences in construction and operation of the fixed lift nets in Wiritasi and Sarang Tiung reflect adaptations to local marine environments and resource availability. Comparable observations have been reported in other geographical regions (Susanto et al., 2020; Fatma et al., 2022), showing that local fishers provided technical insights on material selection, maintenance, operational challenges, and environmental factors affecting fish aggregation, complementing observational data.

Environmental differences determine structural requirements. Sarang Tiung's use of mangrove wood

provides greater resistance to wave motion and marine borers, extending the structure's lifespan. In contrast, Wiritasi's lighter construction facilitates easier installation in shallower waters but resulting in shorter lifespans. While Wiritasi employs 30 anjung poles of smaller diameter, Sarang Tiung's lift net incorporate 24 mangrove poles with larger diameters and increased strength for heavy-duty use. Meanwhile, fishermen in Pangandaran, West Java, utilized "betung" bamboo as the primary material for constructing their lift-nets, owing to its strength and resistance to strong currents, reflecting disparities in local resource accessibility. These findings align with the principle that local material selection

impacts the durability and efficiency of stationary fishing platforms (Alamsah et al., 2021; Husniati and Ghaffar, 2023). Socio-economic imbalances should be considered in regional fisheries management policies aiming to promote equitable development (Ramadhan et al., 2016).

The observed differences in catch variability between Wiritasi and Sarang Tiung likely attributed to environmental and operational factors. Wiritasi's offshore location, characterized by shallow and turbid waters, may be associated with stronger currents and variable plankton conditions, which are likely to influence fish aggregation around the lift-net lighting systems. The mercury lamps used in Wiritasi were effective in attracting pelagic species such as sardines and scads, although catch intensity appeared more variable compared to the LED-based system. In contrast, Sarang Tiung's nearshore setting was characterized by relatively more stable fishing conditions, which was associated with more consistent daily catches during the study period. The clearer and deeper waters allow light to disperse effectively, attracting a wider variety of pelagic species, including sardine, anchovy, and squids (Husniati and Ghaffar, 2023; Notanubun et al., 2024). Sarang Tiung benefits from nearshore fishing grounds requiring less travel and smaller boat, while Wiritasi incurs higher travel costs due to its offshore location and reliance on larger vessel. Distance from shore, depth, lamp type, and local productivity jointly affect the spatial and temporal stability of stationary lift-net fisheries (Sulaiman et al., 2015; Sudirman et al., 2019). The catch composition at both sites dominated by small pelagic species exhibiting positive phototactic responses to artificial light. *S. fimbriata* was consistently the most abundant species across fishing operations.

Light configurations significantly impact catch efficiency and aggregation behavior. Wiritasi uses mercury lamps suitable for shallow waters but with less predictability and high costs, while Sarang Tiung employs LED lamps offering higher luminous efficiency and deeper attraction ranges. Currently, there is no specific restriction on the use of LED lighting in the study area; however, national regulations impose technical limits on fishing lights. The lamp power applied in both fishing grounds was substantially lower than the maximum threshold stipulated in Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia No. 36/2023 ($\leq 2,500$ W), indicating compliance with existing regulatory provisions. Nevertheless, the increasing adoption of high-efficiency LED systems highlights the need for clearer, LED-specific management guidelines to ensure that technological gains do not translate into excessive fishing pressure.

Consistent with our findings, higher light intensity can enhance fish attraction and increase catches, but this is often accompanied by increased operational costs related to fuel consumption and equipment maintenance, reflecting the socio-economic constraints shaping technological adoption in small-scale fisheries (Rudin et al., 2017; Sudirman et al., 2020). This trade-off between energy consumption and catch optimization underscores

the need for cost-effective lighting strategies that improve efficiency without disproportionately increasing operating costs (Solomon and Ahmed, 2016). In this context, the adoption of energy-efficient LED lighting systems, coupled with modular lift-net frame designs, offers a practical approach to improving operational flexibility and reducing long-term expenses. The effectiveness of LED-based lighting for fixed lift-net fisheries has been consistently reported in previous studies (Lebong et al., 2021; Susanto et al., 2020; Fatma et al., 2022), supporting their broader application under appropriate management frameworks.

While CPUE differed significantly between sites ($p < 0.05$), this finding should be interpreted in light of the limited number of fishing operations and associated statistical assumptions. Wiritasi outperformed Sarang Tiung with a higher CPUE of 12.83 kg/haul, compared to Sarang Tiung's 8.46 kg/haul. Catch variability likely correlated with site-specific environmental conditions and gear configurations. Differences in mean daily catches (25.67 kg at Wiritasi vs. 16.92 kg at Sarang Tiung) were statistically validated, highlighting the impact of construction material and lighting system on fish aggregation and efficiency. Overall, Wiritasi contributed about 60.27% of the total catch, which may be associated with the proximity of Wiritasi's fishing ground to primary migratory routes or schooling aggregations of dominant clupeids such as sardines and scads (Fatmawati et al., 2020). While Wiritasi's offshore lift-net fishery achieves higher catches under favorable conditions, Sarang Tiung offers more predictable performance under stable environmental setting, ensuring consistent catch patterns. The CPUEs in the present study was comparatively higher than the CPUEs (1.57–5.92 kg/haul) for fixed lift nets in Pangkajene Islands (Fatimah et al., 2022).

The stark contrast in mean CPUE between Wiritasi and Sarang Tiung warrants a deeper look into the economics of effort. While Wiritasi demonstrates superior catch rates under favorable conditions, the higher operational costs associated with powerful mercury lamps and the shorter lifespan of its construction materials introduce a trade-off. Sarang Tiung, despite a lower CPUE, potentially benefits from greater operational predictability and lower input costs due to its more durable infrastructure and consistent, albeit lower, daily catches. This suggests that high CPUE does not always equate to higher profitability or sustainability. Furthermore, the significantly higher CPUE observed in the present study compared to regional benchmarks (e.g., Fatimah et al., 2022) indicates that both sites are currently exploiting relatively productive fishing grounds. However, sustaining these high catch rates requires careful monitoring to prevent localized overfishing, especially of fast-growing clupeids. This highlights the urgent need to balance the high efficiency suggested by Wiritasi's CPUE with resource conservation and economic viability, emphasizing the strategic value of incorporating energy-efficient LEDs to maintain catch rates while mitigating operational expenditure and environmental impact (FAO, 2024).

The comparative results detailing differences in technology, operation, and performance of lift net fisheries underscore the necessity of site-specific management strategies that integrate ecological, technological, and socio-economic dimensions. This evidence provides critical support for transitioning away from conventional 'one-size-fits-all' approaches toward spatially differentiated fisheries policies. For instance, Sarang Tiung's use of durable mangrove wood offers a structural model for increasing gear lifespan, whereas Wiritasi's high-intensity luring technique demonstrates a method for maximizing catch in specific ecological niches. Therefore, policy recommendations should prioritize subsidizing energy-efficient LED lighting for Wiritasi fishers, accompanied by strict regulatory measures such as catch quotas, limits on light intensity and operating duration, and standardized technical specifications (Ministry of Marine Affairs and Fisheries 36/2023), to decouple high catch rates from high operational costs and to mitigate the risk of overfishing arising from unregulated LED application.

Addressing underlying socio-economic imbalances is essential to ensure equitable access to technology transfer and productive fishing grounds. Based on the findings of this study, several key management and operational recommendations emerge. Fishers are encouraged to selectively adopt proven gear designs, construction materials, and operational practices that enhance efficiency and durability. Optimization of LED light intensity and configuration should be prioritized to balance energy consumption with catch performance while minimizing environmental impacts. The use of modular lift-net frames may further reduce maintenance costs and improve operational flexibility.

In addition, exploratory adjustments in fishing locations, depths, and timing, aligned with periods of higher fish aggregation, may improve catch outcomes. While sardines remain the primary target species due to their economic importance, maintaining catch diversity including squid, can enhance resilience to market fluctuations and stock variability. Finally, strengthening inter-site knowledge exchange among fishing communities, particularly through the sharing of ecological knowledge and net-handling practices, could help standardize and improve fishing performance across the region.

These strategies should be informed by CPUE data and socio-economic indicators to build adaptive capacity against environmental uncertainty, ensuring the long-term viability of the fixed lift-net fisheries.

Conclusion

This study demonstrates that lift-net output varies substantially between fishing grounds, reflecting interactions among species composition, gear configuration, and lighting systems. While Wiritasi achieved higher catch efficiency, the contrast with LED-based operations in Sarang Tiung underscores the importance of transitioning toward more energy-efficient

practices while balancing production with sustainability. For fishers and fisheries managers, promoting energy-efficient lighting, adaptive gear design, and equitable access to technology offers a practical pathway to enhance economic returns while reducing environmental impacts.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

The first author designed the study, analyzed the data, and wrote the manuscript. The second and third authors collected data and conducted measurements. All authors reviewed and approved the final version.

Ethics Approval

No ethics committee approval is required for this study.

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RESEARCH ARTICLE

The First Record of Bopyrid Isopod *Urobopyrus processae* (Richardson, 1904) in the Estuarine Alpheid Shrimp *Alpheus estuariensis* from the Vellar Estuary, Southeast Coast of India

Gothandapani Jana, Ezhilmaran Shamkumar, Muthusamy Thangaraj*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India- 608 502
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India- 608 502
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India- 608 502

<https://orcid.org/0009-0005-3286-7711>
<https://orcid.org/0009-0000-1480-2272>
<https://orcid.org/0000-0001-6055-4900>

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Abstract: In this study, we report the first occurrence of the bopyrid isopod *Urobopyrus processae* in the estuarine alpheid shrimp *Alpheus estuariensis* from the Vellar Estuary, southeast coast of India. Shrimps were collected between November 2024 and April 2025 fortnightly and examined for parasitic infestation. Infestation was identified by bulging of the carapace and the presence of isopods in the branchial chamber or abdomen. In all collected alpheid shrimps (n=412), the parasitic infested animals were 3.39%. Our results also showed a higher prevalence of infestation in female shrimps (71%) than males (29%). A positive correlation ($R^2 = 0.3138$; $p < 0.001$) was found between host weight and parasite weight, indicating synchronized growth. This record not only extends the known distribution range of *U. processae* to Indian waters but also adds a new host species to its profile in the Vellar estuarine ecosystem.

Anahtar kelimeler:

Tabanca karides
Alpheus
Urobopyrus
Isopoda
Parazit
İstila

Hindistan'ın Güneydoğu Kıyısındaki Vellar Nehri Halici'nden Toplanan *Alpheus estuariensis* Türünde Bopyrid İzopod *Urobopyrus processae* (Richardson, 1904)'un İlk Kaydı

Öz: Bu çalışmada, Hindistan'ın güneydoğu kıyısındaki Vellar Nehri Halici'nden toplanan *Alpheus estuariensis* türü karideslerde bopyrid izopod *Urobopyrus processae*'nin ilk kez görüldüğünü bildiriyoruz. Karidesler, Kasım 2024 ile Nisan 2025 tarihleri arasında iki haftada bir toplanmış ve parazit istilası açısından incelenmiştir. İstila, kabuğun şişmesi ve solungaç boşluğunda veya abdomende izopodların varlığı ile belirlenmiştir. Toplanan tüm alpheid karideslerde (n=412), parazit istilasına uğramış bireylerin oranı %3,39 olmuştur. Sonuçlarımız ayrıca dişi karideslerde (%71) erkeklere (%29) göre daha yüksek bir istila baskınlığı göstermiştir. Konak ağırlığı ile parazit ağırlığı arasında pozitif bir korelasyon ($R^2 = 0,3138$; $p < 0,001$) bulunmuştur; bu da senkronize büyümeyi göstermektedir. Bu kayıt, *U. processae*'nin bilinen dağılım aralığını Hint sularına genişletmekle kalmayıp, Vellar halic ekosistemindeki profiline yeni bir konak türü de eklemektedir.

Introduction

Bopyrid isopods, in particular, are obligate parasites (holoparasites) that primarily infest decapod crustaceans as their definitive hosts (Markham, 1986). They are commonly found parasitizing the branchial chambers of penaeid shrimps across the Indo-West Pacific region, from the Red Sea to Japan (Chopra, 1930; Chu and Leong, 1996; Boyko, 2003). The occurrence of *Urobopyrus processae* in the Vellar estuary represents either an overlooked native population or a recent range expansion. Similar biogeographic shifts have been documented in marine parasitic isopods such as *Epipenaeon ingens*, which extended its distribution from the Indo-West Pacific into the Eastern Mediterranean following the opening of the Suez Canal (Markham, 1986). In Indian coastal waters, *E. ingens* was reported by Thomas (1977) on *Penaeus*

semisulcatus from the Palk Bay and Gulf of Mannar, and by Ravichandran et al. (2000) on *P. monodon* from the Parangipettai coast, Tamilnadu. The infestation often results in parasitic castration, where energy normally allocated to gonadal development is diverted to parasite growth, leading to complete or partial suppression of host reproduction. Infected individuals frequently exhibit reduced fecundity, diminished ovarian development, and absence of mature gametes. Additionally, parasite attachment within the branchial chamber may cause gill damage, impairing respiration and reducing oxygen uptake efficiency. Chronic infestation is also associated with growth inhibition, altered molting cycles, reduced feeding efficiency, and increased metabolic stress. These combined effects can lower host survival, decrease population

*Corresponding author: coralholder@yahoo.com

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recruitment, and ultimately impact fisheries productivity, particularly in commercially important shrimp and crab species (Koesharyani et al., 1999; Thatcher and Blumenfeldt, 2001). While bopyrid isopods are common in wild populations of *Macrobrachium* spp., they are relatively rare under aquaculture conditions (Oliveira, 2000). Although these parasites do not pose any direct health risk to humans, their presence in the gill chambers of host shrimps can reduce the fitness and commercial value of the infected individuals (Somers, 1991). In some host populations, high prevalence of bopyrid infestation has been observed—for instance, *Argeia pugettensis*, which parasitizes at least 20 species of commercially important crangonid shrimps (Jarrin and Shanks, 2008).

Urobopyrus processae is a bopyrid isopod with a distribution encompassing the Atlantic Ocean, Caribbean Sea, Gulf of Mexico, and Mediterranean Sea (Markham, 1986; Rasch and Bauer, 2015). This parasite typically infests juvenile shrimps and matures alongside its host in both marine and brackish water ecosystems (Romero-Rodríguez and Román Contreras, 2013). In rare instances, it has been found infesting older, larger hosts, such as amphidromous shrimps during their reproductive migrations to estuarine environments (Conner and Bauer, 2010).

To date, there have been no reports of *U. processae* infecting the estuarine alpheid shrimp, *Alpheus estuariensis*. This study presents the first documented case of infection by *U. processae* in male and female *A. estuariensis*. We also examined the host sex preference by the parasite, correlation between host size and parasite size as well as number of embryos in a brood and female total length. This record extends the distribution of *U. processae* to Indian estuary waters.

Material and Methods

Alpheid shrimps (*Alpheus estuariensis*) were collected from the inter-tidal oyster beds by hand picking in the Vellar Estuary (Lat. 11°29'N; Long. 79°46'E), fortnightly in November 2024 - April 2025. The Vellar Estuary, situated along the southeast coast of India, is classified as a “true estuary” due to its permanently open mouth and continuous exchange with adjacent coastal waters. Based on salinity gradients, it has been divided into four distinct zones: marine, gradient (mixing), tidal, and freshwater regions (Ramamurthy, 1954). The estuary is influenced by semi-diurnal tides, with a maximum tidal amplitude of approximately 1 m, and tidal intrusion extends up to 16 km upstream. Water depth generally ranges from 2.3 to 5.0 m. Hydrodynamic interactions between neritic waters and estuarine conditions promote the active exchange of biotic and abiotic components, supporting high ecological productivity. The system also exhibits pronounced seasonal fluctuations in freshwater inflow and chlorinity, particularly during monsoonal and post-monsoonal periods, which regulate nutrient cycling and elemental fluxes within both the estuarine environment and the adjacent coastal ecosystem (Natarajan et al., 1979).

Totally 412 (Male= 156; Female= 256) individual shrimps were collected. For each specimen, carapace length, weight, sexes were recorded. The parasitic incidence and attachment of parasites in the branchial cavities and abdominal region of the host were noted. Host shrimps were identified morphologically using the identification key by Banner and Banner (1982), while the parasitic isopods were identified based on the taxonomic keys provided by Manning and Chace (1971) and Abele and Kim (1986). Prevalence and mean intensity were calculated according to Bush et al., (1997).

The host shrimp body size was assessed using body weight (mg) and total length (TL, measured to the nearest ± 0.01 mm). Total length was defined as the distance from the tip of the telson to the proximal end of the rostrum and was measured using a digital vernier caliper. Sex determination was based on the presence (male) or absence (female) of large chelate pereopods and the presence of ovigerous pleopode in female. Parasite total length was measured with the precision of 0.01 mm in a digital vernier caliper. The weight of the parasite was obtained with the precision of 0.0001g in a digital weighing balance (Sartorius BT224S). Based on morphological characteristics, the parasites collected were identified as *U. processae*. For brood size estimation, egg masses were carefully removed from the parasites and placed in 100 mL of 5% sodium hypochlorite (NaClO) solution. The samples were gently stirred for 5 min to ensure complete egg separation, followed by rinsing with 50 mL of deionized water. Three randomly selected 1 mL subsamples were examined under a stereomicroscope to count the eggs. Total brood size per female was calculated by multiplying the mean egg count per millilitre by the total sample volume (100 mL). The female parasites with egg, embryo, young ones, and male parasites were photographed by a camera mounted on a microscope (Olympus CX41). One-way ANOVA and Pearson correlation analysis were performed in SPSS v 19.0 software.

Results

The morphological characters of female *U. processae* were as follows: Body slightly asymmetrical, broader than long; colour uniformly white to creamy in preserved specimens. Cephalon with frontal margin produced and slightly upturned; posterior margin broadly rounded. Anterolateral angles each with a distinct black mark, presumably representing ocular remnants. Pereon of eight distinct pereomeres. Pereomeres 1–4 each with prominent ovarian bosses situated anteriorly. Epimera of pereomeres 1–4 represented by narrow lateral plates external to ovarian bosses. Pereomeres 5–7 with epimera expanded into large lateral plates, asymmetrically developed, extending beyond lateral margins of corresponding pereomeres. Pleon of six distinct pleomeres; lateral margins rounded, not produced. Pleotelson absent; terminal pleomere bilobed. Uropods biramous, inserted laterally on terminal pleomere; endopod smaller and more slender than exopod. Pleopods five pairs, biramous,

forming elongate lamellae; endopods smaller than exopods and directed medially; exopods extending beyond lateral margins of pleon. Oostegites five pairs, arising from pereomeres 1–5, forming brood pouch; oostegites not meeting medially, leaving brood pouch partially open, usually containing eggs. Pereopods seven pairs, all present.

The morphological characters of male *U. processae* were as follows: Body elongate, cephalon large, rounded; eyes distinct. Pereon of seven distinct pereomeres, lateral margins rounded. Pleon externally unsegmented, without indication of six pleomeres dorsally or laterally; triangular in outline, apex rounded. Pleopods present and well developed on ventral surface.

Shrimps were considered infested when the bopyrid isopod parasites are present within the branchial chamber or abdominal region. Infested individuals typically exhibited a noticeable deformation on one side of the carapace, or abdominal region which appeared bulged outward due to the parasite's presence (Fig. 1F-H). Female parasites were asymmetrical in shape and most appeared bright yellow in colour, attributed to the presence of egg masses or developing embryo (Fig. 1A,B). Males were significantly smaller in size and were observed attached inside the pouch of the females (Fig. 1D). Adult males were 1.2-1.4 mm in size with eight pairs of appendages (Fig. 1E).

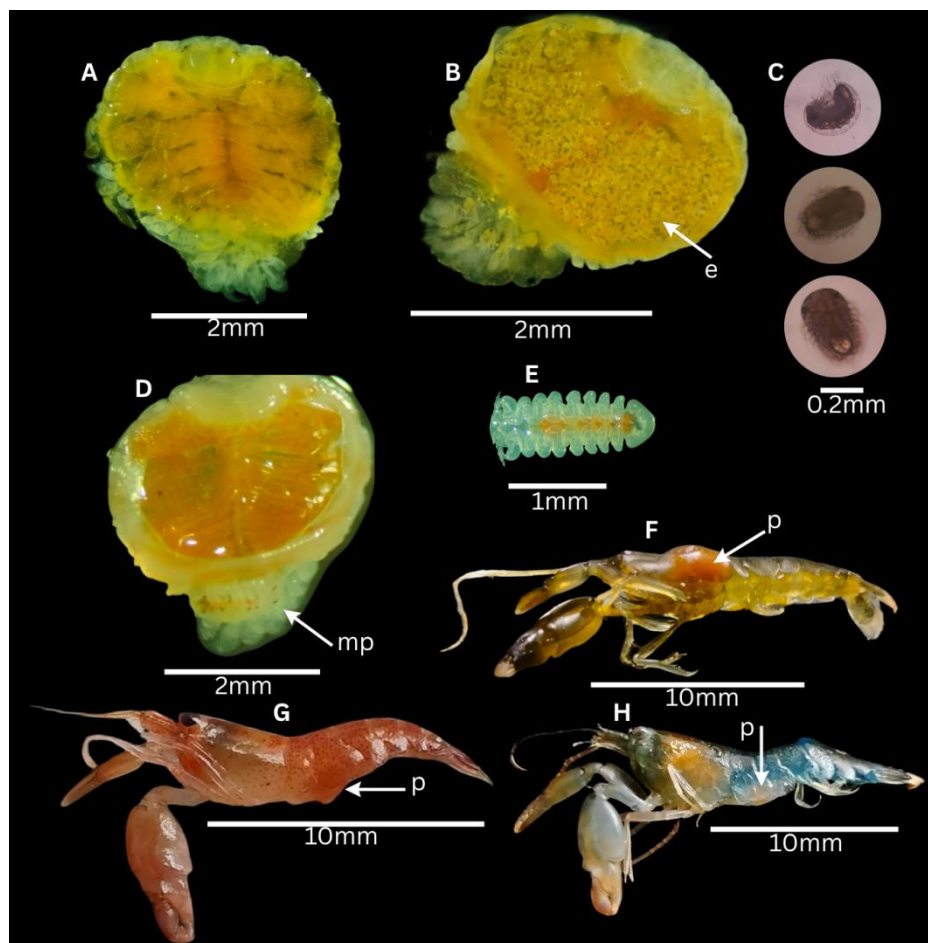


Figure 1. *Urobopyrus processae* parasite. (A) *U. processae* female ventral view; (B) Female *U. processae* incubating embryos; (C) *U. processae* youngones; (D) *U. processae* female with egg and male (mp); (E) *U. processae* adult male ventral view; (F,G,H) *U. processae*(p) infesting the snapping shrimp *Alpheus estuariensis*

In the present study, among the 412 shrimp samples collected, the prevalence of parasitic infestation was 3.39%. We also observed a clear preference for female hosts with a significantly higher infestation rate of 71% compared to males (29%). The host sex preference pattern of *U. processae* is illustrated in Fig. 2. Additionally, a significant positive correlation ($p < 0.001$) was found between host body weight and parasite body weight,

indicating that larger hosts tend to accommodate larger parasites. This relationship is represented in Fig. 3, with a coefficient of determination (R^2) value of 0.3138. Brood size of the parasites ranged from 500 to 1150 embryos, and there was a positive relationship ($p < 0.001$) present between number of parasite embryos brooded and female parasite length with the R^2 value of 0.9702 (Fig. 4).

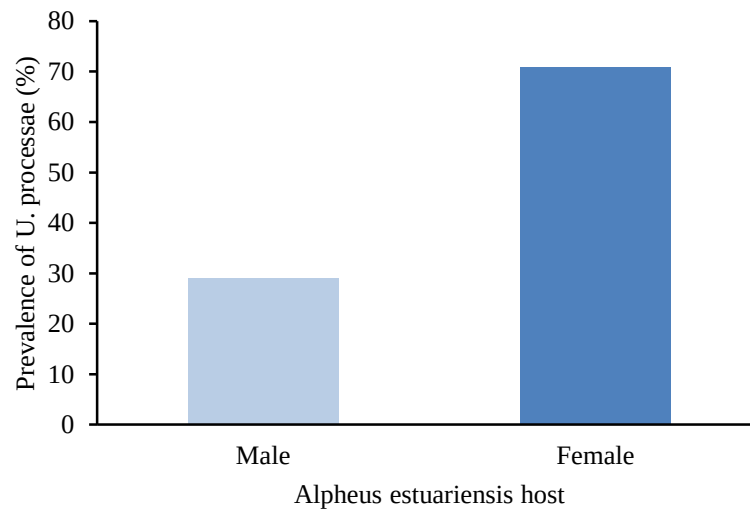


Figure 2. Preference of host sex by *U. processae* parasite

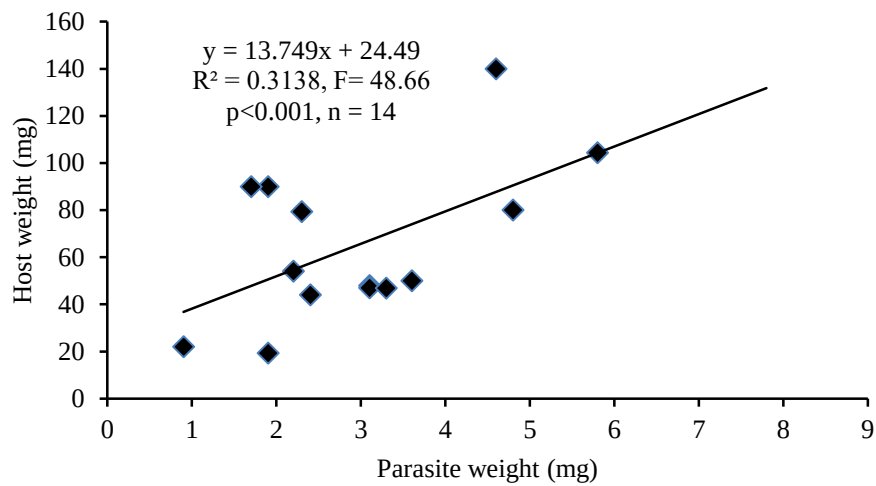


Figure 3. Correlation between host weight and parasite weight

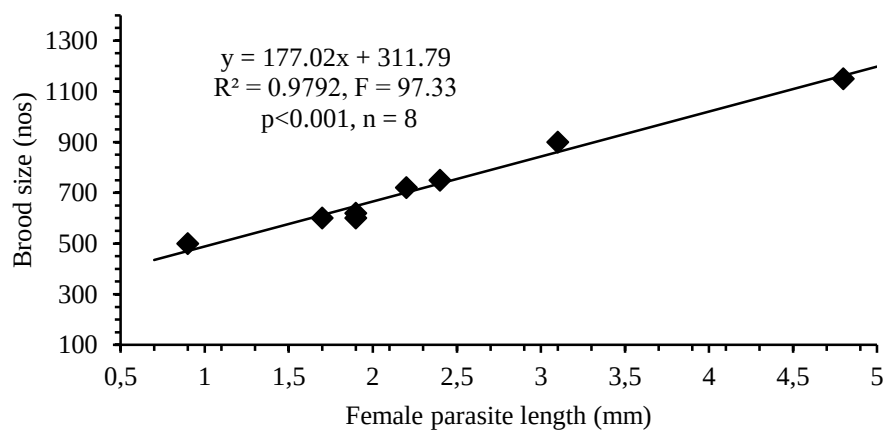


Figure 4. Correlation between number of embryos in a brood and female total length

Discussion

Our findings represent the first recorded incidence of infestation by the bopyrid isopod *U. processae* in the alpheid shrimp *A. estuariensis* in Indian estuarine waters. Earlier, Bourdon (1968) reported an overall infestation rate of 6.4% by a bopyrid parasite in caridean shrimp, *Processa edulis*. In the present study, the infestation rate was 3.39% by the bopyrid isopod *U. processae*. While Bourdon (1968) did not provide information on the sex of infested hosts, our observations revealed a higher infestation rate in female shrimps than males. Our findings also align with observations from *A. symmetricus*, in which females showed higher rates of infestation (Jay, 1989). This pattern is consistent with findings from other studies on bopyrid parasites infesting sexually dimorphic caridean shrimps, where females being the larger sex are more frequently parasitized (Romero-Rodríguez and Román-Contreras, 2013). The preference of *U. processae* for female hosts in our study aligns with this trend, supporting the hypothesis that parasite fecundity is constrained by host size. Larger hosts can accommodate larger female parasites, which in turn can produce more offspring (Miranda and Mantelatto, 2010).

During our investigation, we have observed one male per female parasite, consistent in size with those previously reported in *U. processae* (Rasch and Bauer, 2015). The pronounced sexual dimorphism and the male's presence within the female's brood pouch are characteristic features of bopyrid reproductive biology. Some bopyrid species are known to show a preference for one side of the host's branchial chamber, particularly in non-natantian decapods (Rasch and Bauer, 2015). However, previous studies suggest that branchial bopyrids generally infest both left and right gill chambers with almost equal frequency (Miranda and Mantelatto, 2010). Although we have noted lateral deformation of the carapace in infested individuals, our sample size was insufficient to establish a statistically significant pattern of side preference.

The positive correlation observed between host weight and parasite weight in this study supports the hypothesis that the host and the parasite grow concurrently (Romero-Rodríguez and Román-Contreras, 2013). This pattern aligns with earlier reports of synchronized growth in other bopyrid–host associations (Rasch and Bauer, 2015). The brood size of female *U. processae* is also positively correlated with female parasite size (TL), as documented in previous studies (Beck, 1980a; Romero-Rodríguez and Román-Contreras, 2008, 2013). Consequently, larger hosts, which accommodate larger parasites within their branchial chambers, support larger female parasites capable of producing larger broods. The slight variation in brood size among similarly sized females, contrasted with the high variation among differently sized females, mirrors patterns observed in other bopyrids parasitizing caridean shrimps—for example, *Bopyrina abbreviata* on *Hippolyte zostericola* (Romero-Rodríguez and Román-Contreras,

2013), and *U. processae* on *Ambidexter symmetricus* (Rasch and Bauer, 2015).

Conclusion

The present study documents the first record of the bopyrid isopod *U. processae* infesting the estuarine alpheid shrimp *A. estuariensis* from the Vellar Estuary, southeast coast of India. This finding represents a significant extension of the known geographic distribution of *U. processae* into Indian waters and establishes *A. estuariensis* as a new host species. Although the overall prevalence of infestation was relatively low (3.39%), the marked predominance of infection in females (71%) suggests potential sex-biased parasitism, possibly linked to host size, reproductive condition, or behavioral differences. The observed positive correlation between host weight and parasite weight ($R^2 = 0.3138$) indicates synchronized growth between host and parasite, a characteristic feature of many bopyrid–decapod associations. Such growth coupling may enhance parasite fitness while potentially imposing energetic and reproductive costs on the host. This study contributes baseline data on host–parasite interactions in the Vellar estuarine ecosystem and highlights the need for long-term monitoring to assess seasonal dynamics, reproductive impacts, and potential ecological consequences of bopyrid infestations in other estuarine crustaceans.

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Conflicts of Interest

The authors declare that there are no conflicts of interest or competing interests.

Author Contributions

GJ: Sample collection, identification of species. ES: Sample collection, measurement. MT: Designing of the study, supervision, data analysis, review and editing.

Ethics Approval

Ethics committee approval is not necessary for this study.

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RESEARCH ARTICLE

First Mediterranean Record of the Tintinnid, *Leprotintinnus simplex* (Schmidt, 1902), from İzmir Bay (Aegean Sea, Türkiye)

Levent Yurga

Ege University, Faculty of Fisheries, Department of Marine Biology, 35100, Bornova, İzmir, Türkiye

<https://orcid.org/0000-0003-4033-7567>

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Bay of İzmir

Abstract: *Leprotintinnus simplex* Schmidt, 1902 was recorded for the first time in İzmir Bay during plankton monitoring conducted in September 2025. The species was observed at all three sampling stations, albeit at varying abundances. Morphometric analyses based on 100 individuals revealed a mean lorica length of 124.8 μm (range: 65.0–208.5 μm), a mean lorica oral diameter (LOD) of 19.6 μm (range: 10.0–36.0 μm), and a mean L/LOD ratio of 6.5 (range: 3.8–9.2). The tapering angle of the lorica from the oral to the aboral end averaged 2.4° (range: 0.9°–3.8°). These measurements fall within the range of global morphological variation previously reported for the species. The detection of *L. simplex* in İzmir Bay suggests that the species may have been introduced via maritime transport, most likely through ship ballast water. Previously reported from the Indian Ocean, the South Pacific Ocean, the Atlantic Ocean, and the Gulf of Mexico, the species is here recorded for the first time from the Mediterranean Sea. This study documents the first Mediterranean occurrence of *L. simplex* and provides a concise biogeographical overview of its global distribution.

Anahtar kelimeler:

Tintinnidiidae
Lorika morfolojisi
Mikrozooplankton
Balast suyu girişi
İzmir Körfezi

İzmir Körfezi'nden (Ege Denizi, Türkiye) Tintinnid, *Leprotintinnus simplex* (Schmidt, 1902)'in Akdeniz'deki İlk Kaydı

Öz: *Leprotintinnus simplex* Schmidt, 1902, 2025 yılı Eylül ayında gerçekleştirilen plankton izleme çalışmaları sırasında İzmir Körfezi'nde ilk kez kaydedildi. Tür, üç örnekleme istasyonunda da farklı yoğunluklarda gözlemlendi. 100 bireye dayalı morfolometrik analizler, ortalama lorikanın uzunluğunun 124,8 μm (aralığı: 65,0–208,5 μm), ortalama lorikanın ağız çapının (LOD) 19,6 μm (aralığı: 10,0–36,0 μm) ve ortalama L/LOD oranının 6,5 (aralığı: 3,8–9,2) olduğunu ortaya koydu. Lorikanın ağız ucundan aboral uca doğru inceleme açısı ortalama 2,4° (0,9°–3,8°) olarak bulundu. Bu ölçümler, tür için bildirilen küresel morfolojik varyasyon içinde yer almaktadır. İzmir Körfezi'nde *L. simplex*'in tespit edilmesi, türün deniz yoluyla, büyük olasılıkla gemi balast suları aracılığıyla taşınmış olabileceğini düşündürmektedir. Daha önce Hint Okyanusu, Güney Pasifik Okyanusu, Atlantik Okyanusu ve Meksika Körfezinde saptanan tür ilk kez olarak Akdeniz'de bu çalışmada İzmir Körfezi'nde rastlanmıştır. Bu çalışma, *L. simplex*'in Akdeniz'deki ilk bulunuşunu belgelemekte ve türün küresel dağılımı hakkında biyocoğrafik özet bilgiler sunmaktadır.

Introduction

Ciliates are key components of marine microbial food webs, linking primary producers to higher trophic levels by grazing on bacteria, small flagellates, and diatoms, and in turn serving as prey for larger zooplankton (Stoecker and Sanders, 1985). Among marine ciliates, tintinnids constitute one of the most diverse and ecologically important groups. Their loricae—species-specific in size, shape, and ornamentation—facilitate reliable taxonomic identification (Kofoid and Campbell, 1929, 1939; Dolan, 2000; Lynn, 2008). Approximately 1,000 tintinnid species have been described worldwide since Müller's pioneering work in 1776 (Müller, 1776), now classified into 14 families and 75 genera (Agatha and Strüder-Kypke, 2014). Tintinnids are

particularly abundant in coastal and neritic systems and are influential consumers of phytoplankton and copepod nauplii, contributing significantly to carbon transfer in marine ecosystems (Dolan, 2010; Pitta et al., 2001; Calbet and Landry, 2004).

Although most tintinnid species inhabit marine environments, several tolerate brackish or freshwater conditions (Foissner and O'Donoghue, 1990; Snieszek et al., 1991; Petz and Foissner, 1993; Smith et al., 2018). Their robust loricae enable them to withstand physical stress, turbulence, and chemical gradients, allowing them to survive in harsh environments, including ballast tanks of ships (Pierce and Turner, 1993). For more than a century,

*Corresponding author: levent.yurga@ege.edu.tr

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studies have documented the unintentional introduction of non-native planktonic organisms through maritime transport. Ballast water surveys have revealed substantial numbers of living diatoms, dinoflagellates, ciliates, spores, and cysts transported across oceans (Hallegraeff and Bolch, 1992; McCarthy and Crowder, 2000). The ecological impacts of such introductions have gained increasing attention, as alien planktonic species can become established in receiving environments and alter local community structures (Ostenfeld, 1908; Cohen, 1998).

Within the genus *Leprotintinnus* (Family: Tintinnidiidae), nine species are recognized: *L. bottnicus*, *L. bubianicus*, *L. dogieli*, *L. elongatus*, *L. neriticus*, *L. nordqvistii*, *L. pellucidus*, *L. simplex*, and *L. tubulosus*. The species *Leprotintinnus simplex* Schmidt, 1902 is currently registered under AphiaID number 427571 (WORMS and AlgaeBase, 2026). The species *Leprotintinnus simplex* Schmidt, 1902 is a widely distributed species reported from tropical, subtropical, and temperate coastal regions of the Indian Ocean, Pacific Ocean, South China Sea, Red Sea, and Gulf of Mexico. However, despite more than seven decades of plankton studies in İzmir Bay, this species has never been reported from the region. While long-term monitoring studies in the İzmir Bay have identified numerous tintinnid species (Ergen, 1967; Koray and Özel, 1983a,b; Çolak-Sabancı and Koray, 2001, 2005, 2015), and another congeneric species, *Leprotintinnus nordqvisti*, has been reported from İzmir Bay (Yurga, 2012, 2018) and the Sea of Marmara (Balkis and Koray, 2014), *L. simplex* remained undocumented in the Mediterranean Sea until now.

The present study reports the first occurrence of the tintinnid ciliate, *Leprotintinnus simplex*, in the Mediterranean Sea, specifically from İzmir Bay and reviews its morphological features and distributional patterns in İzmir Bay. This finding expands the known geographic distribution of this species and provides new insights into its morphological variability and potential introduction pathways. Given the increasing role of maritime transport in species dispersal, documenting new records of tintinnids is essential for understanding biogeographic shifts and the ecological dynamics of coastal ecosystems.

Material and Methods

Surface water samples collected from three stations in İzmir Bay in September 2025 were analyzed (Figure 1, Table 1). The species was first detected simultaneously at all three stations during this monitoring period, prompting the present investigation. Physicochemical measurements of seawater were made in situ using digital sensors. Salinity (ppt) was measured using a YSI Pro30 conductivity meter, temperature (°C) using a YSI Pro400, dissolved oxygen (mg/L) using a YSI Pro20, and pH using a YSI EcoSense pH100A meter. In situ chlorophyll-a measurements were performed using a Turner Designs AquaFluor Handheld Fluorometer/Turbidimeter. Surface seawater samples were

collected in 5-liter plastic containers and preserved with 5% Lugol's iodine solution.

After transport to the laboratory, samples were concentrated through a two-stage sedimentation procedure following Utermöhl (1958), transferred into 100-cc glass tubes, and labeled. The samples were subsequently concentrated to 2.5 ml and enumerated under a microscope using the single-drop method (Lackey, 1938). All counts were recorded using spreadsheet software.

Microscopic examinations were conducted with an Olympus BX43F phase-contrast microscope equipped with a high-resolution digital camera. Photomicrographs were taken using the same system, and all measurements were performed with Olympus imaging software. Measurements were conducted on both preserved and live specimens to ensure accuracy and capture potential morphological variation. For the assessment of lorica length, lorica oral diameter (LOD), and lorica width of the tintinnid *L. simplex*—the focal species of this study—100 measurements were obtained from three samples collected across the three stations, and mean values were calculated.

Global literature on *L. simplex* was reviewed, and all available records documenting the species—along with their reported morphometrics, sampling locations, and authors—were compiled. Geographic coordinates for each global observation were incorporated into a dataset, from which a worldwide distribution map was generated, including the new record from the present study (Figure 1).

The map illustrating the sampling stations (Figure 1) and the global distribution map of the species (Figure 4) were prepared using CorelDRAW, a desktop vector-based graphic design software. The figures were exported in PNG format at a resolution of 600 dpi for use in this study.

Using published drawings and photographs provided by researchers who previously illustrated or documented the species, the morphology of *L. simplex* was re-illustrated for comparative purposes (Figure 2). Species identification followed the diagnostic features described by Schmidt (1902), Hada (1938), and Hirota (1975). The re-illustration process incorporated information from Schmidt (1902), Hada (1938), Yamaji (1966), Xu and Song (2005), Abou-Zaid and Hellal (2012), Wang et al. (2014), Zhang et al. (2016), Rakshit et al. (2017), Feng et al. (2018), Santiago et al. (2021), and Hu et al. (2022).

Table 1. Coordinates of the stations

Station	Coordinates
1	38°27'16.64"N 27° 5'32.46"E
2	38°26'03"N 27°02'40"E
3	38°24'4.09"N 27° 4'43.03"E



Figure 1. Sampling stations

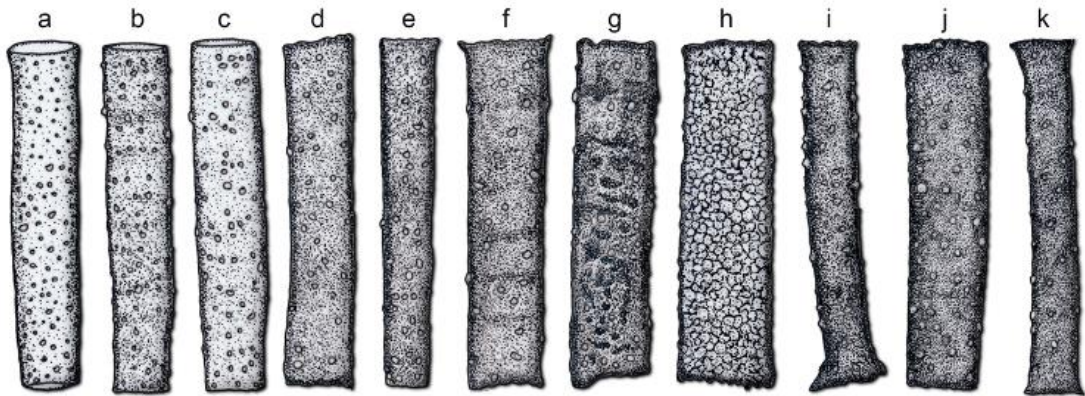


Figure 2. Modified views of the species in previous studies

Modified (not to scale) drawings by Yurga, adapted from a) Schmidt, 1902 b) Hada, 1938 c) Yamaji, 1966 d) Xu and Song, 2005 e) Abou-Zaid and Hellal, 2012 f) Zhang et al., 2016 g) Rakshit et al., 2017 h) Feng et al., 2018 i) Santiago et al., 2021 j) Hu et al., 2022 k) this work, 2026, re-drawn for taxonomic comparison; original sources listed below.

Results

Environmental conditions

The physicochemical parameters measured at the three stations sampled in September were summarized in Table 2. Temperature decreased slightly from the inner to the

outer bay (23.1–22.4 °C). Dissolved oxygen ranged from 6.6 to 7.4 mg L⁻¹, and Secchi depth increased from 110 to 240 cm. Chlorophyll-*a* (Chl-*a*) concentrations ranged from 0.3 to 0.6 µg L⁻¹, while cell abundance of *L. simplex* varied between 53 and 160 cells L⁻¹. No variation in pH or salinity was observed among the stations.

Morphometric and Taxonomic Characterization of *Leptotintinnus simplex* from the İzmir Bay

Leptotintinnus simplex was observed at all three stations identified in this study. The species was detected in varying concentrations in seawater surface samples taken in September 2025. Length, lorica oral diameter (LOD), and lorica width measurements were taken from 100 individuals

of the species. According to microscopic examinations, the species' average lorica length was 124.8 μm (range 65.0–208.5 μm); average LOD 19.6 μm (range 10.0–36.0 μm) and average ratio L/oral diameter is 6.5 (range 3.8 - 9.2). The slight angle of narrowing of the lorica from the oral to the aboral edge of 100 individuals was also measured. The average tapering angle of the lorica was 2.4° (range 0.9–3.8°).

The standard deviation of the mean lorica length was 22.46 μm , with a standard error of 2.25 μm (n=100). The

standard deviation of the mean LOD was 4.40 μm , with a standard error of 0.44 μm . The standard deviation of the mean tapering angle was 0.78°, with a standard error of 0.08° (Table 3). Current systematic information about the species is given in Table 4.

Microscopic images illustrating the morphology and dimensional measurements of unfixed and Lugol's-fixed specimens collected in September 2025 are presented in Figure 3.

Table 2. Physicochemical parameters at the stations

Station	DO (mg/l)	T (°C)	S (ppt)	Secchi (cm)	pH	Chl-a ($\mu\text{g/L}$)	Abundance (cell/L)
1	7.1	23.1	38.1	110.0	8.6	0.6	53
2	6.6	22.9	38.6	190.0	8.6	0.3	29
3	7.4	22.4	37.8	240.0	8.6	0.5	160

DO: Dissolved oxygen; T: Temperature; S: Salinity; Chl-a: Chlorophyll-a

Table 3. Species-specific measurements

	Oral diameter	Lorica length	L/oral diameter	Tapering
Min	10,00	65,00	3,75	0,90
Max	36,00	208,50	9,20	3,80
Ave	19,60	124,80	6,50	2,44
SD	4,40	22,46	1,03	0,78
SE	0,44	2,25	0,10	0,08

Min: Minimum, Max: Maximum, Ave: Average, SD: Standart Deviation, SE: Standart Error

Table 4. Taxonomic information of the species

Taxonomy	
Domain: Eukaryota	Subclass: Oligotrichia
Kingdom: Chromista	Order: Choreotrichida
Subkingdom: Harosa	Suborder: Tintinnina
Infrakingdom: Alveolata	Family: Tintinnidiidae
Phylum: Ciliophora	Genus: <i>Leprotintinnus</i>
Subphylum: Intramacronucleata	Species: <i>L. simplex</i>
Class: Oligotricha	

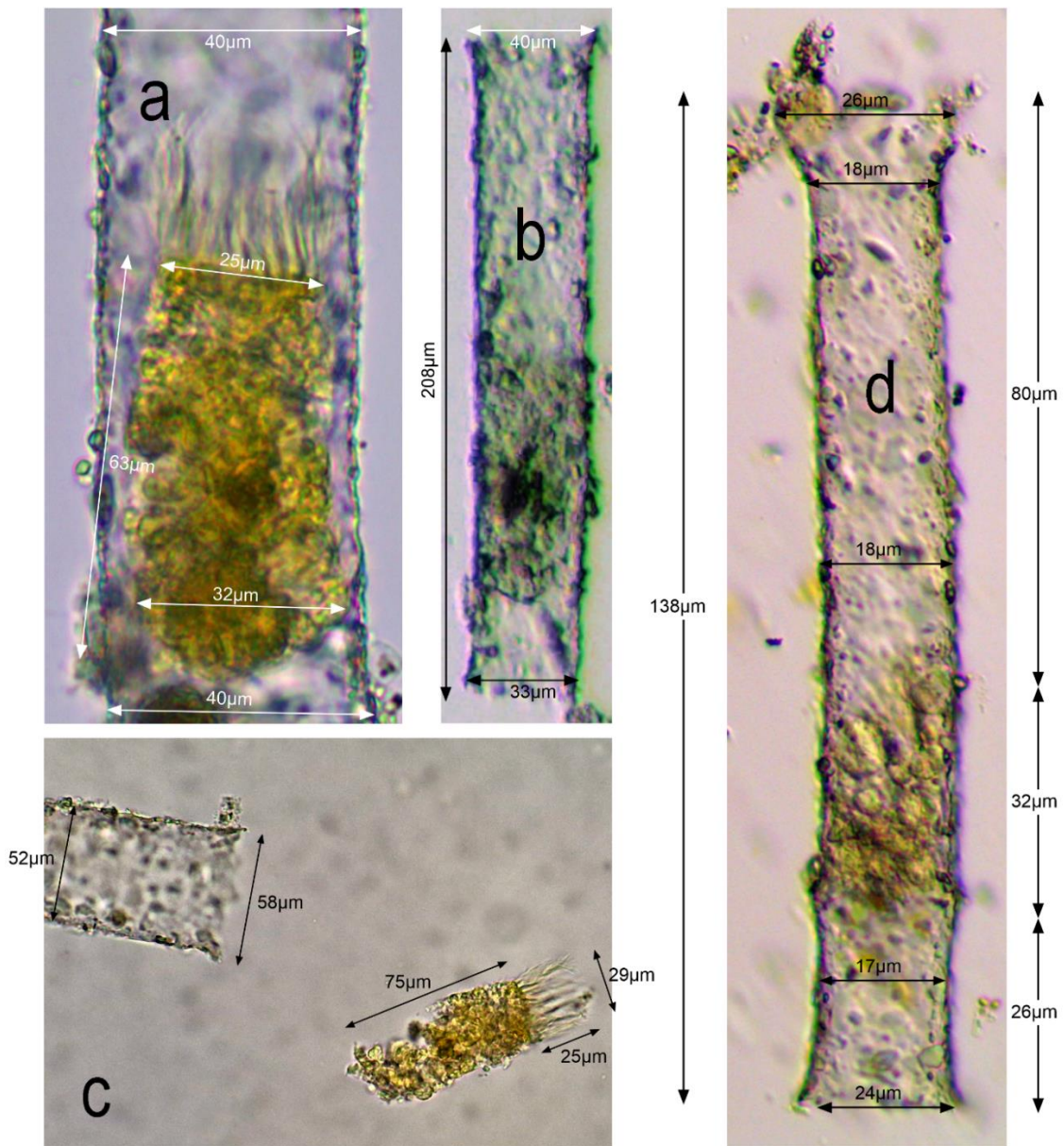


Figure 3. Morphological features and lorica measurements of *L. simplex* from September sampling, illustrated using unfixed and Lugol-fixed material. (a) Cilium within the lorica, (b) Longest lorica recorded in unfixed samples, (c) *L. simplex* cell positioned outside the lorica in fixed material, (d) Morphometric measurements of a Lugol-fixed specimen.

Discussion

The present study documents the first confirmed occurrence of *L. simplex* in the Mediterranean Sea, marking a notable eastward extension of the species' known distribution into the Aegean Sea. The absence of previous records despite more than seven decades of plankton research in the region strongly suggests that *L. simplex* is a recent arrival rather than an overlooked native species. Given the species' known ability to survive in dark, turbulent, low-oxygen environments such as ship ballast tanks, maritime transport represents the most plausible introduction pathway (Hallegraeff and Bolch, 1992; McCarthy and Crowder, 2000; Cohen, 1998).

Globally, *L. simplex* is recognized as a highly adaptable tintinnid with a broad ecological tolerance. Its geographic range spans tropical, subtropical, and warm-temperate marine and estuarine environments across the Indo-Pacific, Red Sea, Arabian Sea, and Gulf of Mexico. This broad distribution reflects its capacity to persist under diverse salinity conditions (0.2–34 psu), wide temperature ranges (approximately 10–30°C or higher), and varying nutrient regimes. Seasonal studies from India, China, and Southeast Asia frequently report strong temporal shifts in abundance, with population increases typically occurring during monsoon or summer periods when environmental fluctuations are pronounced (Biswas et al., 2013; Sarkar, 2015; Wang et al., 2014; Yu et al., 2022).

The global distribution of *L. simplex* is illustrated in Figure 4. This species was first described by Schmidt (1902) from the Gulf of Siam. Subsequent records documented its occurrence in Indonesia (Hada, 1938), Japan (Hirota, 1975), and South Korea (Yoo et al., 1988), confirming its early recognition in the Indo-Pacific region. During the 2000s, the species was increasingly reported from tropical and subtropical coastal environments, including India (Godhantaraman, 2001, 2002; Sarkar, 2015; Rakshit et al., 2016, 2017), China (Xu & Song, 2005; Wang et al., 2014; Chen et al., 2014; Feng et al., 2018; Li et al., 2019), the Red Sea (Abou-Zaid & Hellal, 2012), and the Philippines (Santiago & Ablan-Lagman, 2021). Additional records from the Gulf of Mexico (Snyder et al., 2021), the South China Sea (Kumar et al., 2021), Pakistan (Yasmeen et al., 2025), and the North Pacific Transition Zone (Li et al., 2021) demonstrate its broad distribution across Indo-

Pacific and Atlantic coastal systems. Across these studies, *L. simplex* has been reported from estuarine, brackish, and neritic marine environments, often exhibiting marked seasonal variability and considerable morphometric plasticity (Godhantaraman, 2001, 2002; Sarkar, 2015; Rakshit et al., 2016, 2017; Li et al., 2021). Reported lorica measurements show wide variation, reflecting environmental influences and population-level polymorphism. Recent phylogenetic analyses (Hu et al., 2022) clarified taxonomic ambiguities within the genus and confirmed the distinct status of *L. simplex*, while highlighting previous misidentifications among closely related species. Overall, existing records indicate that *L. simplex* is widely distributed in tropical and subtropical coastal waters, yet prior to the present study it had not been documented from the Mediterranean Sea.



Figure 4. Locations of studies conducted with the species *L. simplex*

Most important studies on tintinnids in the Mediterranean Sea date back to the late nineteenth century (Fol, 1881; Entz, 1885) and continued into the early twentieth century (Entz, 1909; Jørgensen, 1924). Thereafter, the number of investigations increased substantially, particularly from the mid-twentieth century onward (Balech, 1959; Fonda Umani et al., 1985, 1992, 2010; Zingone et al., 1990; Dolan, 2000; Pitta et al., 2001; Modigh and Castaldo, 2002; Gomez and Gorsky, 2003; Dolan et al., 2005, 2009; Calbet & Saiz, 2005; Sitran et al., 2007, 2009; Monti-Birkenmeier et al., 2019; Ouba et al., 2025). In Turkish seas, tintinnid studies have been conducted in the Sea of Marmara (Balkıs, 2004), the Aegean Sea (Balkıs-Özdelice & Wasik, 2005), Edremit Bay (Balkıs-Özdelice & Toklu-Alıçlı, 2009), Mersin Bay (Polat et al., 2019) and Turkish seas (Balkıs and Koray, 2014).

Plankton investigations in İzmir Bay began in the 1950s; however, early studies primarily provided lists at the class and family levels rather than species-level identifications (Nüman, 1955; Acara & Nalbantoğlu, 1960). The first publications to report species-level identifications were those of Ergen (1967) and Geldiay & Ergen (1968). Subsequent decades continued plankton research in the region, with several studies focusing specifically on tintinnids (Koray, 1988; Koray et al., 1999; Yurga, 1992, 2012; Çolak-Sabancı & Koray, 2001, 2005, 2015).

Studies focusing specifically on the genus *Leprotintinnus* in the Mediterranean Sea remain remarkably limited. Within the region, most records concern *Leprotintinnus nordqvisti*, reported from İzmir Bay and the Sea of Marmara (Yurga, 2012, 2018; Balkıs & Koray, 2014), as well as from the Levantine basin along the Lebanese coast (Abboud-Abi Saab et al., 2022). In the

Adriatic Sea, the genus has occasionally appeared in community-based tintinnid surveys; however, these studies did not provide detailed taxonomic or morphometric analyses. To date, no comprehensive species-level revision or focused ecological investigation of *Leptotintinus* has been conducted in the Mediterranean basin. The absence of prior records of *L. simplex* from the region further emphasizes the biogeographical significance of the present finding. Previously reported from coastal regions of the South Pacific and Indian Oceans, the species was detected in the present study in İzmir Bay, marking its first record in the Mediterranean Sea. Overall, this first record of *L. simplex* in the Mediterranean Sea enhances current understanding of the species' biogeography.

When the length measurements of the species obtained from surface samples collected at three stations in İzmir Bay during September were examined, the maximum lorica length was 208.5 μm and the minimum was 65.0 μm . These values are consistent with those reported by other researchers (Table 2). The mean lorica oral diameter (LOD) measured in this study was 19.6 μm , with a maximum of 36.0 μm and a minimum of 10.0 μm . These results are also in agreement with the published ranges, except for the notably larger measurements reported by Hu et al. (2022) and Yu et al. (2022). The mean tapering angle of the lorica was calculated as 2.4°, which likewise corresponds well with values provided by previous authors. The mean lorica width was measured as 18.9 μm , with a maximum of 39.9 μm and a minimum of 9.0 μm . The standard deviation and standard error of the lorica width were 4.28 μm and 0.43 μm , respectively (Table 5).

A very slight downward slope of the lorica of this species has been measured, starting from the mouth opening. The average tapering of the measured samples was calculated as 2.4°. This value is consistent with the measurements of previous researchers on the species (Hada, 1938; Abou-Zaid and Hellal, 2012; Hu et al., 2022). Morphometric measurements obtained from İzmir Bay therefore align well with the global variability reported for the species and further support the identification. The mean lorica length (124.8 μm) and lorica oral diameter (19.6 μm) in this study fall within published ranges, although some regions—such as Yuandang Lake (Hu et al., 2022) and Shenhu Bay (Wang et al., 2014)—exhibit notably larger morphotypes. Such variation is characteristic of *L. simplex*, which is known to display pronounced morphological plasticity associated with environmental gradients, especially temperature and salinity. The tapering angle measured in this study (mean 2.4°) also corresponds closely with previously reported values (Hada, 1938; Abou-Zaid and Hellal, 2012; Hu et al., 2022), further supporting accurate identification.

It has been reported that the species increases more in low salinity conditions after monsoon rains (Biswas et al., 2013; Sarkar, 2015; Rakshit and Sarkar, 2016; Yu et al., 2022). In this study, the species was identified in samples taken after the rains in the bay. The abundance of certain tintinnid species, which function as consumers of primary producers (planktonic organisms) and secondary producers

such as copepods (Dolan, 2010), has been shown to vary in relation to seawater temperature (Pierce & Turner, 1993; Pitta et al., 2001; Calbet & Landry, 2004). Previous studies have reported that *L. simplex* may attain higher abundances following rainfall events compared to co-occurring species (Biswas et al., 2013; Sarkar, 2015). Seasonal variability has also been documented, with lorica length decreasing during winter months when temperature and salinity decline (Rakshit et al., 2016). Spearman rank correlation analyses have indicated a positive relationship between *L. simplex* abundance and increasing pH, as well as a preference for more turbid waters (Kumar et al., 2021). Furthermore, the species has been reported to respond positively to chlorophyll-a concentrations and water temperature, while exhibiting a negative correlation with salinity (Yu et al., 2022).

The species occurred at relatively low densities in İzmir Bay compared with other regions where it may reach abundances exceeding 10^3 cells L^{-1} or even 10^6 cells m^{-3} during peak periods (Sarkar, 2015; Rakshit et al., 2017; Yasmeen et al., 2025). In contrast, the maximum abundance observed here (160 cells L^{-1}) indicates that the species is not yet established as a dominant ciliate in the local plankton community. Instead, its presence appears sporadic, potentially linked to post-rainfall conditions, as many studies have shown enhanced growth of *L. simplex* under reduced salinity and increased nutrient input.

The introduction of this non-native species into the bay is most likely associated with maritime transport, particularly through the ballast tanks of vessels of varying sizes entering the harbor for commercial and other purposes. It is well established that aquatic invertebrates and vertebrates, including their larval stages, as well as various planktonic organisms, can be transported via ships' ballast water (Ostenfeld, 1908; Hallegraeff & Bolch, 1992; Hallegraeff, 1998; Hamer et al., 2001; Yurga, 2021). Although only a small proportion of transported species are known to form harmful algal blooms (HABs) (Anil et al., 2002; Sonak et al., 2018), the establishment and bloom of introduced taxa may be facilitated in areas affected by anthropogenic marine pollution (Nixon, 1995). Furthermore, global warming is expected to enhance the survival and spread of such non-native species by creating increasingly favorable environmental conditions (Smayda, 1990; Hallegraeff, 1993, 2010).

Given its ecological flexibility, *L. simplex* may persist and possibly expand within İzmir Bay if environmental conditions become favorable. Continued monitoring is, therefore, essential to assess long-term establishment, seasonal dynamics, and potential ecological impacts. While tintinnids typically play beneficial roles in microbial food webs, the arrival of a non-native species raises important questions regarding competition with resident microzooplankton, alterations in grazing dynamics, and broader ecosystem consequences.

The presence of this species in İzmir Bay emphasizes the need for continued monitoring, as newly introduced protists may undergo delayed population expansion after

initial detection. Given the species' demonstrated tolerance to fluctuating salinity and its ability to dominate microzooplankton assemblages in other regions, future increases in abundance—particularly during autumnal or post-rainfall periods—should be anticipated and monitored. Long-term observations will be essential to determine whether *L. simplex* becomes an established component of

the bay's microzooplankton community or remains an intermittently occurring, non-proliferating alien species. A table showing the length and width of the lorica, the locations where the species was found, and the tapering angles from previous studies on the species has been prepared chronologically according to the years in which the authors conducted their studies (Table 5).

Table 5. Data from studies on the species that include morphological data

Author	Length (µm)	Width (µm)	Location	Country	The Lorica Oral Diameter (LOD) (µm)	Tapering degree
Schmidt, 1902	204.0	41.0	Gulf of Siam. Koh Chang Island	Thailand		
Hada, 1938	205.0	38.0	Semarang shores. Jawa	Indonesia		2.0°
Yoo et al., 1988	200.0		Chinhae Bay	South Korea	38.0	
Abou-Zaid & Hellal, 2012	205.0		Hurghada coasts	Egypt	33.0	2.0°
Biswas et al., 2013	184.8-57.3		Dhamakhali	India	18.3-46.9	
Wang et al., 2014	130.0-380.0	40.0-75.0	Shenhu Bay	China		
Rakshit and Sarkar, 2016	82.6		Hooghly estuary	India	24.4	
Rakshit et al., 2016	79.7	17.5	Hooghly River Estuary	India		
Dash et al., 2017	100.0-113.0		Digha. Bay of Bengal	India		
Kumar et al., 2021	187.5-322.9		South China Sea	Malaysia	31.2-41.6	
Hu et al., 2022	170.0-425.0	39.8	Coast of Xiamen	China	57.0-68.0	2°-4°
Yu et al., 2022			Bohai Bay	China	52.0-56.0	
Yurga (this work)	65.0-208.5	9.0-34.9	Izmir Bay	Türkiye	10.0-36.0	2.4°

Conclusion

This study provides the first confirmed record of *L. simplex* in the Mediterranean Sea, extending the species' known geographical distribution into the eastern Aegean Sea. Morphometric measurements obtained from 100 individuals revealed a wide range of lorica dimensions, consistent with the global variability documented across tropical, subtropical, and warm-temperate regions. The absence of any prior record in more than seventy years of plankton research in the bay strongly suggests that *L. simplex* is a recent introduction, most likely transported via ship ballast water rather than being a previously overlooked native species.

The ecological traits of *L. simplex*—including its broad tolerance to temperature and salinity, pronounced morphological plasticity, and ability to persist in both marine and brackish environments—indicate substantial

adaptive potential. However, its relatively low abundance in İzmir Bay, compared with the high densities reported from the Indian subcontinent, China, and the Red Sea, suggests that the species has not yet established a stable or expanding population in this region.

The first occurrence of *L. simplex* in İzmir Bay, on the Turkish coast of Aegean Sea, highlights the ecological significance of continued plankton monitoring programs, particularly for the early detection of non-native protists and the evaluation of their potential impacts on local microbial food webs. Long-term seasonal observations will be critical for determining whether *L. simplex* becomes a recurrent component of the ciliate community, increases in abundance under specific environmental conditions, or undergoes further morphological shifts as it adapts to the local ecosystem.

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Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author Contributions

Levent Yurga: Onboard works, Sample collecting, Laboratorial works, Microscope and Photography, Drawings, Supervision, Software, Data analyzes, Writing, Measurements, Data entry

Ethics Approval

Ethics committee approval is not required in this study.

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RESEARCH ARTICLE

Comparative Analysis of Catch Efficiency and Discard Ratio in Monofilament versus Multifilament Bottom Trammel Nets: A Case Study from Çanakkale, Türkiye

Oğuzhan Ayaz^{1*}, Uğur Altınağaç²

¹School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale 17100, Türkiye

²Department of Fisheries and Fish Processing Technology, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

<https://orcid.org/0000-0001-8458-4872>

<https://orcid.org/0000-0002-3638-9834>

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Abstract: This study aimed to evaluate the catch efficiency and chondrichthyan bycatch of monofilament and multifilament bottom trammel nets which are extensively used in small-scale commercial fishing in the Çanakkale region. The field trials of the study were carried out between September 2024 and March 2026. Within the scope of the study, a total of 8 units (800 m) of trammel nets were used. The inner panels of the trammel nets were rigged with a 84 mm stretch mesh size, and all other technical characteristics of the monofilament and multifilament net were identical. A total of 65 fishing trials were carried out with these nets. The nets were deployed for 36 hours during each trial. As a result of the fishing experiments, a total of 981 individuals weighing 622.3 kg, representing 20 species, were captured. Species with commercial value were classified as target species, while those without commercial value were classified as discard. When the economic species caught in the nets were compared on a numerical basis, multifilament trammel nets caught 1.22 times more individuals than monofilament trammel nets. In contrast, for discarded catch, monofilament trammel nets caught 1.62 times more individuals than those caught by multifilament trammel nets, and these differences were statistically significant ($P < 0.05$). In addition, the ratio of priority conservation species within the total catch in terms of the number of individuals was calculated as 23.2% in monofilament trammel nets and 12.5% in multifilament trammel nets. In the comparison made using repeated measures analysis of variance, the difference between the nets was found to be statistically significant. The study determined that the preference for monofilament material is considered unnecessary for the nets used in the fishing of species such as cuttlefish, lobster, and scorpionfish in the region, as multifilament trammel nets are more efficient than monofilament trammel nets in catching target species. Additionally, considering that a large part of the discard consists of conservation-priority chondrichthyan fish species, it was determined that the use of monofilament material in trammel nets is not suitable in terms of sustainable fisheries. It was established that the preference for multifilament trammel nets would enhance bycatch mitigation.

Anahtar kelimeler:

Av verimliliği
Fanyalı dip uzatma ağları
İskarta av
Monofilament ağ
Multifilament ağ

Monofilament ve Multifilament Fanyalı Dip Uzatma Ağlarının Av Verimi ve İskarta Av Açısından Karşılaştırılması; Çanakkale, Türkiye Örneği

Öz: Bu çalışmada, Çanakkale bölgesinde küçük ölçekli ticari balıkçılıkta yaygın şekilde kullanılan monofilament ve multifilament materyalden donatılmış fanyalı dip uzatma ağlarının av verimi ve iskarta av miktarlarının değerlendirilmesi amaçlanmıştır. Arazi çalışmaları Eylül 2024 ile Mart 2026 tarihleri arasında gerçekleştirilmiştir. Çalışma kapsamında, toplam 8 boy (800 m) fanyalı ağ kullanılmıştır. Bu ağlar 84 mm tor göz açıklığına sahip olup, material farkı dışında diğer tüm teknik özellikleri bakımından birbirleriyle aynı olacak şekilde donatılmıştır. Söz konusu ağlarla toplam 65 deneme gerçekleştirilmiş ve her bir denemede ağlar 36 saat süreyle suda bekletilmiştir. Denemeler sonucunda, 20 türe ait toplam 981 birey ve 622,3 kg balık avlanmıştır. Ekonomik değere sahip tüm türler hedef tür olarak, ekonomik değeri olmayan türler ise iskarta (discard) olarak sınıflandırılmıştır. Ağlarda yakalanan ekonomik türler karşılaştırıldığında, multifilament fanyalı ağların monofilament fanyalı ağlara göre 1,22 kat daha fazla av yaptığı belirlenmiştir. İstatistiksel analiz sonuçlarına göre de ip ağların av miktarının misina ağlara kıyasla anlamlı derecede yüksek olduğu tespit edilmiştir. İskarta av açısından ise misina ağların, ip ağlara göre 1,62 kat daha fazla av gerçekleştirdiği belirlenmiştir. Buna ek olarak, toplam av içerisindeki koruma öncelikli türlerin birey sayısı bazındaki oranı misina ağlarda %23,2, ip ağlarda ise %12,5 olarak hesaplanmıştır. Tekrarlı ölçümler varyans analizi ile yapılan karşılaştırmada, bu sayısal veriler arasındaki farkın istatistiksel olarak anlamlı olduğu bulunmuştur. Çalışma sonucunda, bölgede mürekkep balığı, ıstakoz, böcek ve iskorpit gibi türlerin avcılığında kullanılan ağlarda monofilament materyal tercihinin gerekli olmadığı, multifilament materyalden donatılmış fanyalı dip uzatma ağların hedef türlerin avlanmasında daha yüksek verimlilik sağladığı belirlenmiştir. Ayrıca, iskarta avın önemli bir kısmının koruma öncelikli kıkırdaklı balık türlerinden oluştuğu dikkate alındığında, fanyalı dip uzatma ağlarında daha az av yaptığı belirlenen monofilament ağların av baskısını azaltması sürdürülebilir balıkçılık açısından da uygun olmadığı ortaya konmuştur. Bu nedenle multifilament materyalden donatılmış fanyalı dip uzatma ağların tercih edilmesinin iskarta avın azaltılmasına katkı sağlayacağı sonucuna ulaşılmıştır.

*Corresponding author: oguzhanayaz53@gmail.com

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Introduction

The coasts of Çanakkale are among the regions where bottom trammel nets are extensively used by small-scale commercial fishers. (Ayaz et al., 2008). In recent years, the use of these nets has increased particularly in cuttlefish fisheries, where multifilament materials are generally preferred. On the other hand, studies have shown that sharks, including endangered species, are also caught in these nets, which are generally deployed at depths between 10 and 100 meters in the region (Cilasın et al., 2015). Consequently, the use of bottom trammel nets may pose a threat to sharks, rays, and other chondrichthyans (Ayaz et al., 2010). Therefore, improving our understanding of the interactions between these species and fishing gear is important for the development of ecologically sustainable fisheries management practices. Chondrichthyans inhabiting Turkish seas are mostly of Atlantic-Mediterranean and cosmopolitan origin, but also include species endemic to the Mediterranean and Red Sea migrants (Kabasakal, 2021). According to their distribution in Turkish seas, there are 9 species in the Black Sea, 36 in the Marmara Sea, 59 in the Aegean Sea, and 62 in the Mediterranean (Bengil and Başusta, 2018). The long lifespans, slow growth, low fecundity, and late attainment of sexual maturity of chondrichthyans make them more sensitive to possible ecosystem changes and overfishing pressure (Dulvy et al., 2014). The global population decline in fish stocks has directed commercial fishers and fisheries management toward new target species caught as bycatch and toward alternative strategies for their utilization (Kelleher, 2005). As a result, sharks and rays are being exploited at high rates today (Stevens et al., 2000). According to the Turkish Statistical Institute (TURKSTAT) data for the last decade in Türkiye, there is a significant decrease in ray and shark landings (TURKSTAT, 2023). Although chondrichthyans, whose reproductive capacity is limited compared to bony fishes, are bycatch species in areas where commercial fishing is practiced, they are significantly affected by fishing pressure (Bengil and Başusta, 2018). For this reason, many chondrichthyan species are classified by the International Union for Conservation of Nature (IUCN) as vulnerable, endangered, or data deficient.

Recently, awareness of sensitivity toward these species has been increasing in Türkiye, and with the regulation of commercial fisheries (Communique No. 4/1), for species such as *Squatina oculata* (Bonaparte, 1837), *Squatina squatina* (Linnaeus, 1758), *Squatina aculeata* (Cuvier, 1829), *Rhinobatos rhinobatos* (Linnaeus, 1758), *Rhinobatos cemiculus* (Geoffroy Saint-Hilaire, 1817), *Oxynotus centrina* (Linnaeus, 1758), *Mobula mobular* (Bonaparte, 1824), *Mobula japonica* (Müller and Henle, 1841), *Alopias vulpinus* (Bonnaterre, 1788), *Isurus*

oxyrinchus (Rafinesque, 1810), *Raja clavata* (Linnaeus, 1758), *Squalus blainville* (Daudin, 1809) and Myliobatidae species have been added to *Carcharhinus plumbeus* (Savi, 1844), *Cetorhinus maximus* (Garman, 1888), *Galeorhinus galeus* (Linnaeus, 1758), *Lamna nasus* (Bonnaterre, 1788) and *Squalus acanthias* (Linnaeus, 1758), all types of fishing have been banned in Turkish waters. However, these species continue to be caught as bycatch. Considering the scarcity of studies conducted in Türkiye, further research is of increasing importance for their effective conservation and management.

According to a study conducted in the Gulf of Izmir (Aydın et al., 2008) the discard rates were 77.8% for the monofilament material and 22.8% for those using multifilament material in red mullet fishing. In a study conducted in Sinop (Ozdemir et al., 2005) the catch composition of trammel nets with 18 mm mesh size rigged with monofilament and multifilament materials, as well as gillnets, the researchers determined that the trammel net with monofilament material was more efficient in whiting (*Merlangius merlangus euxinus* Linnaeus, 1758) fishing. However, no significant difference was found in the catch of by-catch species.

With the re-legalization of the use of monofilament nets under Communique No. 5/1 published by the Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture (Anonymous, 2020), it has been observed that small-scale fishers have started to use nets rigged with both the outer and the inner panels entirely of monofilament material instead of multifilament trammel nets (MuTNs). While this shift brings a significant transformation in fishing practices, it necessitates a scientific evaluation of the impacts of such nets on bycatch species and catch efficiency.

In this study, two different bottom trammel nets consisting entirely of monofilament and multifilament materials, were compared in terms of the catch efficiency and discarded catch rates. The findings are expected to contribute to sustainable fisheries management and provide a basis for evaluating the ecological effects of fisheries regulations.

Material and Methods

The trials of this study were carried out between September 2024 and March 2026 in the regions of Morto Bay (1), Tekke Bay (2), Çukurçeşme Bay (3), Çifte Burunlar, and Alçıtepe (4), located within the borders of Çanakkale province (Figure 1). These locations were selected due to extensive use of bottom trammel nets by commercial fishers in this area.

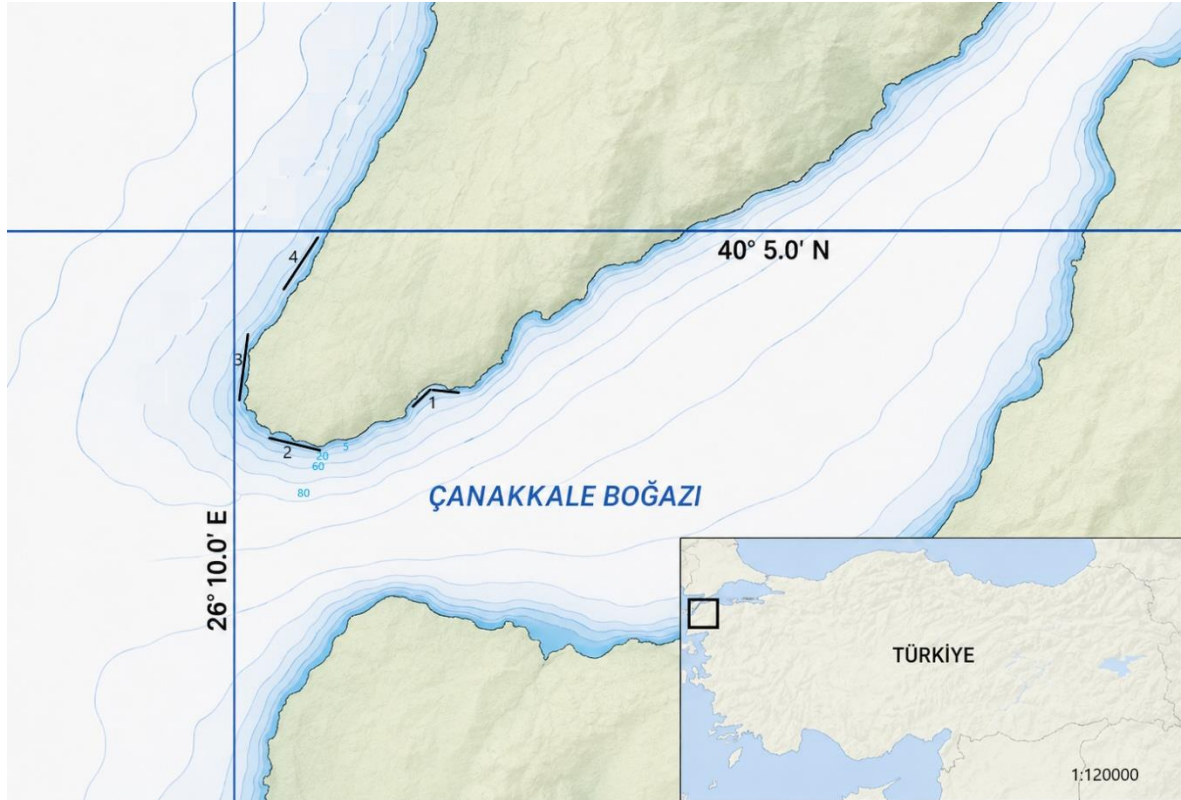


Figure 1. Study area

Trials within the scope of the study were carried out with a 7-meter-long commercial fishing vessel named "REMZİ KAPTAN 17," which has an engine power of 90 hp.

In the trials, a total of 8 units (100 m) of bottom trammel nets, consisting of 4 units of monofilament and 4 units of multifilament nets were used. To prevent variables such as region and depth between trials, each multifilament and monofilament trammel net (MoTNS) was connected to each other. One unit of multifilament net and one unit of monofilament net deployed together were considered as a single trial. The nets were set in the water before dark in the evening, using the method employed by commercial fishers, and were deployed in the water for a total of 36 hours. During this period, diving was performed under suitable weather conditions to visualize the fish caught in the nets underwater. Live specimens listed as endangered were released after measuring their length and weight. Other species and dead shark species were separated into different boxes according to the net materials and trial groups, and necessary morphological measurements were performed later.

The trammel nets were equipped with a 84 mm stretch mesh size and constructed using monofilament and

multifilament materials, while all other technical characteristics were kept identical. The technical characteristics of the nets are given in Figures 2 and 3.

When determining the target catch, all species with economic value were included in the target catch by considering the legal catch sizes. Economic species without a legal catch size were included in the target catch by determining the size at which they are commercially valued, based on interviews with fishers. Catches involving priority conservation species and species lacking economic value were classified as bycatch and discarded catch. Catch Per Unit Effort (CPUE) was calculated per 100 m of net per operation. The operational data obtained in the field studies were subjected to analysis to determine whether the difference was statistically significant on a numerical and weight basis according to the materials. The comparison of the total catch obtained according to the materials in each operation on a numerical basis was performed using one-way repeated measures analysis of variance (ANOVA).

The whole video-photographic archive is deposited in the Benthic Ecology Laboratory of Messina University, and available upon request..

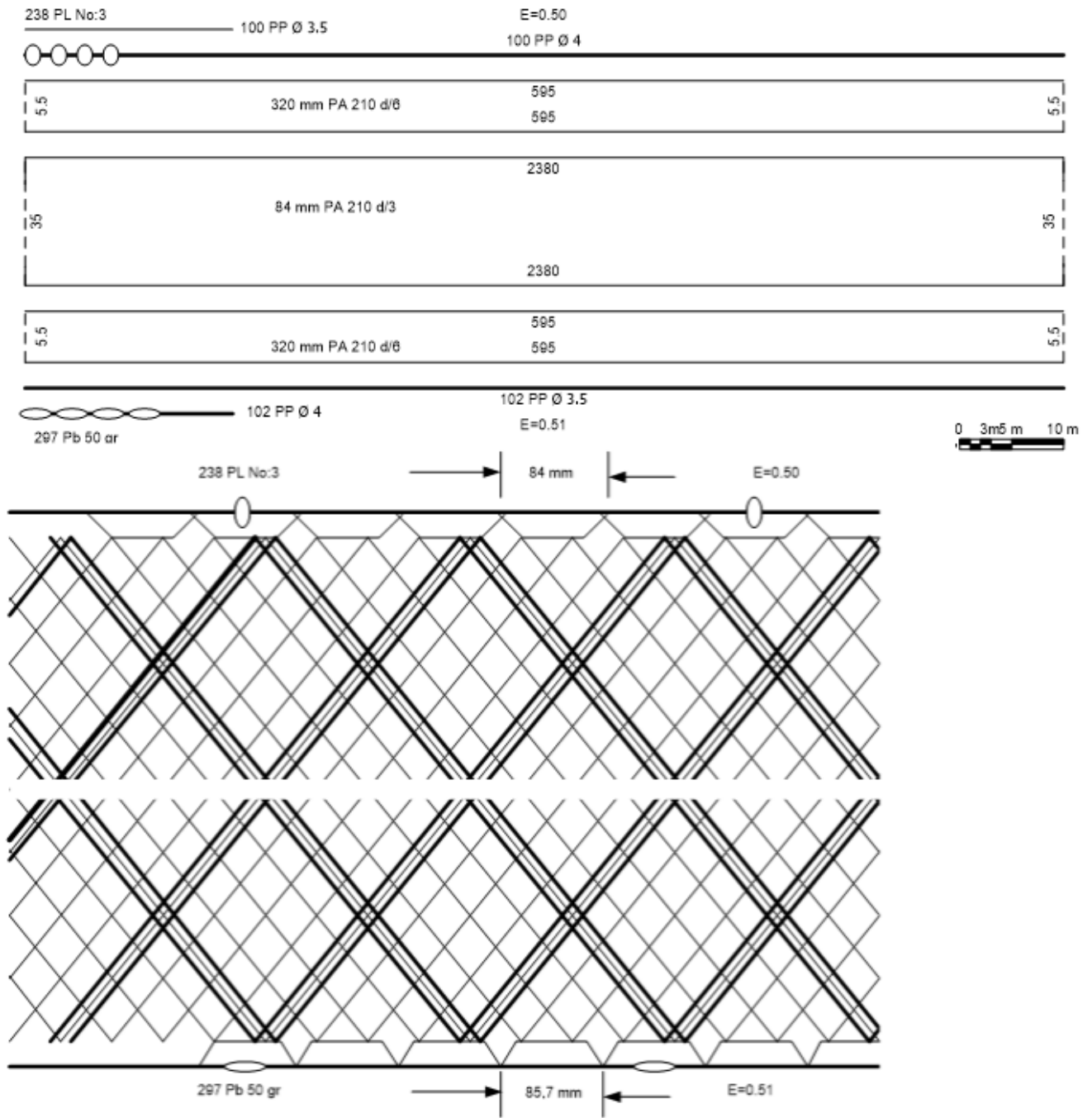


Figure 2. Technical plan of multifilament bottom trammel nets

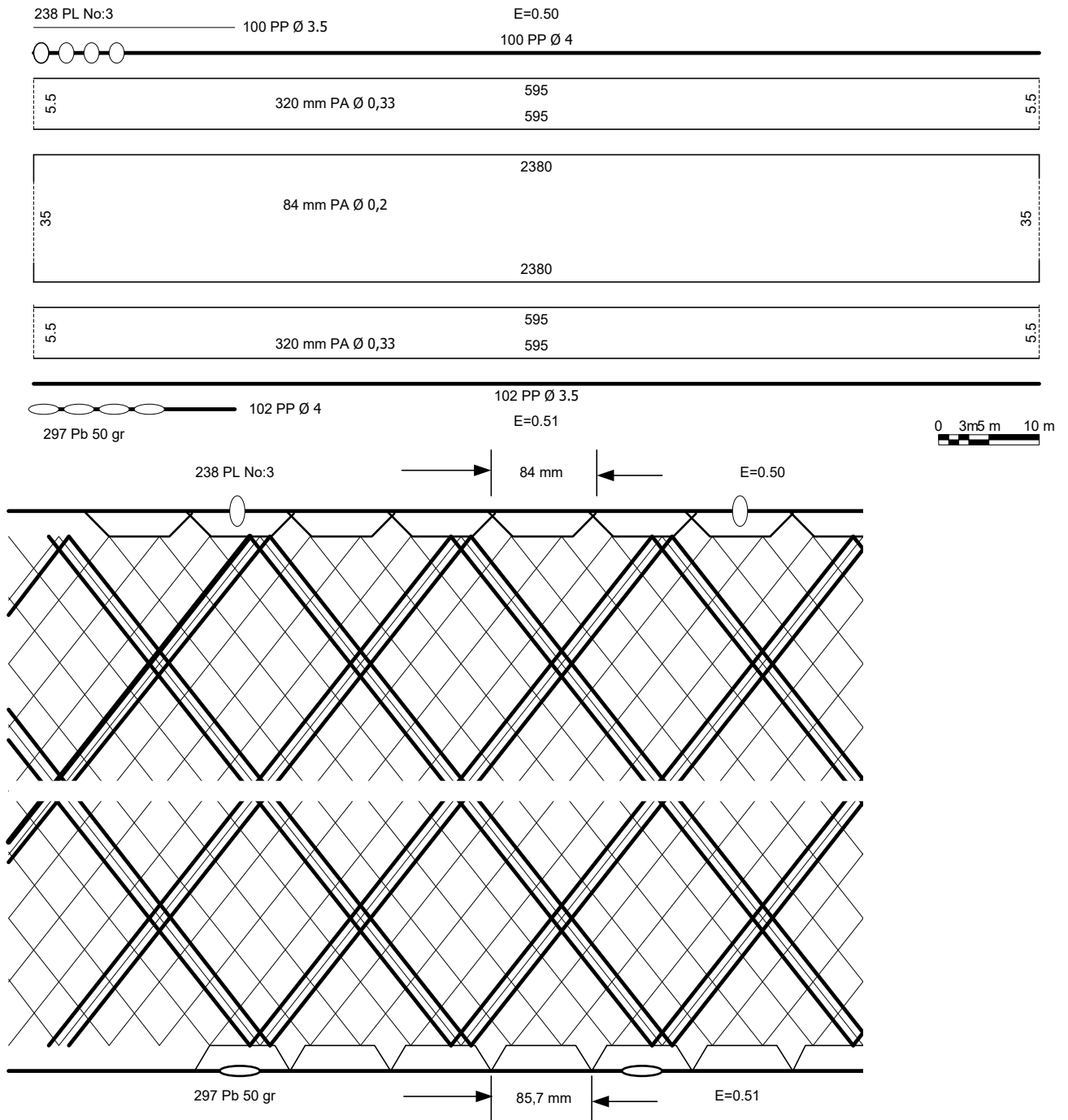


Figure 3. Technical plan of monofilament bottom trammel nets

Results

A total of 20 species were caught during the trials. The number and weight of the target species caught are given in Table 1, and discarded species are given in Table 2. During the study, 981 individuals and 622.3 kg of fish were caught with the trial nets. When compared, multifilament bottom trammel nets with 84 mm stretch mesh size caught a total of 506 individuals (279.8 kg), while monofilament bottom trammel nets caught 475 individuals (342.5 kg) (Tables 1, 2). However, when the catch amounts of target species were examined, 430 individuals (165.1 kg) were caught with multifilament trammel nets, and 352 individuals (137.9 kg) were caught with monofilament trammel nets (Table 1). During the study, in terms of number, black scorpionfish (*Scorpaena porcus* Linnaeus, 1758) ranked first with 468 individuals, followed by common cuttlefish (*Sepia officinalis* Linnaeus, 1758) with 252 individuals, and smoothback angelshark (*Squatina oculata*). In terms of weight, smoothback angelshark (*Squatina oculata*) ranked first with 242.9 kg, followed by common cuttlefish (*Sepia officinalis*) with 185.9 kg and black scorpionfish (*Scorpaena porcus*) with 98.8 kg (Tables 1, 2). While 16 species were caught with multifilament trammel nets, 20 species were caught with monofilament trammel nets. Among the priority conservation species, 2 individuals of common eagle ray (*Myliobatis aquila* Linnaeus, 1758)

were caught by monofilament trammel nets, whereas multifilament trammel nets did not catch any individuals of this species. Priority conservation chondrichthyan species accounted for 23.2% of the total catch in monofilament trammel nets, and 12.5% in multifilament trammel nets. Monofilament trammel nets increased the catch rate of priority conservation species by approximately 1.85 times compared to multifilament trammel nets: 94 individuals of priority conservation species were caught in monofilament trammel nets, and 43 individuals in multifilament trammel nets. Specifically, it was determined that individuals of *Squatina oculata* and *Squatina aculeata*, which are classified as "Critically Endangered", were caught in higher numbers using monofilament trammel nets (Table 2). When target species such as common cuttlefish (*Sepia officinalis*), black scorpionfish (*Scorpaena porcus*), common sole (*Solea solea* Linnaeus, 1758), and common pandora (*Pagellus erythrinus* Linnaeus, 1758) were evaluated, multifilament trammel nets caught 1.22 times more than those caught by monofilament trammel nets. Statistically, multifilament trammel nets caught more than monofilament trammel nets ($F = 10.68$; $df = 1.61$, $P < 0.05$). In the discarded catch, monofilament trammel nets caught 1.62 times more individuals than multifilament trammel nets (Table 3) which was statistically significant ($F = 7.28$; $df = 1.28$, $P < 0.05$).

Table 1. The amount of target catches in the bottom trammel nets

Scientific name	Common name	MuTNs		MoTNs	
		N	Weight (g)	N	Weight (g)
<i>Sharpsnout seabream</i>	Sheephead bream	4	1066	2	471
<i>Diplodus vulgaris</i>	Common two-banded seabream	6	932	2	390
<i>Pagellus bogaraveo</i>	Blackspot seabream	3	149	4	312
<i>Pagellus erythrinus</i>	Common pandora	1	155	7	1376
<i>Sarpa salpa</i>	Salema	8	6395	3	1768
<i>Scorpaena porcus</i>	Black scorpionfish	264	56166	204	42594
<i>Sepia officinalis</i>	Common cuttlefish	133	98335	119	87614
<i>Solea solea</i>	Common sole	7	1229	5	1370
<i>Spondyllosoma cantharus</i>	Black seabream	4	652	4	649
<i>Umbrina cirrosa</i>	Shi drum	0	0	2	1332
Total		430	165079	352	137876

A significant part of the chondrichthyan species caught in this the study belonged to the high-risk group in the International Union for Conservation of Nature (IUCN) Red List category. The smoothback angelshark (*Squatina oculata*) and the sawback angelshark (*Squatina aculeata*) were evaluated as "Critically Endangered," the rough ray (*Raja radula*) as "Endangered," common stingray

(*Dasyatis pastinaca*), and the common eagle ray (*Myliobatis aquila*) as "Vulnerable." Most of these species are benthic and show high sensitivity to trammel nets. Therefore, assessing the effect of net material on bycatch rates was important not only from the perspective of catch efficiency but also for the conservation of these species.

Table 2. The amount of discarded catches in the bottom trammel nets

Scientific name	Common name	IUCN Red List Status	MuTNs		MoTNs	
			N	Weight (g)	N	Weight (g)
<i>Squatina aculeata</i>	Sawback angelshark	Critically Endangered	5	2019	21	6828
<i>Squatina oculata</i>	Smoothback angelshark	Critically Endangered	31	75002	55	167947
<i>Raja radula</i>	Rough ray	Endangered	3	780	9	1769
<i>Dasyatis pastinaca</i>	Common stingray	Vulnerable	4	1333	7	1749
<i>Myliobatis aquila</i>	Common eagle ray	Vulnerable	0	0	2	1446
<i>Conger conger</i>	European conger	Least Concern	1	552	1	557
<i>Scyliorhinus canicula</i>	Lesser spotted dogfish	Least Concern	0	0	1	2105
<i>Serranus scriba</i>	Painted comber	Least Concern	1	193	3	569
<i>Torpedo marmorata</i>	Marbled electric ray	Least Concern	20	30214	15	18716
<i>Uranoscopus scaber</i>	Stargazer	Least Concern	11	4657	9	2878
Total			76	114750	123	204564

Table 3. Catch quantities and catch per unit effort for monofilament and multifilament bottom trammel nets

	MuTNs		MoTNs	
	N	Weight (g)	N	Weight (g)
Target Catch Quantity	430	165079	352	137876
Discarded Catch Quantity	76	114750	123	204564
Target Catch Efficiency (CPUE)	6.61±0.27	2540±105.4	5.42±0.23	2121±92.6
Discarded Catch Efficiency (CPUE)	1.17±0.05	1765±88.2	1.89±0.08	3147±142.8

In this study, Catch Per Unit Effort (CPUE) was calculated by dividing the total number and weight of target and discarded catch by the number of fishing operations, counting one monofilament and one multifilament trammel net as a single trial. CPUE values were expressed as individuals per 100 m per operation (N/100 m/operation $\pm$ SE) and weight per 100 m per operation (kg/100 m/operation $\pm$ SE) (Table 3). Based on the results of the trials, the percentage distribution of target and discarded catches in monofilament and multifilament trammel nets, expressed in terms of the number of individuals, is presented in Figures 4 and 5, respectively.

In multifilament trammel nets, the target catch rate was 85%, and the discarded catch rate was 15%. In monofilament trammel nets, these values were 74% and 26%, respectively.

Dives were conducted under suitable weather conditions to capture underwater images of the trial nets and caught individuals. In the underwater photographs, it is clearly distinguishable that multifilament trammel nets are much more visible than monofilament trammel nets. Sample images are provided in Figures 6 and 7.

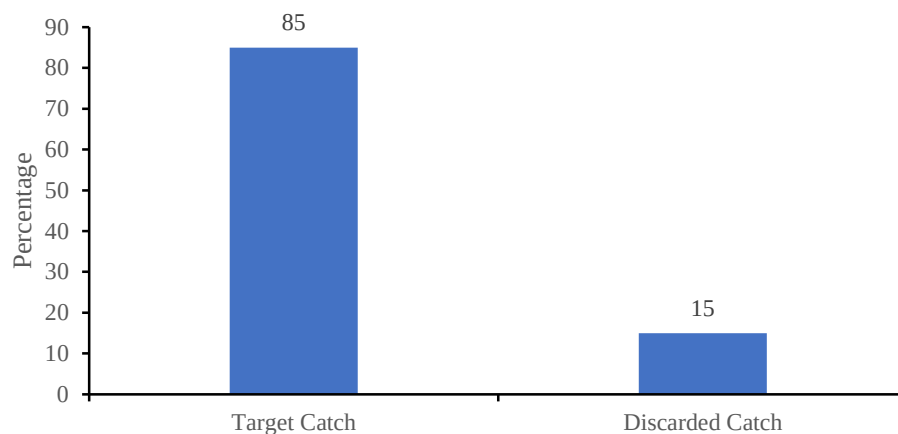


Figure 4. Catch distribution in multifilament trammel nets

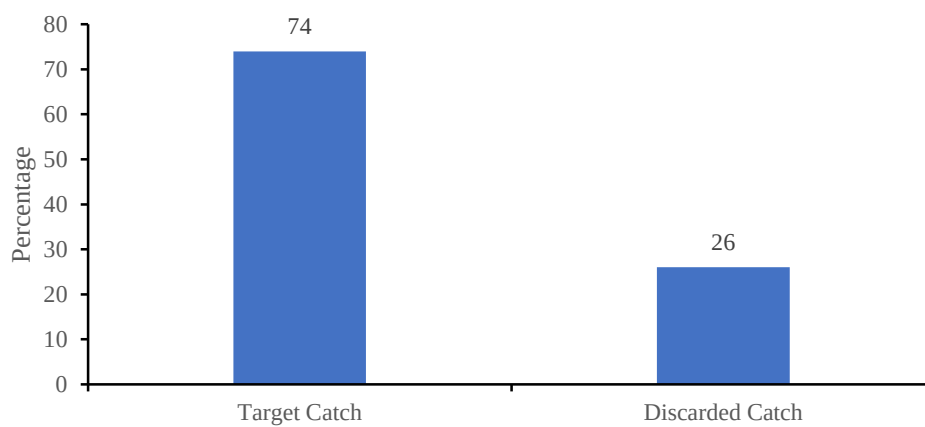


Figure 5. Catch distribution in monofilament trammel nets



Figure 6. Rough Ray (*Raja radula*) in multifilament trammel net



Figure 7. Sawback Angelshark (*Squatina aculeata*) in monofilament trammel net

Discussion

In the trials, multifilament trammel nets caught 16 species, while monofilament trammel nets caught 20 species of fish. In addition, 94 individuals categorized as priority conservation species (Otero et al., 2019) were caught in monofilament trammel nets consisting of 55 *Squatina oculata*, 21 *Squatina aculeata*, 9 *Raja radula*, 7 *Dasyatis pastinaca* and 2 *Myliobatis Aquila*, whereas a total of 43 individuals were caught in multifilament trammel nets, including 31 *Squatina oculata*, 5 *Squatina aculeata*, 3 *Raja radula*, and 4 *Dasyatis pastinaca*. This result indicated that the net material has a significant impact on the catch rate of these species.

While a limited number of studies have addressed species diversity in Turkish inland fisheries using nets of varying materials, there is a lack of comprehensive research on nets fully constructed with both monofilament and multifilament materials. In our study, it is thought that this difference affects the fishing of priority conservation species, especially within the bycatch. This difference may be related to the lower underwater visibility of monofilament trammel nets compared to that of multifilament trammel nets underwater (Ayaz et al., 2026).

As a result of the statistical comparison, multifilament trammel nets caught more target species than monofilament trammel nets. On a numerical basis, target species were caught 1.22 times more by multifilament trammel nets than those by monofilament trammel nets. In the discarded catch, monofilament trammel nets caught 1.62 times more than those caught by multifilament trammel nets. On a weight basis, while multifilament trammel nets caught 1.2 times more target species than monofilament trammel nets, monofilament trammel nets

caught 1.78 times more discarded catch than multifilament trammel nets.

Thomas et al. (2003) conducted a study on the coasts of India with three different net types: plain multifilament, monofilament, and multifilament trammel nets. As a result of the trials, they stated that for the amount of target shrimp, the net with monofilament material caught 1.5 times more than the plain multifilament net, and the multifilament trammel net caught 2 times more than the monofilament net. This finding highlights the importance of selecting net types according to the target species. In the trials, the target species catch rate was 74% in monofilament trammel nets and 85% in multifilament trammel nets. In a study conducted in the region, İbin and Ayaz (2022) calculated the target catch rate as 20% for 20 mm gillnets. Similarly, for a gillnet with the same characteristics and a 20 mm mesh size, this value was calculated as 13% (Ayaz et al., 2010). It is thought that this difference arises because nets with small mesh sizes catch more bycatch species in regions with high species diversity, and also not all economic species were included in the target catch group. As a result of the study, it was determined that multifilament trammel nets provided higher efficiency in target species fishing compared to monofilament trammel nets and significantly reduced the bycatch rate.

In another study conducted in the same region between 2008 and 2010 using multifilament trammel nets, bycatch was 12% higher than target catches on a weight basis (Cilasın et al., 2015). In our study, the target catch obtained with multifilament nets was 30% higher than the discard weight. This difference is thought to be related to the seasonal differences during which the experiments were conducted.

A study conducted in the İzmir Bay reported that multifilament nets accounted for 19.1% of the catch distribution, whereas monofilament nets accounted for 80.9% of the total catch (Aydın and Metin, 2008). In our study, however, monofilament trammel nets had an approximately 16% lower catch rate in the target catch. This difference is thought to be due to variations in the water conditions in the environments where the experimental nets were used.

The experimental nets used in the study were deployed for a total of 36 hours. Although this soaking duration may be considered long compared with that used in many other regions, fishermen in the Çanakkale region typically leave bottom trammel nets in the water for a similar period. Almost all priority conservation species caught in the nets were found dead. The long soaking time is thought to have contributed to this outcome.

Conversely, monofilament trammel nets markedly increased the catch rate of chondrichthyan fish species which are categorized as critically endangered and endangered by the International Union for Conservation of Nature. While the ratio of priority conservation species within the total catch was calculated as 23.2% in monofilament trammel nets, this ratio was 12.5% in multifilament trammel nets. These findings revealed that net material should be evaluated not only in terms of economic efficiency but also within the framework of ecosystem-based fisheries management. These findings suggest that regulatory authorities should reconsider the unrestricted use of monofilament trammel nets, particularly in regions where priority conservation chondrichthyan species are known to occur. Implementing material-based regulations, seasonal restrictions, or spatial management measures could significantly reduce the incidental capture of vulnerable and critically endangered species. In this context, encouraging or mandating the use of multifilament trammel nets may represent a practical and immediately applicable mitigation strategy. There is a need for studies aimed at material-based regulations for bycatch mitigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

O.A.: Conceptualization, sample collection, identification of species, data analysis, writing original draft. U.A.: Sample collection, identification of species, review and editing. Authors have read and agreed to the published version of the manuscript.

Ethics Approval

All processes and experimental protocols have been approved by Çanakkale Onsekiz Mart University, Animal Experiments Local Ethics Committee (No: 2024/03-02), (No: 2025/03-01) under the General Directorate of Agricultural Research and Policy surveillance.

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RESEARCH ARTICLE

Health Risks of Heavy Metals in Surface Waters of Mogan Lake (Ankara, Türkiye) for Recreational and Residential Users

Semih Sayın^{1*}, Serap Pulatsü²

¹Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture, Ankara, Türkiye

²Ankara University, Faculty of Agriculture Department of Fisheries and Aquaculture, 06100, Ankara, Türkiye

<https://orcid.org/0009-0002-6029-7451>

<https://orcid.org/0000-0001-5277-417X>

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Abstract: Mogan Lake is an important recreational center for the capital city Ankara, not only because of its ecological value but also because of the various recreational activities it offers. In this study, the levels of certain heavy metals (As, Cd, Cr, Cu, Ni, Pb, Hg, Zn, Fe) were determined in surface waters at three selected stations in Mogan Lake for individuals (adults and children) who are exposed to heavy metals detected in the lake water and use it for both residential and recreational purposes, health risks via ingestion and dermal contact were estimated as follows: a) Non-carcinogenic health risks (Hazard Quotient – HQ, Hazard Index – HI, and Total Hazard Index - THI), and b) Carcinogenic health risks (Total Cancer Risk – TCR = CR_{ingestion} + CR_{dermal}) and Cancer Risk Index (CR). According to the research findings; a) Exposure to heavy metals does not pose non-carcinogenic health risks (THI < 1), b) The hazard index values for children (HI_{ingestion} and HI_{dermal}) are higher than those for adults, c) Based on the TCR values for As, exposure to this metal through ingestion and dermal absorption does not pose a health risk for recreational users (TCR < 1×10⁻⁴), while for residents, although it does not constitute a significant cancer risk, it is not entirely negligible (1.13×10⁻⁶ – 1.43×10⁻⁶). In this context, monitoring especially arsenic (As) levels in the lake water should be considered an essential component of lake management, both to protect the health of residents and to ensure the sustainability of the lake's recreational potential.

Anahtar kelimeler:

Kanserojenik olmayan sağlık riskleri
Kanserojenik sağlık riskleri
Kanser risk indeksi
Yüzey suları
Mogan Gölü

Mogan Gölü (Ankara, Türkiye) Yüzey Sularındaki Ağır Metallerin Rekreatif ve Konut Kullanıcıları İçin Sağlık Riskleri

Öz: Mogan Gölü, yalnızca ekolojik değeri açısından değil, aynı zamanda sunduğu çeşitli rekreatif aktiviteler açısından da başkent Ankara için önemli bir dinlenme merkezidir. Bu çalışmada, Mogan Gölü'nde seçilen üç istasyon yüzey sularında belirli ağır metallerin (As, Cd, Cr, Cu, Ni, Pb, Hg, Zn, Fe) seviyeleri belirlenmiştir. Göl suyunun konut ve rekreatif amaçlı kullanımı yoluyla bu ağır metallerle maruz kalan yetişkinler - çocuklar için yutma ve deri teması yoluyla oluşan sağlık riskleri tahmin edilmiştir. Bu kapsamda; a) Kanserojenik olmayan sağlık riskleri (Tehlike Katsayısı – HQ, Tehlike İndeksi - HI, Toplam Tehlike İndeksi - THI), b) Kanserojenik sağlık riskleri (TCR = CR_{yutma} + CR_{dermal}) ve Kanser Risk İndeksi (CR) saptanmıştır. Araştırma bulgularına göre; a) Mogan Gölü yüzey sularındaki ağır metallerin olumsuz kanserojenik olmayan sağlıksal etkilerinin olmadığı (THI < 1), b) Çocuk tehlike indeksi değerlerinin (HI_{ingestion} - HI_{dermal}) yetişkinlere göre daha yüksek olduğu, c) As'e ilişkin TCR değerine göre bu metale yutma ve dermal absorpsiyon yolu ile maruziyetin rekreatif kullanıcılar açısından sağlıksal bir tehdit oluşturmadığı (TCR < 1×10⁻⁴), yerleşik sakinlerin sağlığı açısından ise ciddi bir kanser riski oluşturmamakla birlikte riskin tamamen ihmal edilebilir seviyede (1.13×10⁻⁶ - 1.43×10⁻⁶) olmadığı tespit edilmiştir. Bu bağlamda, gerek yerleşik sakinlerin sağlığının korunması ve gerekse gölün rekreatif potansiyelinin devamlılığı açısından, göl sularında özellikle As düzeylerinin izlenmesi, göl yönetiminin önemli bir parçası olarak dikkate alınmalıdır.

Introduction

Heavy metals, one of the factors causing water pollution, have a wide range of sources compared to other pollutants and reach aquatic ecosystems not only through various anthropogenic sources such as industrial wastewater, mining activities, and untreated municipal

wastewater, but also via natural processes such as weathering and erosion (Pulatsü & Topçu, 2015; Kianersi et al., 2025). Heavy metals in aquatic ecosystems can pose significant health risks to both humans (Briffa et al., 2020; Khushbu et al., 2022) and aquatic organisms (Vajargah,

*Corresponding author: semsayin06@gmail.com

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2021; Banaee, 2024) due to their toxicity, low biodegradability, and potential for bioaccumulation.

As a result of intense anthropogenic pressure, water pollution caused by heavy metals threatens aquatic environments and, consequently, human health. Heavy metals can have adverse health effects by posing both non-carcinogenic and carcinogenic risks to humans. Heavy metals can enter the human body through the consumption of contaminated food, inhalation, drinking polluted water, and dermal contact from agricultural, pharmaceutical, manufacturing, residential, and industrial sources. Exposure to contaminated water can cause acute or chronic health effects depending on the types of pollutants, the duration, and the level of exposure. Long-term exposure to heavy metals may lead to organ damage, developmental disorders, reproductive issues, and an increased risk of cancer (Mukherjee et al., 2021; Patel et al., 2021; Saravanan et al., 2024).

Rivers and lakes have been increasingly investigated for heavy metal pollution, and as these studies deepen, conventional methods for characterizing and assessing heavy metal contamination are becoming progressively inadequate to meet the needs of ecological environmental protection. Consequently, biotoxicological and human health assessments (Zhaoyong et al., 2015; Wang et al., 2024) are being used more frequently as complementary approaches to evaluate the impacts of heavy metal pollution in these aquatic systems. In this context, a common practice in health risk assessment is to determine the risk levels of heavy metals in water bodies based on established models and standards (USEPA, 2004; ATSDR, 2018; Yu et al., 2022).

Mogan Lake is one of the natural assets of Türkiye's capital city, Ankara, and an important recreational and tourism area for the urban population. Located in the Gölbaşı district, approximately 25 km from the city center, commercial fishing is prohibited in the lake. However, activities such as birdwatching, recreational fishing, rowing, sailing, and nature photography are carried out. The lake shores are heavily used for picnicking, especially on weekends. Intensive dry farming is practiced in the southern part of the lake and around the Çökek Marsh. Rapid and unplanned urbanization processes within the lake basin, along with increasing mining activities, have led the lake to be subjected to significant pollution pressure. The main causes of pollution in Mogan Lake include domestic wastewater, agricultural chemicals, pollutants transported by inflow waters, and solid waste (Anonymous, 2026). In this context, several studies have been conducted to determine the spatial and seasonal variations of heavy metals in the lake water-sediment system (Küçükosmanoğlu & Filazi, 2020; Binici et al., 2021; Binici & Pulatsü, 2022). This study, however, focuses on whether the surface waters of the lake pose a risk to human health in relation to heavy metal contamination. In this context, for surface waters at three selected stations in Mogan Lake, across two different periods and for residential and recreational receptors (adults and children), (a) non-carcinogenic health risk was

evaluated using Hazard Quotient (HQ; via ingestion and dermal absorption), Hazard Index (HI), and Total Hazard Index (THI), and (b) carcinogenic health risk was assessed using Total Cancer Risk (TCR).

Material and Methods

Material

Study area

Mogan Lake is a natural dam lake formed behind alluvial barriers. With an average surface area of 5.6 km², Mogan Lake discharges into Eymir Lake, which has an area of approximately 1.25 km² in the northeast, through a wetland-marsh transitional zone extending 2 km from the southern part of the lake, via both surface and subsurface flow, and with an average elevation difference of about 5 meters. The Mogan-Eymir lakes and surrounding wetland-marsh areas constitute important bird habitats in Türkiye, having been proposed as a Ramsar site and serving as breeding, resting, and overwintering areas for 227 bird species. Of these species, 40 breed in the region, 30 are observed throughout the year, and the remainder are recorded during migration periods or only around the lake area (Anonymous, 2026).

Selection of the research stations

In this study, surface water samples were collected from stations selected to represent the pollution sources of Mogan Lake, based on previous studies. In this context, the characteristics of the selected stations are as follows:

Station I: The station where wastewater originating from quarry activities is intensely discharged;

Station II: The station is located closest to densely populated residential areas;

Station III: The station selected within the region where agricultural lands are predominant.

The study area and the locations of the selected stations are shown in Figure 1.

Methods

Surface water samples from Mogan Lake were collected at a depth of 30 cm during the dry period (August 2025) and the rainy period (December 2025), and subsequently transported to the laboratory under dark and cold conditions.

Laboratory work

Heavy metal analyses of the water samples were performed in an accredited laboratory with four replicates. For heavy metal (Hg, As, Cd, Cr, Pb, Ni, Cu, Zn, Fe) analyses, TS EN ISO 17294-1.2 was used. Non-carcinogenic health risks (Hazard Quotient (HQ; through ingestion and dermal absorption), Hazard Index (HI), Total Hazard Index (THI)) were estimated using Equations (1–6), while carcinogenic health risks (TCR = CR_{ingestion} + CR_{dermal}) were calculated using Equations (7–17); additionally, the USEPA (2024) Regional Screening Level

Calculator, developed to validate HQ and CR values for heavy metals, was also utilized. The equations used within the scope of the study are presented below, and the values

and references considered in the calculations are provided in Table 1.

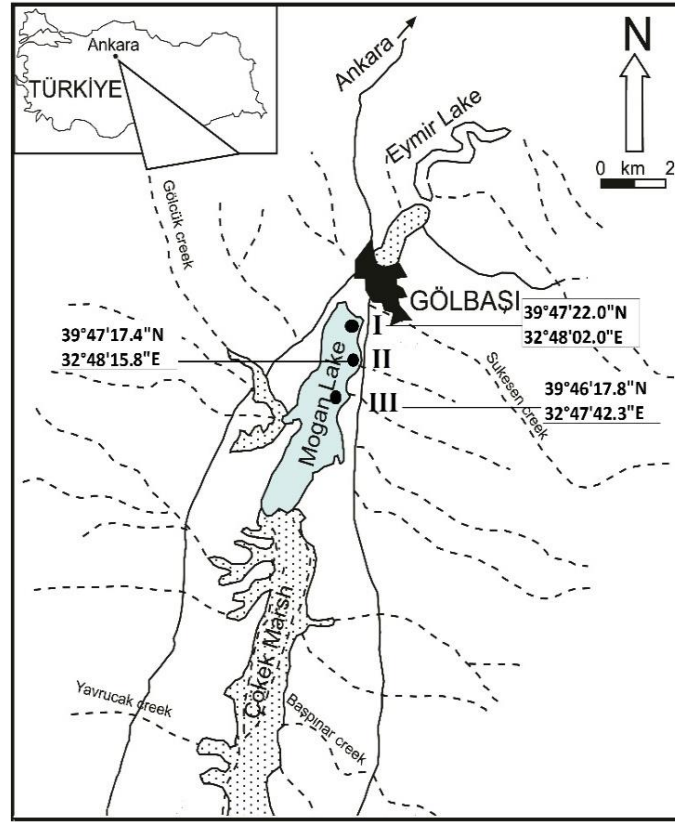


Figure 1. Mogan Lake and selected stations in the study area. Index map shows the geographical location of the study area within Ankara, Türkiye.

Non-carcinogenic risks for residential receptors

$$HQ_{\text{ingestion}} = \frac{Cw \times IRW_{\text{res}} \times EF_{\text{res}} \times ED}{BW \times AT_{\text{res}} \times RfDo \times 10^3} \quad (1)$$

$$HQ_{\text{ingestion}} = \frac{CCw \times SA \times Kp \times ET_{\text{res}} \times EV \times EF_{\text{res}} \times ED}{BW \times AT_{\text{res}} \times RfDo \times GIABS \times 10^6} \quad (2)$$

Non-carcinogenic risks for recreational receptors

$$HQ_{\text{ingestion}} = \frac{Cw \times IRW_{\text{rec}} \times EF_{\text{rec}} \times ED \times EV \times ET_{\text{rec}}}{BW \times AT_{\text{rec}} \times RfDo \times 10^3} \quad (3)$$

$$HQ_{\text{ingestion}} = \frac{Cw \times SA \times Kp \times ET_{\text{rec}} \times EV \times EF_{\text{rec}} \times ED}{BW \times AT_{\text{rec}} \times RfDo \times GIABS \times 10^6} \quad (4)$$

$$HI = \sum HQ_s \quad (5)$$

$$THI = HI_{\text{ingestion}} + HI_{\text{dermal}} \quad (6)$$

When HI and THI are < 1, there are no non-carcinogenic health effects; however, if HI and THI are > 1, non-carcinogenic risk/adverse effects on human health are a cause for concern. (USEPA,2004).

Carcinogenic risks for residential receptors

$$CR_{\text{ingestion}} = \frac{Cw \times IFW_{\text{res}} \times CSFo}{BAT \times 103} \quad (7)$$

$$IFW_{\text{res}} = \frac{EF_{\text{res}} \times EDa \times IRW_{\text{res-a}}}{BWa} + \frac{EF_{\text{res}} \times EDc \times IRW_{\text{res-c}}}{BWc} \quad (8)$$

$$CR_{\text{dermal}} = \frac{Cw \times Kp \times 0.001 \times ET_{\text{event-res}} \times DFW_{\text{res}} \times CSFo}{AT \times GIABS \times 10^3} \quad (9)$$

$$ET_{\text{event-res}} = \frac{ET_{\text{res-a}} \times EDa + ET_{\text{res-c}} \times EDc}{ED} \quad (10)$$

$$DFW_{\text{res}} = \frac{Eva \times EF_{\text{res}} \times EDa \times SAa}{BWa} + \frac{Evc \times EF_{\text{res}} \times EDc \times SAC}{BWc} \quad (11)$$

Carcinogenic risks for recreational receptors:

$$CR_{\text{ingestion}} = \frac{Cw \times IFW_{\text{rec}} \times CSFo}{AT \times 103} \quad (12)$$

$$IFW_{\text{rec}} = \frac{Eva \times ET_{\text{rec-a}} \times EF_{\text{rec}} \times EDa \times IRW_{\text{rec-a}}}{BWa} + \frac{Evc \times ET_{\text{rec-c}} \times EF_{\text{rec}} \times EDc \times IRW_{\text{rec-c}}}{BWc} \quad (13)$$

$$CR_{\text{dermal}} = \frac{C_w \times K_p \times 0.001 \times E_{\text{Tevent-rec}} \times DFW_{\text{rec}} \times CS_{\text{Fo}}}{AT \times GI_{\text{ABS}} \times 103} \quad (14)$$

$$E_{\text{Tevent-rec}} = \frac{E_{\text{Trec-a}} \times ED_a + E_{\text{Trec-c}} \times ED_c}{ED} \quad (15)$$

$$DFW_{\text{rec}} = \frac{E_{\text{Va}} \times E_{\text{Frec}} \times ED_a \times SA_a}{BW_a} + \frac{-E_{\text{Vc}} \times E_{\text{Frec}} \times ED_c \times SA_c}{BW_c} \quad (16)$$

$$TCR = CR_{\text{ingestion}} + CR_{\text{dermal}} \quad (17)$$

$CR > 1.0 \times 10^{-4}$ is considered unacceptable, $1.0 \times 10^{-4} < CR < 1.0 \times 10^{-6}$ is considered an acceptable range

depending on the exposure conditions, $CR < 1.0 \times 10^{-6}$ is considered not to have significant health effects (USEPA, 1989).

Statistical analyses:

Within the scope of the study, the Kruskal–Wallis test was used to determine whether there was a statistically significant difference between adult and child health risk indices across stations during the dry and rainy periods (Kolassa, 2020). All statistical analyses were performed using SPSS 22.

Table 1. Variables used in health risk estimation of recreational (RC) and residential (RS) receptors; **A)** Definitions, symbols, units and values, **B)** Dermal permeability coefficient, reference dose, slope factor and gastrointestinal absorption coefficient for each element

A

Definitions	Symbols	Units	Values
Element concentration	C_w	µg/L	
Body weight-adult	BW_a	kg	70 ^a
Body weight-child	BW_c	kg	15 ^a
RS/RC exposure duration	ED	years	26 ^a
RS/RC exposure duration-child	ED_c	years	6 ^a
RS/RC exposure duration-adult	ED_a	years	20 ^a
RS exposure frequency	EF_{res}	days/year	350 ^a
RC exposure frequency	EF_{rec}	days/year	135 ^b
RS exposure time-adult	$ET_{\text{res-a}}$	hours/event	0.71 ^a
RS exposure time-child	$ET_{\text{res-c}}$	hours/event	0.54 ^a
RC exposure time-adult	$ET_{\text{rec-a}}$	hours/event	2 ^b
RC exposure time-child	$ET_{\text{rec-c}}$	hours/event	2 ^b
RS/RC events-adult	EV_a	per day	1 ^c
RS/RC events-child	EV_c	per day	1 ^c
RS/RC skin surface area-adult	SA_a	cm ²	19652 ^a
RS/RC skin surface area-child	SA_c	cm ²	6365 ^a
RS water intake ratio-adult	$IRW_{\text{res-a}}$	L/day	2.5 ^a
RS water intake ratio-child	$IRW_{\text{res-c}}$	L/day	0.78 ^a
RC water intake ratio-adult	$IRW_{\text{rec-a}}$	L/day	0.11 ^d
RC water intake ratio-child	$IRW_{\text{rec-c}}$	L/day	0.12 ^d
RS averaging time-adult	$AT_{\text{res-a}}$	days	365 x ED = 9490 (non-carcinogenic) ^a
RS averaging time-child	$AT_{\text{res-c}}$	days	365 x ED _c = 2190 (non-carcinogenic) ^a
RC averaging time-adult	$AT_{\text{rec-a}}$	days	365 x ED _a (non-carcinogenic) ^a
RC averaging time-child	$AT_{\text{rec-c}}$	days	365 x ED _c (non-carcinogenic) ^a
RS water ingestion rate	IFW_{res}	L/kg	Age-adjusted ^e
RC water ingestion rate	IFW_{rec}	L/kg	Age-adjusted ^e
RS water dermal contact factor	DFW_{res}	cm ² - event/kg	Age-adjusted ^e
RC water dermal contact factor	DFW_{rec}	cm ² - event/kg	Age-adjusted ^e
RS water exposure time	$ET_{\text{event-res}}$	hours/event	Age-adjusted ^e
RC water exposure time	$ET_{\text{event-rec}}$	hours/event	Age-adjusted ^e
Life time	LT	years	70 ^a
Averaging time	AT	days	365 x LT = 25550 (carcinogenic) ^a

B

Element	Dermal permeability constant (K_p ; cm/h)	Oral reference dose (RfD _o ; mg/kg-day)	Oral slope factor (CSF _o ; mg/kg-day)	Gastrointestinal absorption (GIABS; unitless)
As	0.001 ^c	0.0003 ^e	1.5 ^e	1 ^e
Cr	0.002 ^c	0.003 ^e	0.5 ^e	0.025 ^e
Cu	0.001 ^c	0.04 ^e		1 ^e
Fe	0.001 ^c	0.7 ^e		1 ^e
Ni	0.0002 ^c	0.02 ^e	1.7 ^f	0.04 ^e
Pb	0.0001 ^c	0.0014 ^e	8.5 ^g	1 ^e
Zn	0.0006 ^c	0.3 ^e		1 ^e

a-USEPA (2014), b- Site-specific, c) USEPA (2004), d) USEPA (2011), e) USEPA (2024), f - Kumar et al. (2019), g- Mohammadi et al. (2019)

Results

In the dry and rainy periods of the study, the potential non-carcinogenic risks (HQ) and the total potential non-carcinogenic risks caused by different exposure pathways (HI) for adults and children, based on three stations and separately for recreational and residential receptors, are presented in Tables 2–5. Our findings indicated that, Hg and Zn were below the detection limits at all stations during the dry period, while in the rainy period, Cd, Hg, and Pb at Stations I and II, and Cd, Hg, Pb, and Zn at Station III were found to be below the detection limits.

For recreational receptors, in both the dry and rainy sampling periods, child HQ_{ingestion} values and child THI values were found to be higher than those of adults. Likewise, in both periods, HQ_{ingestion} values were higher than dermal exposure (HQ_{dermal}) values. During the dry period, within the HQ_{ingestion} risk values, As contributed the most (maximum values for child and adult were 1.05×10^{-3} and 2.10×10^{-4} , respectively), while within the HQ_{dermal} risk values Cd showed the highest contribution (child: 4.07×10^{-4} ; adult: 2.69×10^{-4}). Based on THI values in the dry period, Station I, where quarry-related wastewater reaches the system, exhibited a higher risk compared to the other stations (child: 1.67×10^{-3} ; adult: 5.94×10^{-4}) (Table 2). While THI values for the dry period were higher than those of the wet period at Stations I and II, they showed similar trends at Station III (Table 3).

During the rainy period, As made a significant contribution to both HQ_{ingestion} and HQ_{dermal} risk values, while Cd, which had the highest contribution to HQ_{dermal} in the dry period, was not detected in this period. In addition, Station III, which has the highest likelihood of receiving agricultural runoff, showed comparatively lower risk levels than the other stations (child: 1.55×10^{-3} ; adult: 4.00×10^{-4}) (Table 3).

For recreational receptors, no statistically significant differences were observed in health risk indices among stations for both adults and children during the dry period

($p > 0.05$). In the wet period, however, health risk indices for both adults and children showed statistically significant differences among stations ($p < 0.05$). According to post hoc tests, these differences were observed between adult and child health risk indices measured at Station I and those obtained from Stations II and III, as well as between measurements from Station II and those from Station III. Nevertheless, all values being below 1 indicate that non-carcinogenic health risks for recreational receptors are at negligible levels.

For residential receptors, during the dry sampling period, HQ_{ingestion}, HQ_{dermal}, and THI values for children were found to be higher than those for adults across all stations. Among the investigated metals, As within HQ_{ingestion} (maximum values for child and adult were 8.99×10^{-3} and 6.18×10^{-3} , respectively) and Cd within HQ_{dermal} (child and adult maximum values were 2.85×10^{-4} and 1.91×10^{-4} , respectively) contributed more significantly to the total non-carcinogenic health risk compared to other metals. The higher THI values observed at Station II, which is closest to residential areas, compared to the other stations, support the conclusion that residential areas pose a potential risk for both metals (Table 4).

For this category, during the rainy sampling period, HQ_{ingestion}, HQ_{dermal}, and THI_{ingestion-dermal} values for children were found to be higher than those for adults at all stations, similar to the dry period (Table 5). In addition, As remained the dominant contributor to both HQ_{ingestion} and HQ_{dermal}. However, it is noteworthy that Cd, which showed the highest contribution to HQ_{dermal} during the dry period, was not detected in the rainy period. Based on total hazard index values in the rainy period, surface waters at Station III, which is closest to agricultural activity areas, exhibited higher risk levels compared to the other stations for both children and adults. Considering Tables 4–5, HQ_{ingestion} values were consistently higher than HQ_{dermal} values. Furthermore, the absence of Cd and Pb during the rainy

period appears to be a contributing factor to the substantially lower THI (child–adult) values observed at Stations II and III compared to those of the dry period. For

residential receptors, no statistically significant differences were found among stations in terms of $HI_{\text{ingestion}}$ values for both adults and children during the dry period ($p > 0.05$).

Table 2. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for dry season for recreational receptors

Stations	Metals	Concentration ($\mu\text{g/L}$) (mean $\pm$ SD)	$HQ_{\text{ingestion}}$		HQ_{dermal}	
			Child	Adult	Child	Adult
I	As	53.15 $\pm$ 1.22	1.05 $\times 10^{-3}$	2.06 $\times 10^{-4}$	1.36 $\times 10^{-4}$	8.97 $\times 10^{-5}$
	Cd	1.62 $\pm$ 0.14	1.92 $\times 10^{-5}$	3.77 $\times 10^{-6}$	4.07 $\times 10^{-4}$	2.69 $\times 10^{-4}$
	Cr	2.61 $\pm$ 1.92	5.15 $\times 10^{-6}$	1.01 $\times 10^{-6}$	2.18 $\times 10^{-5}$	1.44 $\times 10^{-5}$
	Cu	1.65 $\pm$ 0.39	2.44 $\times 10^{-7}$	4.79 $\times 10^{-8}$	2.59 $\times 10^{-6}$	1.71 $\times 10^{-6}$
	Fe	15.78 $\pm$ 2.27	1.33 $\times 10^{-7}$	2.62 $\times 10^{-8}$	3.54 $\times 10^{-8}$	2.34 $\times 10^{-8}$
	Ni	3.35 $\pm$ 0.16	9.92 $\times 10^{-7}$	1.95 $\times 10^{-7}$	6.58 $\times 10^{-6}$	4.35 $\times 10^{-6}$
	Pb	4.37 $\pm$ 0.70	1.85 $\times 10^{-5}$	3.63 $\times 10^{-6}$	3.26 $\times 10^{-7}$	2.16 $\times 10^{-7}$
$HI_{\text{ingestion}} - HI_{\text{dermal}}$			1.09 $\times 10^{-3}$	2.15 $\times 10^{-4}$	5.74 $\times 10^{-4}$	3.80 $\times 10^{-4}$
THI		Child	1.67 $\times 10^{-3}$			
		Adult	5.94 $\times 10^{-4}$			
II	As	54.09 $\pm$ 1.94	1.07 $\times 10^{-3}$	2.10 $\times 10^{-4}$	1.38 $\times 10^{-4}$	9.13 $\times 10^{-5}$
	Cd	1.54 $\pm$ 0.09	1.83 $\times 10^{-5}$	3.59 $\times 10^{-6}$	3.88 $\times 10^{-4}$	2.57 $\times 10^{-4}$
	Cr	2.00 $\pm$ 3.00	3.94 $\times 10^{-6}$	7.74 $\times 10^{-7}$	1.67 $\times 10^{-5}$	1.11 $\times 10^{-5}$
	Cu	1.56 $\pm$ 0.18	2.31 $\times 10^{-7}$	4.53 $\times 10^{-8}$	2.45 $\times 10^{-6}$	1.62 $\times 10^{-6}$
	Fe	11.81 $\pm$ 0.88	9.98 $\times 10^{-8}$	1.96 $\times 10^{-8}$	2.65 $\times 10^{-8}$	1.75 $\times 10^{-8}$
	Ni	4.06 $\pm$ 0.25	1.20 $\times 10^{-6}$	2.36 $\times 10^{-7}$	7.96 $\times 10^{-6}$	5.26 $\times 10^{-6}$
	Pb	4.71 $\pm$ 0.54	1.99 $\times 10^{-5}$	3.91 $\times 10^{-6}$	3.52 $\times 10^{-7}$	2.33 $\times 10^{-7}$
$HI_{\text{ingestion}} - HI_{\text{dermal}}$			1.11 $\times 10^{-3}$	2.18 $\times 10^{-4}$	5.53 $\times 10^{-4}$	3.66 $\times 10^{-4}$
THI		Child	1.66 $\times 10^{-3}$			
		Adult	5.84 $\times 10^{-4}$			
III	As	53.71 $\pm$ 0.94	1.06 $\times 10^{-3}$	2.08 $\times 10^{-4}$	1.37 $\times 10^{-4}$	9.07 $\times 10^{-5}$
	Cd	1.22 $\pm$ 0.07	1.45 $\times 10^{-5}$	2.84 $\times 10^{-6}$	3.07 $\times 10^{-4}$	2.03 $\times 10^{-4}$
	Cr	1.53 $\pm$ 2.12	3.02 $\times 10^{-6}$	5.94 $\times 10^{-7}$	1.28 $\times 10^{-5}$	8.49 $\times 10^{-6}$
	Cu	0.36 $\pm$ 0.15	5.38 $\times 10^{-8}$	1.06 $\times 10^{-8}$	5.71 $\times 10^{-7}$	3.78 $\times 10^{-7}$
	Fe	155.68 $\pm$ 53.43	1.32 $\times 10^{-6}$	2.59 $\times 10^{-7}$	3.49 $\times 10^{-7}$	2.31 $\times 10^{-7}$
	Ni	4.65 $\pm$ 0.43	1.37 $\times 10^{-6}$	2.70 $\times 10^{-7}$	9.11 $\times 10^{-6}$	6.03 $\times 10^{-6}$
	Pb	0.40 $\pm$ 0.21	1.69 $\times 10^{-6}$	3.33 $\times 10^{-7}$	2.99 $\times 10^{-8}$	1.98 $\times 10^{-8}$
$HI_{\text{ingestion}} - HI_{\text{dermal}}$			1.08 $\times 10^{-3}$	2.12 $\times 10^{-4}$	4.67 $\times 10^{-4}$	3.09 $\times 10^{-4}$
THI		Child	1.55 $\times 10^{-3}$			
		Adult	5.22 $\times 10^{-4}$			

Table 3. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for rainy season for recreational receptors

Stations	Metals	Concentration ($\mu\text{g/L}$) (mean $\pm$ SD)	HQ _{ingestion}		HQ _{dermal}	
			Child	Adult	Child	Adult
I	As	58.22 $\pm$ 1.88	1.15x10 ⁻³	2.26x10 ⁻⁴	1.49x10 ⁻⁴	9.83x10 ⁻⁵
	Cr	0.98 $\pm$ 0.74	1.93x10 ⁻⁶	3.80x10 ⁻⁷	8.20x10 ⁻⁶	5.42x10 ⁻⁶
	Cu	0.52 $\pm$ 0.57	7.75x10 ⁻⁸	1.52x10 ⁻⁸	8.22x10 ⁻⁷	5.44x10 ⁻⁷
	Fe	267.33 $\pm$ 86.61	2.26x10 ⁻⁶	4.44x10 ⁻⁷	5.99x10 ⁻⁷	3.97x10 ⁻⁷
	Ni	5.13 $\pm$ 0.35	1.52x10 ⁻⁶	2.98x10 ⁻⁷	1.01x10 ⁻⁵	6.66x10 ⁻⁶
	Zn	1.61 $\pm$ 0.31	3.18x10 ⁻⁸	6.25x10 ⁻⁹	5.06x10 ⁻⁹	3.35x10 ⁻⁹
HI _{ingestion} - HI _{dermal}			1.15x10 ⁻³	2.27x10 ⁻⁴	1.68x10 ⁻⁴	1.11x10 ⁻⁴
THI			Child	1.32x10 ⁻³		
			Adult	3.38x10 ⁻⁴		
II	As	63.20 $\pm$ 1.15	1.25x10 ⁻³	2.45x10 ⁻⁴	1.61x10 ⁻⁴	1.07x10 ⁻⁴
	Cr	1.77 $\pm$ 0.15	3.49x10 ⁻⁶	6.86x10 ⁻⁷	1.48x10 ⁻⁵	9.81x10 ⁻⁶
	Cu	0.73 $\pm$ 0.67	1.08x10 ⁻⁷	2.13x10 ⁻⁸	1.15x10 ⁻⁶	7.61x10 ⁻⁷
	Fe	420.57 $\pm$ 36.96	3.56x10 ⁻⁶	6.98x10 ⁻⁷	9.43x10 ⁻⁷	6.24x10 ⁻⁷
	Ni	5.88 $\pm$ 0.19	1.74x10 ⁻⁶	3.42x10 ⁻⁷	1.15x10 ⁻⁵	7.64x10 ⁻⁶
	Zn	1.48 $\pm$ 0.21	2.92x10 ⁻⁸	5.74x10 ⁻⁹	4.65x10 ⁻⁹	3.07x10 ⁻⁹
HI _{ingestion} - HI _{dermal}			1.26x10 ⁻³	2.47x10 ⁻⁴	1.90x10 ⁻⁴	1.26x10 ⁻⁴
THI			Child	1.45x10 ⁻³		
			Adult	3.72x10 ⁻⁴		
III	As	67.51 $\pm$ 1.47	1.33x10 ⁻³	2.62x10 ⁻⁴	1.72x10 ⁻⁴	1.14x10 ⁻⁴
	Cr	2.41 $\pm$ 0.20	4.76x10 ⁻⁶	9.36x10 ⁻⁷	2.02x10 ⁻⁵	1.34x10 ⁻⁵
	Fe	612.04 $\pm$ 35.08	5.17x10 ⁻⁶	1.02x10 ⁻⁶	1.37x10 ⁻⁶	9.08x10 ⁻⁷
	Ni	6.31 $\pm$ 0.18	1.87x10 ⁻⁶	3.66x10 ⁻⁷	1.24x10 ⁻⁵	8.18x10 ⁻⁶
HI _{ingestion} - HI _{dermal}			1.34x10 ⁻³	2.64x10 ⁻⁴	2.06x10 ⁻⁴	1.36x10 ⁻⁴
THI			Child	1.55x10 ⁻³		
			Adult	4.00x10 ⁻⁴		

Table 4. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for dry season for residential receptors

Stations	Metals	Concentration (µg/ L) (mean±SD)	HQ _{ingestion}		HQ _{dermal}	
			Child	Adult	Child	Adult
I	As	53.15±1.22	8.83x10 ⁻³	6.07x10 ⁻³	9.49x10 ⁻⁵	6.35x10 ⁻⁵
	Cd	1.62±0.14	1.62x10 ⁻⁴	1.11x10 ⁻⁴	2.85x10 ⁻⁴	1.91x10 ⁻⁴
	Cr	2.61±1.92	4.34x10 ⁻⁵	2.98x10 ⁻⁵	1.53x10 ⁻⁵	1.02x10 ⁻⁵
	Cu	1.65±0.39	2.05x10 ⁻⁶	1.41x10 ⁻⁶	1.81x10 ⁻⁶	1.21x10 ⁻⁶
	Fe	15.78±2.27	1.12x10 ⁻⁶	7.72x10 ⁻⁷	2.48x10 ⁻⁸	1.66x10 ⁻⁸
	Ni	3.35±0.16	8.36x10 ⁻⁶	5.74x10 ⁻⁶	4.60x10 ⁻⁶	3.08x10 ⁻⁶
	Pb	4.37±070	1.56x10 ⁻⁴	1.07x10 ⁻⁴	2.28x10 ⁻⁷	1.53x10 ⁻⁷
HI _{ingestion} - HI _{dermal}			9.21x10 ⁻³	6.32x10 ⁻³	4.02x10 ⁻⁴	2.69x10 ⁻⁴
THI	Child	9.61x10 ⁻³				
	Adult	6.59x10 ⁻³				
II	As	54.09 ± 1.94	8.99x10 ⁻³	6.18x10 ⁻³	9.66x10 ⁻⁵	6.47x10 ⁻⁵
	Cd	1.54 ± 0.09	1.54x10 ⁻⁴	1.06x10 ⁻⁴	2.71x10 ⁻⁴	1.82x10 ⁻⁴
	Cr	2.00 ± 3.00	3.32x10 ⁻⁵	2.28x10 ⁻⁵	1.17x10 ⁻⁵	7.83x10 ⁻⁶
	Cu	1.56 ± 0.18	1.94x10 ⁻⁶	1.33x10 ⁻⁶	1.71x10 ⁻⁶	1.15x10 ⁻⁶
	Fe	11.81 ± 0.88	8.41x10 ⁻⁷	5.78x10 ⁻⁷	1.85x10 ⁻⁸	1.24x10 ⁻⁸
	Ni	4.06 ± 0.25	1.01x10 ⁻⁵	6.95x10 ⁻⁶	5.57x10 ⁻⁶	3.73x10 ⁻⁶
	Pb	4.71 ± 0.54	1.68x10 ⁻⁴	1.15x10 ⁻⁴	2.46x10 ⁻⁷	1.65x10 ⁻⁷
HI _{ingestion} - HI _{dermal}			9.36x10 ⁻³	6.43x10 ⁻³	3.87x10 ⁻⁴	2.59x10 ⁻⁴
THI	Child	9.75x10 ⁻³				
	Adult	6.69x10 ⁻³				
III	As	53.71 ± 0.94	8.93x10 ⁻³	6.13x10 ⁻³	9.60x10 ⁻⁵	6.42x10 ⁻⁵
	Cd	1.22 ± 0.07	1.22x10 ⁻⁴	8.38x10 ⁻⁵	2.15x10 ⁻⁴	1.44x10 ⁻⁴
	Cr	1.53 ± 2.12	2.55x10 ⁻⁵	1.75x10 ⁻⁵	8.99x10 ⁻⁶	6.01x10 ⁻⁶
	Cu	0.36 ± 0.15	4.54x10 ⁻⁷	3.12x10 ⁻⁷	4.00x10 ⁻⁷	2.68x10 ⁻⁷
	Fe	155.68 ± 53.43	1.11x10 ⁻⁵	7.62x10 ⁻⁶	2.44x10 ⁻⁷	1.63x10 ⁻⁷
	Ni	4.65 ± 0.43	1.16x10 ⁻⁵	7.95x10 ⁻⁶	6.38x10 ⁻⁶	4.27x10 ⁻⁶
	Pb	0.40 ± 0.21	1.43x10 ⁻⁵	9.80x10 ⁻⁶	2.10x10 ⁻⁸	1.40x10 ⁻⁸
HI _{ingestion} - HI _{dermal}			9.11x10 ⁻³	6.26x10 ⁻³	3.27x10 ⁻⁴	2.19x10 ⁻⁴
THI	Child	9.44x10 ⁻³				
	Adult	6.48x10 ⁻³				

However, measurements of HI_{dermal} (child and adult) and THI (child and adult) showed statistically significant differences across stations. This variation was observed between Station I and Station III, as well as between Station II and Station III. During the rainy period, the differences in health risk indices among stations for both adults and children were also found to be statistically significant ($p < 0.05$). According to post hoc (pairwise comparison) tests, these statistical differences were identified between the adult and child health risk indices

estimated for Station I and the values obtained from Stations II and III. Similarly, statistically significant differences ($p < 0.05$) were observed between the adult and child health risk indices of Station II and the estimated values for Station III. However, since all values were below the threshold of 1, exposure to these metals does not pose a significant non-carcinogenic health risk for residential receptors.

Table 5. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for rainy season for residential receptors

Stations	Metals	Concentration ($\mu\text{g/L}$) (mean $\pm$ SD)	HQ _{ingestion}		HQ _{dermal}	
			Child	Adult	Child	Adult
I	As	58.22 $\pm$ 1.88	9.68x10 ⁻³	6.65x10 ⁻³	1.04x10 ⁻⁴	6.96x10 ⁻⁵
	Cr	0.98 $\pm$ 0.74	1.63x10 ⁻⁵	1.12x10 ⁻⁵	5.74x10 ⁻⁶	3.84x10 ⁻⁶
	Cu	0.52 $\pm$ 0.57	6.53x10 ⁻⁷	4.48x10 ⁻⁷	5.75x10 ⁻⁷	3.85x10 ⁻⁷
	Fe	267.33 $\pm$ 86.61	1.90x10 ⁻⁵	1.31x10 ⁻⁵	4.20x10 ⁻⁷	2.81x10 ⁻⁷
	Ni	5.13 $\pm$ 0.35	1.28x10 ⁻⁵	8.78x10 ⁻⁶	7.04x10 ⁻⁶	4.71x10 ⁻⁶
	Zn	1.61 $\pm$ 0.31	2.68x10 ⁻⁷	1.84x10 ⁻⁷	3.54x10 ⁻⁹	2.37x10 ⁻⁹
	HI _{ingestion} - HI _{dermal} Child		9.73x10 ⁻³ 9.84x10 ⁻³	6.68x10 ⁻³	1.18x10 ⁻⁴	7.88x10 ⁻⁵
II	THI Adult		6.76x10 ⁻³			
	As	63.20 $\pm$ 1.15	1.05x10 ⁻²	7.21x10 ⁻³	1.13x10 ⁻⁴	7.55x10 ⁻⁵
	Cr	1.77 $\pm$ 0.15	2.94x10 ⁻⁵	2.02x10 ⁻⁵	1.04x10 ⁻⁵	6.95x10 ⁻⁶
	Cu	0.73 $\pm$ 0.67	9.13x10 ⁻⁷	6.27x10 ⁻⁷	8.05x10 ⁻⁷	5.39x10 ⁻⁷
	Fe	420.57 $\pm$ 36.96	3.00x10 ⁻⁵	2.06x10 ⁻⁵	6.60x10 ⁻⁷	4.42x10 ⁻⁷
	Ni	5.88 $\pm$ 0.19	1.47x10 ⁻⁵	1.01x10 ⁻⁵	8.08x10 ⁻⁶	5.41x10 ⁻⁶
	Zn	1.48 $\pm$ 0.21	2.46x10 ⁻⁷	1.69x10 ⁻⁷	3.25x10 ⁻⁹	2.18x10 ⁻⁹
III	HI _{ingestion} - HI _{dermal} Child		1.06x10 ⁻² 1.07x10 ⁻²	7.27x10 ⁻³	1.33x10 ⁻⁴	8.89x10 ⁻⁵
	THI Adult		7.35x10 ⁻³			
	As	67.51 $\pm$ 1.47	1.12x10 ⁻²	7.71x10 ⁻³	1.21x10 ⁻⁴	8.07x10 ⁻⁵
	Cr	2.41 $\pm$ 0.20	4.01x10 ⁻⁵	2.76x10 ⁻⁵	1.41x10 ⁻⁵	9.47x10 ⁻⁶
	Cu	0.00 $\pm$ 0.00	0.00x10 ⁰	0.00x10 ⁰	0.00x10 ⁰	0.00x10 ⁰
	Fe	612.04 $\pm$ 35.08	4.36x10 ⁻⁵	2.99x10 ⁻⁵	9.61x10 ⁻⁷	6.43x10 ⁻⁷
	Ni	6.31 $\pm$ 0.18	1.57x10 ⁻⁵	1.08x10 ⁻⁵	8.66x10 ⁻⁶	5.79x10 ⁻⁶
	HI _{ingestion} - HI _{dermal} Child		1.13x10 ⁻² 1.15x10 ⁻²	7.77x10 ⁻³	1.44x10 ⁻⁴	9.66x10 ⁻⁵
	THI Adult		7.87x10 ⁻³			

In many studies on this subject, CR values exceeding the range of 10^{-6} to 10^{-4} are considered to represent the probability of developing cancer over a 70-year lifetime. In this study, five heavy metals—As, Cd, Cr, Ni, and Pb—were considered in the estimation of TCR values. For both the dry and rainy periods, the carcinogenic risk values estimated for surface waters were below 1×10^{-4} , indicating that there is no carcinogenic health threat for recreational receptors. During the dry period, As was identified as the dominant contributor to $CR_{\text{ingestion}}$, a component of carcinogenic risk (TCR), at all stations; this was followed by Pb at Stations I and II and by Ni at Station III. For CR_{dermal} , As was again the primary contributing metal, followed by Cd at Stations I and II and Ni at Station III (Figure 2).

Considering residential receptors, in both the dry and rainy periods, carcinogenic risk values associated with metals other than As (Cr, Ni, Cd, and Pb) were found to be below the target risk level of 1.0×10^{-4} ; therefore, no

adverse effects on human health are expected. TCR values for As were found to be in the range of $1.0 \times 10^{-4} < CR < 1.0 \times 10^{-6}$, indicating an acceptable level depending on exposure conditions. The maximum TCR value for As was recorded at Station I in the dry period (1.13×10^{-6}), while in the rainy period it was observed at Station III (1.43×10^{-6}). Both values indicate a “very low but acceptable risk level.” In both the dry and wet periods, As was identified as the dominant contributor to $CR_{\text{ingestion}}$ and CR_{dermal} at all stations. In the dry period, the second major contributor to TCR after As was Pb, whereas in the rainy period, it was Ni (Figure 2). Statistical analysis revealed that TCR values showed significant differences both between periods and among stations ($p < 0.001$). TCR values were higher in the wet period compared to the dry period, and the highest carcinogenic risk value was observed at Station III, where agricultural runoff is likely to be the primary source of contamination.

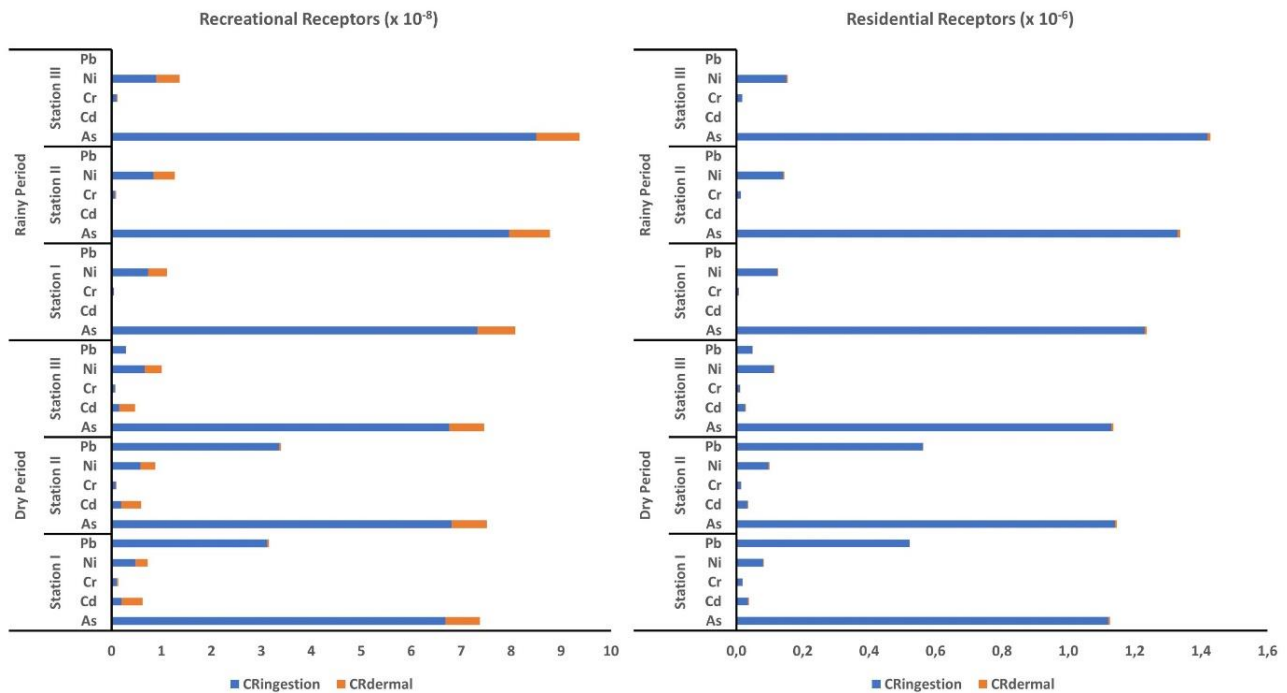


Figure 2. Variation of carcinogenic risk values for recreational (A) and residential (B) receptors depending on stations and periods

Discussion

Heavy metals, which are among the factors causing water pollution, are considered systemic toxic substances that can lead to multiple organ damage as well as teratogenic and carcinogenic effects. In particular, heavy metal risk levels, which are a critical issue affecting the safety of drinking water resources, have been addressed in various studies (Saleem et al., 2019; Fahimah et al., 2024; Wang et al., 2024). It has also been reported that the waters of Ulubat Lake (Şener et al., 2023) and Lake İznik (Şener et al., 2025) in Türkiye may cause both

carcinogenic and non-carcinogenic health problems. In this study, the eutrophic and shallow Mogan Lake was selected as the study area; however, in terms of water quality, it is far from being a drinking water source and primarily serves residential and recreational functions.

Numerous studies in recent years have demonstrated that the assessment of heavy metal-related health risks in rivers and lakes has become an essential component of aquatic environmental management. The concentrations of Cr, Ni, Cu, Zn, As, Cd, Hg, Pb, and Fe in the surface

waters of Mogan Lake were determined, and non-carcinogenic health risks were estimated for residential and recreational receptors. Within the scope of the study, the estimated total non-carcinogenic health risk values for residential and recreational receptors (children and adults) during both dry and rainy periods were found to be below the threshold value of 1. Accordingly, it can be stated that exposure to the aforementioned metals is unlikely to pose a potential health risk to residents or recreational users of the lake. However, the estimated total hazard index ($THI > 1$) values for the Sukesen, Başpınar, and Gölcük streams feeding Mogan Lake indicate a high level of non-carcinogenic health risk (Pulatsü & Latifi, 2023). In this study, particularly during the rainy period, THI values for residential receptors showed statistically significant variation across sampling stations for both adults and children ($p < 0.05$), reaching their maximum at Station III, where agricultural wastewater inputs are received (child: 1.15×10^{-2} ; adult: 7.87×10^{-3}), and at Station II, which is located closest to residential areas (child: 1.07×10^{-2} ; adult: 7.35×10^{-3}). Under current conditions, although exposure to heavy metals in lake surface waters does not pose a significant non-carcinogenic health risk for residential receptors, it is likely that human health risk values may be adversely affected in the future if wastewater inputs originating from residential settlements and agricultural activities in the watershed continue.

The results of this study are consistent with the findings reported for ten ponds located in the Saros Gulf (Türkiye), which is used as a source of irrigation and drinking water (Tokatlı & Varol, 2021), as well as for studies conducted at Doğancı Dam (Bursa, Türkiye) (Fural et al., 2022), where no non-carcinogenic health risk was identified for either adults or children. In the present study, the hazard index (HI) was calculated as the sum of the total potential health risks posed by heavy metals, derived from hazard quotients (HQs) through ingestion and dermal contact exposure pathways. In this context, for both considered periods, ingestion hazard quotient ($HQ_{\text{ingestion}}$) values were found to be higher than dermal absorption hazard quotient (HQ_{dermal}) values for both recreational and residential receptors. Our findings are consistent with those of previous studies (Custodio et al., 2020; Varol & Tokatlı, 2023; Dippong et al., 2024), which reported that non-carcinogenic health risk values associated with metal ingestion are higher than those resulting from dermal exposure. For the four streams feeding Mogan Lake, the calculated $HQ_{\text{ingestion}}$ values for both adults and children, based on Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn concentrations, were also found to be higher than the corresponding HQ_{dermal} values (Pulatsü & Latifi, 2023).

It has been reported that non-carcinogenic risk levels are higher for children compared to adults, indicating that children are exposed to a greater level of potential risk (Sharafi & Salehi, 2025; Pulatsü & Latifi, 2023). Similarly, in our study, for both recreational and residential receptors, child- $HQ_{\text{ingestion}}$ and child-THI values were found to be higher than those of adults during both dry and rainy sampling periods (Tables 2–5). This result is

consistent with findings reported for the upstream–downstream sections of Keban Dam Reservoir (Türkiye) (Varol, 2019) and for water samples collected from both surface and deep zones of the reservoir (Canpolat et al., 2020), where higher HI values were observed for children compared to adults in both residential and recreational exposure scenarios. In addition, it has been reported that child hazard index values ($HI_{\text{ingestion}}$ and HI_{dermal}) for the streams feeding Mogan Lake are also higher than those for adults (Pulatsü & Latifi, 2023). Based on the findings for Mogan Lake, although non-carcinogenic health risks were found to be negligible, it can be stated that children's health is disproportionately more at risk compared to that of adults.

In our study, arsenic (As) was identified as the heavy metal contributing most significantly to the ingestion pathway of the hazard index (HI) for both residential and recreational receptors during the dry period. This finding is consistent with Kumar et al. (2019), who reported that As is the primary contaminant based on hazard quotients associated with ingestion and dermal exposure pathways for both adults and children. Furthermore, the significant contribution of As to the ingestion pathway has also been reported in studies conducted in Keban Dam Lake (for both residential and recreational receptors; Varol, 2019) and in the Meriç River Delta (Tokatlı & Ustaoglu, 2020).

In our study, non-carcinogenic health indices for both recreational and residential receptors were found to be higher during the rainy period compared to the dry period. This result indicates that seasonal hydrological processes (i.e., precipitation) may alter the distribution of environmental pollutants and exposure dynamics. Increased rainfall and surface runoff are thought to enhance the transport of heavy metals into aquatic environments, thereby increasing the potential for receptor exposure through ingestion and dermal contact pathways. In this context, it is likely that the rainy period poses a greater threat to human health, particularly at stations receiving agricultural and residential wastewater inputs. However, conducting a greater number of sampling campaigns in both dry and wet seasons would strengthen the conclusion that seasonal risk variations should be taken into account for individuals living in residential areas and using recreational zones.

In our study, HQ_{dermal} values for both recreational and residential receptors were found to be below 1 (for both children and adults) during the dry period, and Cd was identified as the heavy metal with the highest contribution to this risk factor. In the rainy period, however, As was the metal contributing most significantly to dermal exposure for both residential and recreational receptors. Our findings are not consistent with Varol (2019), who reported that Cr made the highest contribution to the HI for both residential and recreational receptors via dermal exposure in Keban Dam Reservoir. However, they are in agreement with Pulatsü and Latifi (2023), who reported that As contributed most significantly to HQ_{dermal} values in the streams feeding the lake. In this context, arsenic appears to be the most important toxic element responsible

for non-carcinogenic health concerns in the surface waters of Mogan Lake.

In our study, the $CR_{\text{ingestion}}$ exposure risk value, which is one of the components of total carcinogenic risk values, was found to be higher than CR_{dermal} , and this result is in line with the finding of Pulatsü and Latifi (2023), who calculated that $CR_{\text{ingestion}}$ exposure for chromium, nickel, and arsenic in Mogan Lake inflow waters is more risky than CR_{dermal} exposure. Within the scope of the study, among the metals considered in terms of carcinogenic health risks (As, Cd, Cr, Ni, Pb), exposure to As was found to be below the target risk level of 1×10^{-4} and does not pose a hazard in terms of the health of recreational receptors. Our finding is consistent with the results reported by Canpolat et al. (2020) and Varol et al. (2021) for Keban Dam Reservoir.

For residential receptors, exposure to As in terms of carcinogenic health risks was determined to be at an “acceptable risk” level ($1.13 \times 10^{-6} - 1.43 \times 10^{-6}$). As, identified as the metal with the highest risk for residential receptors, is known to be the only carcinogen that can cause cancer through both respiratory and gastrointestinal exposure pathways. Considering its toxicity, the importance of arsenic exposure for public health (Mukherjee et al., 2021; Patel et al., 2021; Anonymous, 2024) and the need for further research and a continuous monitoring program to support effective public health management have been emphasized (Cheung et al., 2020). In our study, Pb and Ni, following As, were identified as metals with a higher potential to pose carcinogenic risk compared to Cr.

As a result, exposure to heavy metals (As, Cd, Cr, Cu, Ni, Pb, Hg, Zn, Fe) in the surface waters of Mogan Lake via ingestion and dermal pathways was found not to cause non-carcinogenic risks for recreational and residential receptors (both adults and children). For recreational and residential receptors during the dry period, arsenic (As) was identified as the primary contributor to the total risk (HI) for the $HQ_{\text{ingestion}}$ pathway, while chromium (Cr) was identified as the primary contributing metal for the HQ_{dermal} pathway. For recreational and residential receptors across both dry and wet periods, based on $HI_{\text{ingestion}}$ and HI_{dermal} values, hazard index values for children were found to be higher than those for adults, indicating that children are exposed to greater health risks from the considered heavy metals. The total carcinogenic risk (TCR) value for arsenic (As) in the surface waters of Mogan Lake suggests that exposure to this metal does not pose a serious cancer risk for residential receptors; however, the risk cannot be considered entirely negligible. In this context, long-term adverse health effects associated with arsenic exposure in residential receptors appear likely. In order to protect the health of local residents in the Mogan Lake basin and to sustain the lake’s recreational potential, monitoring of heavy metal dynamics at the lake/basin scale would be appropriate.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

All authors took part in designing the research, collecting and writing the manuscript.

Ethics Approval

There are no ethical issues with the publication of this manuscript.

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RESEARCH ARTICLE

Rapid Identification of Ectoine-Producing Halophiles Using Newly Designed Degenerate Primers and LC-MS/MS

Ebru Tekin^{1*}, Mustafa Ateş²

Ege University, Institute of Science, Biology, 35100, Bornova, İzmir, Türkiye
Ege University, Faculty of Science, Department of Biology, 35100, Bornova, İzmir, Türkiye

<https://orcid.org/0000-0003-2623-1977>
<https://orcid.org/0000-0002-9305-3480>

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Halophilic bacteria
LC-MS/MS.

Abstract: Ectoine is a valuable compatible solute widely used in pharmaceutical, cosmetic, and biotechnological applications due to its protective and stabilizing properties. This study aimed to identify ectoine-producing bacterial strains isolated from diverse saline environments, including the İzmir Çamaltı Saltern, İzmir Bird Paradise, and commercial Himalayan salt. A PCR-based screening approach was applied using in silico primer design. Newly designed degenerate primers targeting the *ectB-ectC* gene region was used in this study. Primer design was performed for each strain by analyzing conserved genomic regions between the adjacent *ectB* and *ectC* genes using representative type strains retrieved from NCBI. Gradient PCR conditions, particularly annealing temperature and extension time, were optimized individually for each isolate to improve amplification efficiency and specificity. For confirmation of genetic screening, ectoine production was assessed in 15% salinity medium and quantified using LC-MS/MS analysis and ectoine standard. A total of eight bacterial isolates were purified and screened for the presence of the *ectB-ectC* region. Among them, three isolates -*Kocuria* sp. CT-6, *Oceanobacillus* sp. HT-9, and *Halomonas* sp. CS-5- were found positive for the target region. A representative *ectB-ectC* positive *Halomonas* sp. CS-5 strain was selected for further analysis as a study-specific model and cultivated under 15% salinity conditions. Ectoine production was confirmed as 0.395 g/L (mean±SD, n=3, SD=0) in the culture reached the stationary phase at 48.5 h, total dry cell weight was 1.77 g/L corresponding to 22.3% (w/w) of dry cell weight by LC-MS/MS analysis. The study demonstrates that the combined application of PCR-based screening and analytical confirmation provides a potentially useful preliminary screening approach for identifying ectoine-producing microorganisms. This approach supports the selection of promising strains for further biotechnological evaluation.

Anahtar kelimeler:

Ektoin
In silico primer dizaynı
Dejenere primerler
Halofilik bakteri
LC-MS/MS.

Yeni Tasarlanmış Dejenere Primerler ve LC-MS/MS Kullanılarak Ektoin Üreten Halofillerin Hızlı Tanısı

Öz: Ektoin, koruyucu ve stabilize edici özellikleri nedeniyle farmasötik, kozmetik ve biyoteknolojik uygulamalarda yaygın olarak kullanılan önemli bir biyoyoumlu moleküldür. Bu çalışmanın amacı, İzmir Çamaltı Tuzlası, İzmir Kuş Cenneti ve ticari Himalaya tuzu gibi farklı tuzlu ortamlardan izole edilen ektoin üreten bakteriyel suşları belirlemektir. Bu amaçla, in silico primer tasarımına dayalı PCR temelli bir tarama yaklaşımı uygulanmıştır. Çalışmada, *ectB-ectC* gen bölgesini hedefleyen yeni tasarlanmış dejenere primerler kullanılmıştır. Primer tasarımı, NCBI veri tabanından elde edilen temsilci tip suşlar kullanılarak, komşu *ectB* ve *ectC* genleri arasındaki korunmuş genomik bölgelerin analiz edilmesiyle gerçekleştirilmiştir. Amplifikasyon verimini ve özgüllüğünü artırmak amacıyla, özellikle bağlanma sıcaklığı ve uzama süresi gibi gradient PCR koşulları her izolat için ayrı ayrı optimize edilmiştir. Genetik tarama sonuçlarının doğrulanması amacıyla, ektoin üretimi %15 tuzluluk içeren besiyerinde değerlendirilmiş ve ektoin standardı kullanılarak LC-MS/MS analizi ile miktar tayini yapılmıştır. Toplam sekiz bakteriyel izolat saflaştırılmış ve *ectB-ectC* bölgesinin varlığı açısından taranmıştır. Bunlardan üç izolat (*Kocuria* sp. CT-6, *Oceanobacillus* sp. HT-9 ve *Halomonas* sp. CS-5) hedef gen bölgesi açısından pozitif bulunmuştur. *ectB-ectC* gen bölgesi pozitif olan temsilci olarak *Halomonas* sp. CS-5 suşu, çalışmaya özgü bir model olarak seçilmiş ve %15 tuzluluk koşullarında kültüre edilmiştir. LC-MS/MS analizi sonucunda, kültürün 48,5 saatte durağan faza ulaşmasıyla ektoin üretimi 0,395 g/L (ortalama±SD, n=3, SD=0) olarak doğrulanmıştır. Aynı zamanda toplam kuru hücre ağırlığı 1,77 g/L olarak belirlenmiş ve bu değer kuru hücre ağırlığının %22,3'üne (w/w) karşılık gelmiştir. Bu çalışma, PCR tabanlı genetik tarama ile analitik doğrulamanın birlikte uygulanmasının, ektoin üreten mikroorganizmaların belirlenmesinde potansiyel olarak yararlı bir ön tarama yaklaşımı sunduğunu göstermektedir. Bu yaklaşım, ileri biyoteknolojik değerlendirmeler için umut verici suşların seçimini desteklemektedir.

*Corresponding author: ebrutekn@yahoo.com

Introduction

Ectoine ((S)-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) is a natural osmolyte that exhibits strong moisturizing, membrane-stabilizing, and anti-inflammatory properties. It has been demonstrated in preclinical and clinical studies to be effective and safe in the treatment of diseases including atopic dermatitis, rhinosinusitis, dry eye disease, rhinitis sicca, and allergic conjunctivitis. Ectoine has been shown to improve cell membrane barrier function, reduce inflammation, and enhance hydration, thus offering a safe and beneficial alternative to conventional topical therapies. In addition, advances in microbial production and biotechnological process optimization have increased the potential for sustainable ectoine manufacturing and expanded its commercial applications in pharmaceutical and cosmetic products (Cánovas et al., 1998; Bazazzadegan et al. 2017; Bilstein et al., 2021; Xu et al., 2023; Ferreira et al., 2026).

A recent study on ectoine also demonstrated that it exerts anti-cellular senescence effects and ensures skin safety in H₂O₂-induced oxidative stress models using HaCaT cells and EA.hy926 endothelial cells. These effects are primarily mediated through modulation of the p53/p21 and p16 signaling pathways, highlighting ectoine's role in reducing oxidative damage. Ectoine was shown to downregulate the expression of components of the p53/p21 and p16 signaling pathways, thereby suppressing cell cycle arrest (Li et al., 2026).

Saline environments are increasingly recognized as rich reservoirs of halophilic and halotolerant microorganisms capable of producing ectoine. These microorganisms thrive in high-salinity conditions by accumulating compatible solutes to maintain cellular osmotic balance. Ectoine, a cyclic amino acid derivative, is well known for its exceptional protective properties against osmotic stress, temperature fluctuations, and desiccation (Cánovas et al., 1998; Bazazzadegan et al., 2017; Bilstein et al., 2021; Xu et al., 2023). These environments exert selective pressure that favors the evolution of robust adaptive mechanisms, making isolated strains potentially advantageous as natural producers (Galinski et al., 1985; Kanapathipillai et al., 2005; Schubert et al., 2007; Schwibbert et al., 2011; He et al., 2015; Chen et al., 2018; Liu et al., 2021; Hu et al., 2024).

Ectoine is synthesized via a well-characterized biosynthetic pathway involving three key enzymes encoded by the *ectABC* operon (Cánovas et al., 1998). These enzymes catalyze the conversion of aspartate semialdehyde into ectoine through a series of intermediate steps (Xu et al., 2023).

A wide range of microorganisms, including members of the genera *Halomonas*, *Chromohalobacter*, *Oceanobacillus*, and *Bacillus*, have been reported to produce ectoine. Among these, *Halomonas* species are considered particularly promising candidates due to their robustness and high production capacity under saline conditions. However, ectoine production is highly dependent on environmental parameters such as salinity,

nutrient composition, and cultivation conditions (Sauer and Galinski, 1998; Bazazzadegan et al. 2017; Cantera et al., 2023; Wang et al., 2025).

Although several studies have focused on optimizing ectoine production using genetically engineered strains or controlled fermentation strategies, the identification of novel ectoine-producing microorganisms from natural saline environments remains an important area of research. Such environments represent rich and largely unexplored reservoirs of biotechnologically valuable microbial diversity (Sauer and Galinski, 1998; Cantera et al., 2023; Wang et al., 2025).

Traditional approaches for identifying ectoine-producing bacteria rely mainly on cultivation-based methods and phenotypic characterization, which are often time-consuming and may not reliably reflect the metabolic potential of isolates (Pastor et al., 2010). In recent years, molecular techniques such as PCR-based detection of ectoine biosynthesis genes (*ectA*, *ectB*, and *ectC*) have been employed to assess the genetic potential of halophilic bacteria. For instance, in a previous study, a genus-level PCR assay targeting the *ectC* gene was developed, enabling rapid identification of ectoine-producing *Halomonas* strains and overcoming the limitations of conventional phenotypic identification methods (Zanjirband et al., 2008).

In parallel, analytical techniques such as LC-MS/MS have been used to confirm actual ectoine production at the metabolite level (Radha et al., 2026). However, these approaches are generally applied independently rather than as part of an integrated workflow. Despite extensive research on ectoine biosynthesis and its molecular pathways, rapid screening strategies that combine genetic detection of the *ectB-ectC* region with analytical techniques such as LC-MS/MS for confirming metabolite production remain limited. The existence of biosynthetic genes does not always reflect actual metabolite production (Choi et al., 2025). To address this gap, only a few studies have integrated degenerate primer-based detection of the region between the *ectB* and *ectC* genes with LC-MS/MS analysis in a unified workflow for the rapid identification and confirmation of ectoine-producing halophilic bacteria (Widderich et al., 2014).

In this context, the present study aims to identify ectoine-producing bacterial strains isolated from diverse saline environments, including the İzmir Çamaltı Saltern, İzmir Bird Paradise, and commercial Himalayan salt. A key objective is to develop a rapid molecular screening approach based on previously established PCR methods, using newly designed degenerate primers targeting the *ectB-ectC* gene region. For this purpose, primers were specifically designed for each strain by analyzing the conserved genomic regions between the adjacent *ectB* and *ectC* genes, identified in representative type strains retrieved from NCBI. Gradient PCR conditions, particularly annealing temperature and extension time, were optimized individually for each isolate to improve

amplification efficiency and specificity. The findings provide a framework for efficient screening and identification of ectoine-producing bacteria. Additionally, ectoine production was evaluated under 15% salinity conditions, and ectoine was quantified using LC-MS/MS analysis and ectoine standard.

Material and Methods

Sampling sites and bacterial isolates

The halophilic and halotolerant bacterial strains used in this study were previously isolated from three different saline sources: İzmir Çamaltı Saltern (salt bulk and saline water), İzmir Bird Paradise (saline pond), and commercial Himalayan salt during the PhD study of the corresponding author. Isolation and preliminary characterization of the strains were performed in earlier studies. A total of eight isolates (CT-3, CT-4, CT-5, CT-6, CS-4, CS-5, HT-9, and KC-101) were included in the present work (Tekin, 2015; Tekin and Ateş, 2025).

Isolation and cultivation

Bacterial isolation was carried out using HM medium containing 17.8% (w/v) NaCl at pH 7.2. Samples (0.5 g solid salt or 0.5 mL saline water) were inoculated into liquid medium and incubated statically at 30 °C for 72 h. Serial dilutions were prepared and spread onto HM agar plates containing 5% (w/v) total salts. Distinct colonies were selected based on morphological characteristics and purified through repeated streaking. Pure cultures were preserved in HM medium supplemented with 30% (v/v) glycerol and stored at -20 °C (Ventosa et al., 1982; Mata et al., 2002; Tekin, 2015).

Genomic DNA extraction

Genomic DNA was extracted from actively growing cultures using the PureLink Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. Briefly, 1.5 mL of culture was centrifuged at 10,000 rpm for 10 min, and the resulting pellet was processed for DNA isolation. DNA quality and integrity were evaluated by agarose gel electrophoresis (1% w/v agarose in 1× TBE buffer) (Weisburg et al., 1991; Zanjirband et al., 2008; Tekin, 2015).

16S rRNA gene amplification and sequencing

Universal primers 27F and 1542R were used for partial amplification of the 16S rRNA genes (Table 3). Genomic DNA were amplified in a total volume of 50 µL with i-Star Taq Intronbio kit containing PCR buffer, MgCl₂, dNTPs, primers, and Taq DNA polymerase. PCR reactions were carried out under the following thermal cycling conditions. Initial denaturation were performed at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 59 °C for 20 s, and extension at 72 °C for 60 s, with a final extension step at 72 °C for 5 min. PCR products were observed by agarose gel electrophoresis and sequenced using an Applied Biosystems 3130XL platform. Sequence analysis was performed using BLAST against the NCBI GenBank database (Felsenstein, 1985; Saitou

and Nei, 1987; Weisburg et al., 1991; Suzuki et al., 1998; Tamura et al., 2004; Zanjirband et al. 2008; Obradovic et al., 2013; Tamura et al., 2013).

Design of degenerate primers targeting *ectB-ectC* gene region

Degenerate primers targeting the conserved region spanning the *ectB-ectC* gene cluster involved in ectoine biosynthesis were designed in this study based on multiple sequence alignments of homologous gene sequences retrieved from the NCBI GenBank database (Zanjirband et al., 2008). Conserved regions were identified by MEGA software (version 6.06) (Tamura et al., 2013). Primer design was performed in this study using Primer3Plus, where a panel of candidate primer sets was generated to account for sequence variability within the conserved target region. The final primer sets used in this work were selected based on experimental PCR screening performed in this study. In silico evaluation of primer candidates was conducted to assess GC content, melting temperature (T_m) and potential secondary structures, including hairpin and dimer formation. Primer specificity was further verified using NCBI Primer-BLAST against the non-redundant nucleotide database. The synthesized primers (Alpha DNA) were experimentally validated in this study, and those yielding specific amplification were used for downstream screening of ectoine-producing strains (Table 3) (Suzuki et al., 1998; Ye et al., 2012).

Amplification of *ectB-ectC* genes by PCR

Gradient PCR was performed to amplify the *ectB-ectC* gene region using the designed degenerate primers (Table 3). Each reaction (20 µL) contained PCR buffer, dNTP mix, forward and reverse primers, Taq DNA polymerase, and template DNA. PCR conditions were optimized based on primer-specific annealing temperatures. Amplified products were analyzed by electrophoresis on 2% (w/v) agarose gels stained with RedSafe. DNA bands were visualized under UV illumination (Suzuki et al., 1998; Zanjirband et al., 2008; Obradovic et al., 2013).

Ectoine production

A representative positive strain (*Halomonas* sp. CS-5) was selected for extraction and subsequent LC-MS/MS analysis to confirm the analytical workflow. The strain was cultivated in production media containing 15% (w/v) NaCl. The medium composition included glucose (2 g/L), NH₄Cl (0.2 g/L), MgSO₄·7H₂O (0.35 g/L), K₂HPO₄ (0.18 g/L), FeSO₄·7H₂O (0.0005 g/L), monosodium glutamate (1.1 g/L), Tris (0.2 g/L), and L-arginine (0.2 g/L), adjusted to pH 7.5. Amino acids were sterilized by filtration (0.45 µm), while other components were autoclaved. All experiments were conducted in triplicate (Van-Thuoc et al., 2010).

Growth analyses

Growth experiments were performed by inoculating 2 mL of active culture into 80 mL of production medium in 250 mL Erlenmeyer flasks. Cultures were incubated at 37 °C at 100 rpm with shaking. Optical density measurements

were recorded at 520 nm, 600 nm, and 660 nm at different time points (0, 1.5, 23, 46, and 48.5 h) (Van-Thuoc et al., 2010; Microsoft 2025). All experiments were conducted in triplicate.

Extraction of intracellular ectoine

Cells were harvested at the final time point (48.5 h) by centrifugation at 10,000 rpm for 5 min at 4 °C. Pellets were lyophilized and subjected to enzymatic digestion using lysozyme (50 mg/mL) at 37 °C for 30 min. Subsequently, cells were lysed using digestion buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) at 55 °C for 1 h. Ectoine extraction was performed using methanol:chloroform:water (10:5:4, v/v/v), followed by phase separation and centrifugation. The aqueous phase was collected, re-extracted, and filtered through a 0.22 µm syringe filter prior to analysis. The study was repeated three times and the mean values and standard deviation was calculated accordingly (Kunte et al., 1993; Mishra et al., 2019; Microsoft, 2025; Van-Thuoc et al., 2010).

LC-MS/MS Analyses

Intracellular ectoine was extracted from cell pellets of *Halomonas* sp. CS-5 and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to Zhang et al. (2009). The extracts were centrifuged at high speed, and the clear supernatant was collected for LC-MS/MS analysis. LC-MS/MS analyses were done by using a triple quadrupole mass spectrometer coupled to a liquid chromatography system. This chromatographic separation was coupled with a mass spectrometer with multiple reaction monitoring (MRM) and positive electrospray ionization (ESI⁺) mode. Ectoine was identified by comparison with an authentic standard

(Sigma-Aldrich, Cat. No. 81619, Lot No. BCBF0987V), based on retention time (0.35 min) and the characteristic precursor-to-product ion transition of m/z 142.8 ($[M+H]^+$) to 96.8. (Galinski et al., 1985). Quantification was performed using ectoine standards, and peak areas were integrated using instrument software (Zhang et al., 2009). All analyses were performed in triplicate (Mishra et al., 2019).

Intracellular Ectoine Content

Dry cell weight (DCW) was determined gravimetrically, and the mean $\pm$ SD was used to calculate total biomass concentration (g/L). Intracellular ectoine content was quantified by LC-MS/MS analysis. The intracellular ectoine content was expressed as percentage (w/w) and calculated using the following equation:

$$\text{Ectoine (\%, w/w)} = \frac{\text{Ectoine (mg)} \times 100}{\text{DCW (g)} \times 1000}$$

(Zhang et al., 2009).

Results

The isolated and identified bacterial species are summarized in Table 1 and Figures 1-2. The characteristics of the isolates are presented in Table 2. Strains obtained from İzmir Çamaltı Saltern and salt samples were previously identified as *Bacillus* sp. CT-3, *Pantoea* sp. CS-4, *Oceanobacillus* sp. HT-9, and *Halomonas* sp. KC-101, which are obligate halophiles. In contrast, *Kocuria* sp. CT-6, *Virgibacillus* sp. CT-5, and *Halomonas* sp. CS-5 were classified as halotolerant strains (Tekin et al., 2015; Tekin and Ateş, 2025).

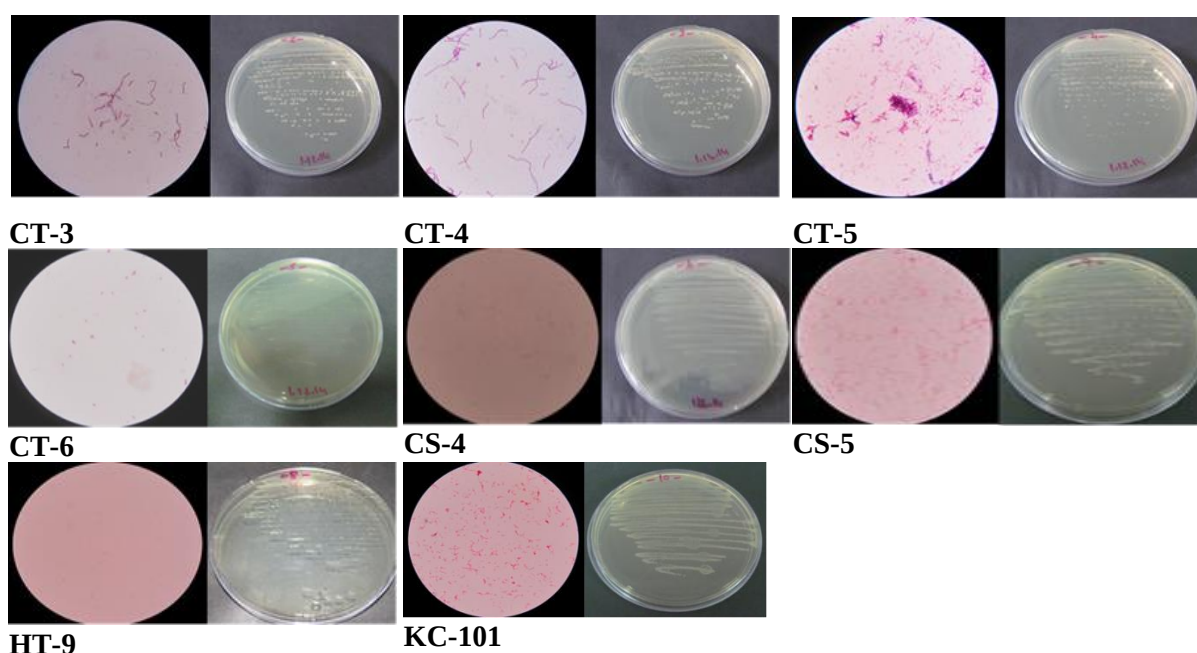


Figure 1. The gram staining and colony view results of the purified strains were CT-3(2), CT-4(3), CT-5(4), CT-6(5), CS-4(6), CS-5(7), HT-9(8), KC-101(10), respectively.

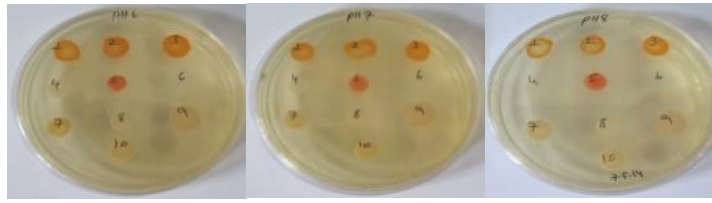


Figure 2. The morphology, colour and texture of the purified strains in different pH containing media.

Table 1. Isolated species of halotolerant or halophilic bacteria

Salt Source	Salt Type	Collection Date	Bacterial load	Code	Number of selected isolates
İzmir Bird Paradise Saline Pond	Seawater	03/03/2011	9×10^5 CFU/mL	KC	1
İzmir Çamaltı Saltern Salt Bulk	Salt bulk	02/11/2011	$3,0 \times 10^2$ CFU/mL	CT	4
İzmir Çamaltı Saltern Saline Pond	Saline water	02/11/2011	$2,3 \times 10^7$ CFU/mL	CS	2
Himalayan Salt	Commercial salt	02/11/2011	$1,8 \times 10^3$ CFU/mL	HT	1

Table 2. Comparison of selected phenotypic and biochemical characteristics of the isolates. 1, *Bacillus sp.* CT-3 strain; 2, *Bacillus sp.* CT-4 strain; 3, *Virgibacillus sp.* CT-5 strain; 4, *Kocuria sp.* CT-6 strain; 5, *Pantoea sp.* CS-4 strain; 6, *Halomonas sp.* CS-5 strain; 7, *Oceanobacillus sp.* HT-9 strain; 8, *Halomonas sp.* KC-101 strain

Characteristics	1	2	3	4	5	6	7	8
Cell shape	Bacillus	Bacillus	Bacillus	Coccus	Bacillus	Short bacillus	Long bacillus	Medium bacillus
Gram reaction	+	+	+	+	-	-	+	-
Motility	+	+	+	-	+	+	Not tested	+
Pigmentation	Orange	Orange	Cream	Red	Cream	Yellow	White	Light cream
Temperature range (°C)	4-50	4-50	20-55	4-37	4-50	4-45	4-45	4-55
pH range	5-11	5-11	5-11	5-11	5-11	5-11	5-11	5-11
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-
Hydrolysis tests:								
Casein	+	+	±	-	-	-	±	-
Starch	+	+	+	+	+	+	+	+
Tributyryn	-	-	-	-	-	-	-	-
DNA	+	+	+	+	+	+	+	+
Tyrosine	-	-	-	-	+	-	-	-

Table 2 Continued

Characteristics	1	2	3	4	5	6	7	8
Phenylalanine deamination	-	-	-	-	-	-	-	-
Arginine decarboxylation	-	-	-	-	-	-	-	-
Lysine deamination	-	-	-	-	-	-	-	-
Ornithine decarboxylation	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	-	-	+
H ₂ S production	-	-	±	-	+	-	-	+
Optimum NaCl (%)	3.5-19	3.5-19	7.5-21	3.5-10	7.5-19	7.5-25	7.5-25	7.5-21
Salt tolerance range (%)	3-22	3-22	0-22	0-19	2.5-19	0-25	2-25	1.5-25
Acid and gas production:								
Sucrose	+++	+	-	+++	++	++	++G	+++
Citrate	±	++	-	±	+	+	++	±
Glycerol	+++	++	-	+	++	++	+++-	+++
Arbutin	-	++	-	+G	+	±	++G	-
Fructose	+	+	+	+	+	+	++	+++
Glucose	+++	++	-	±	±	+++	+++-	+++
Mannitol	++	++	++	++	+	+	+	++
Trehalose	+++	++	-	+	+	+	++	+++
Salicin	+	+	-	+++	+	+	++	+++
Maltose	+++	+	-	+++	++	+++	+++-	+++
Lactose	+G	+	-	+	-	+	++	-
L-Arabinose	+	+	+	+	++	++	++	++
D-Cellobiose	++G	++	-	±	±	±	++	++
D-Ribose	+	+	+	+	+	+	++	+++
D-Galactose	+	+	+	+	++	+	+	++
D-Mannose	+G	-	+	-	+	+	++	+
Bacto-inositol	+	+	+	+G	+	+	+	+
D-Sorbitol	+	+	-	+	+	±	-	+++
Melibiose	±	+	Not tested		+	+	++	Not tested
D-Melezitose	+	+	+	+	++	++	++	++
D-Raffinose	±	±	-	+	+	+	++	±

Y → Not tested, G → Gas positive, +++ → Acid positive, ++ → Acid negative, growth positive, + → Weak growth, ± → Very weak growth, - → No growth

16S rRNA gene sequencing and identification

Partial 16S rRNA gene regions were amplified using conventional PCR for each strain. The obtained sequences

were analyzed using the NCBI GenBank database. The closest phylogenetic relatives of the isolates are presented in Figure 3 and Table 3-4.

Table 3. List of primers (regions between *ectB* and *ectC* genes) designed

Primer	5'3' sequence	² nmol	³ Tm	Source
F2	ATGACACAACTGATATGAG	96,023	54	Designed in this study
R2	TTATTCTTCGCTTGTATCAA	80,906	52	Designed in this study
F3	AAGCTAGATACAGAGTG	87,033	48	Designed in this study
R3	ACCAAAGGAGGATTAAA	68,405	46	Designed in this study
F4	ATTAACGCTGCCCCGTCTTGA	79,229	60	Designed in this study
R4	CCCTTCACCTTCGATGCAGT	102,260	62	Designed in this study
F5	TTCGTAACCTCGAAGAA	74,796	48	Designed in this study
R5	CGTGAAGACACAAGCAA	72,495	50	Designed in this study
27F	AGAGTTTGATCCTGGCTCAG	79,547	60	Weisburg et al., 1991
1542R	AAGGAGGTGATCCAGCCGCA	75,758	64	Weisburg et al., 1991

¹HPSF = high purity salt-free primer, ²nmol = primer concentration, ³Tm temperature = Tm values were determined according to the formula $2(A+T)+4(C+G)$ (alpha-DNA)."

Table 4. The specifically designed primers for *ectB-ectC* gene regions

Species	Primer set	Optimized Annealing Temperature	Extension	Final extension
<i>Bacillus alcalophilus</i>	F2–R2	47 °C (20 sec)	72 °C (60 sec)	72 °C (5 min)
<i>Virgibacillus pantothenicus</i>	F3–R3	47 °C (20 sec)	72 °C (60 sec)	72 °C (5 min)
<i>Oceanobacillus</i>	F4–R4	59 °C (20 sec)	72 °C (60 sec)	72 °C (5 min)
<i>Halomonas</i>	F5–R5	47 °C (20 sec)	72 °C (60 sec)	72 °C (5 min)
<i>Kocuria</i>	F5–R5	46 °C (20 sec)	72 °C (60 sec)	72 °C (5 min)

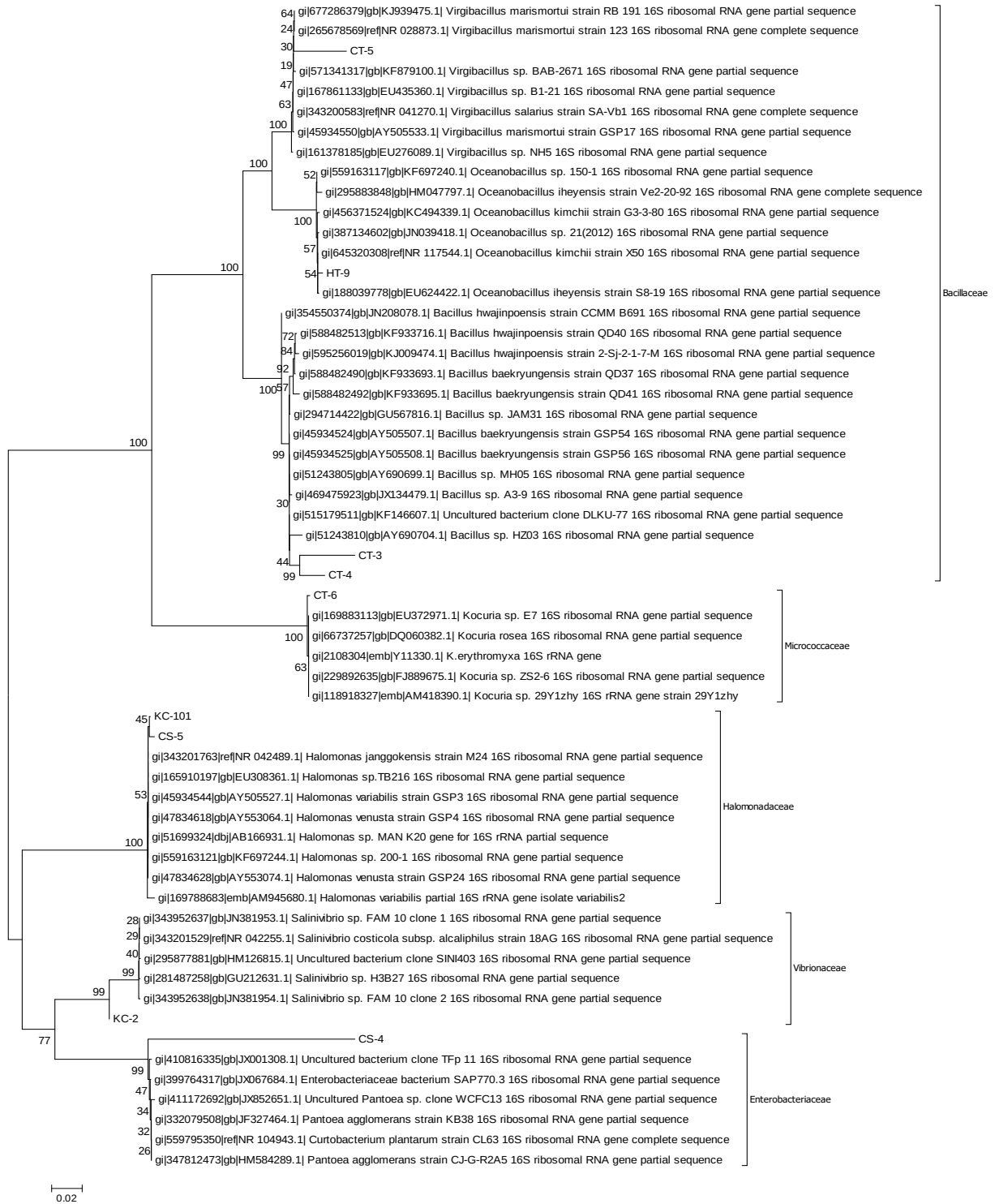


Figure 3. The phylogenetic tree illustrating the relationships among taxa was constructed using the Neighbor-Joining method (Tamura et al., 2004).

A bootstrap analysis with 1000 replicates was performed, and the percentages of replicate trees in which the associated taxa clustered together are shown next to the branches (Saitou and Nei, 1987). The tree was drawn to scale, and branch lengths represent evolutionary distances. Maximum composite likelihood method was used for calculation the evolutionary distances and are scaled as the number of nucleotide substitutions per 100 nucleotide positions. The final dataset consisted of 1295 positions. A total of 58 strain's nucleotide sequences were used in the analysis. Regions with less than 95% coverage were eliminated. Positions containing less than 5% alignment gaps, missing data, and ambiguous bases were eliminated using partial deletion. The analysis was performed using MEGA6 software (Tamura et al., 2013).

Amplification of genes for *ectB-ectC* regions

Genomic DNA was extracted from all isolates, and DNA quality was confirmed by spectrophotometric analysis. Based on 16S rRNA gene identification, closely related species known to possess conserved ectoine

biosynthesis gene regions (*ectB-ectC*) -including *Bacillus alcaliphilus*, *Virgibacillus pantothenicus*, *Halomonas sp.*, and *Oceanobacillus iheyensis*- were selected from GenBank for primer design (Table 3-4).

Gradient PCR was performed using specifically designed degenerate primers. Agarose gel electrophoresis revealed that only three isolates showed positive amplification for the *ectB-ectC* gene region (~2072 bp). Clear DNA bands corresponding to the expected size were observed for *Halomonas sp.* CS-5, *Kocuria sp.* CT-6, and *Oceanobacillus sp.* HT-9 (Table 5). No amplification was detected for the remaining strains. Based on these results, *Halomonas sp.* CS-5 was selected for further ectoine production studies.

Growth analyses

Growth measurements were performed in triplicate at OD₅₂₀, OD₆₀₀, and OD₆₆₀ nm. Among these, OD₅₂₀ consistently showed the highest absorbance values and was therefore selected for subsequent growth analyses (Figures 4-5).

Table 5. Actual codes, similarities, and evaluation of the isolates

Code	NCBI Similarity	Gene Length(bp)	Closest Species name	Ectoine* producers	Isolated Species	NCBI GenBank Accession Number
CT-3	94%	1533	<i>Bacillus sp.</i> MH05	–	<i>Bacillus sp.</i> CT-3	KP238664
CT-4	98%	1507	<i>Bacillus sp.</i> MH05	–	<i>Bacillus sp.</i> CT-4	KP238665
CT-5	95%	1533	<i>Virgibacillus sp.</i> B1-21	–	<i>Virgibacillus sp.</i> CT-5	KP238666
CT-6	99%	1472	<i>Kocuria sp.</i> ZS2-6	+	<i>Kocuria sp.</i> CT-6	KP238667
CS-4	87%	1522	<i>Pantoea agglomerans</i> strain KB38	–	<i>Pantoea sp.</i> CS-4	KP238668
CS-5	99%	1469	<i>Halomonas sp.</i> TB216	+	<i>Halomonas sp.</i> CS-5	KP238669
HT-9	98%	1519	<i>Oceanobacillus kimchii</i> strain X50	+	<i>Oceanobacillus sp.</i> HT-9	KP238670
KC-101	99%	1473	<i>Halomonas sp.</i> TB216	–	<i>Halomonas sp.</i> KC-101	KP238672

*The designed primers were used to genetically determine whether the isolates are ectoine producers.

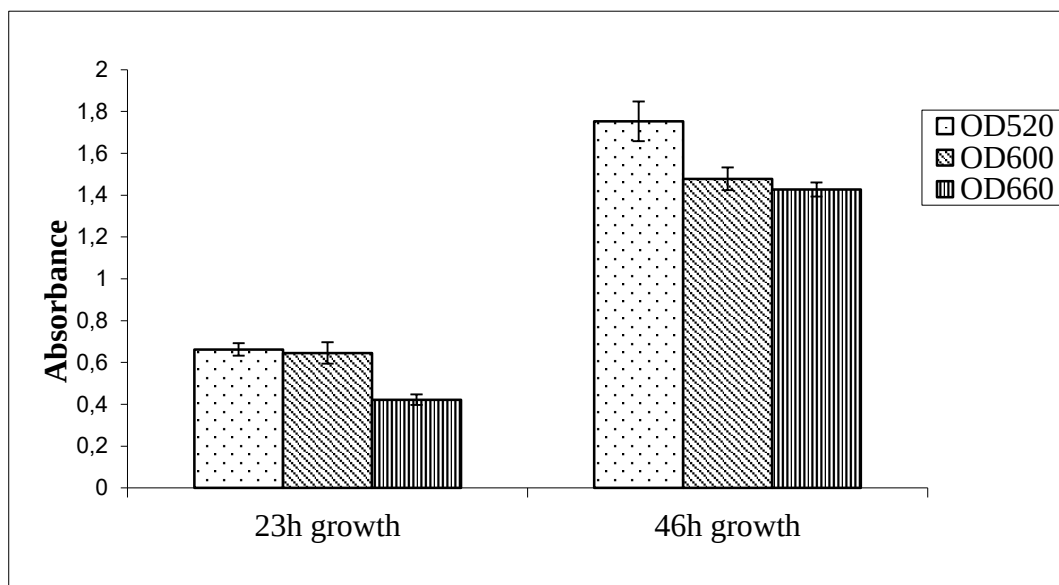


Figure 4. Optical density measurements of *Halomonas sp.* CS-5 cultures 15% (w/v) NaCl measured at three specific wavelengths (520 nm, 600 nm, and 660 nm) using UV-VIS spectroscopy.

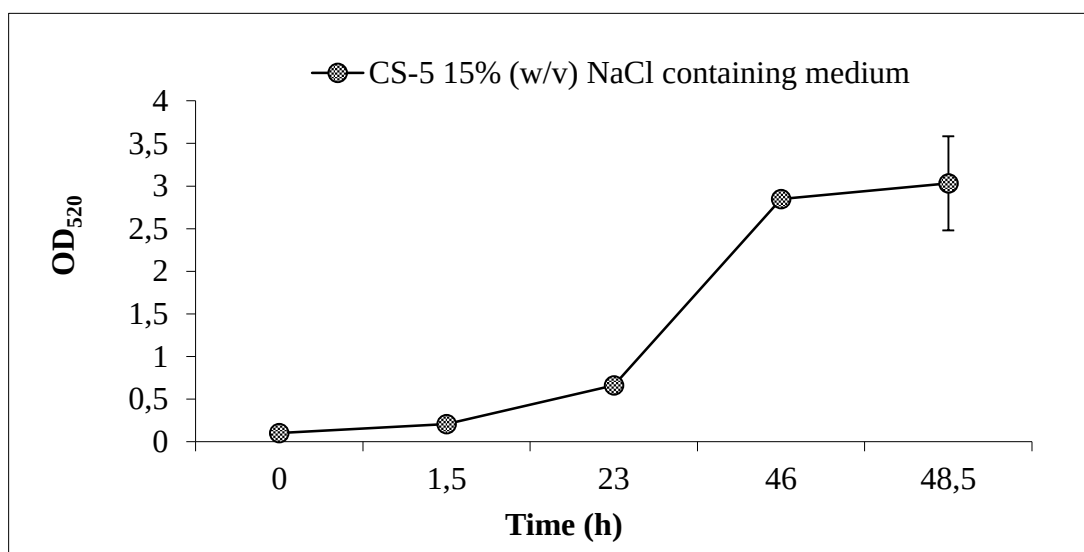


Figure 5. Optical density measurements of *Halomonas sp.* CS-5

LC-MS/MS analysis and ectoine quantification

Intracellular ectoine produced by *Halomonas sp.* CS-5 was extracted and analyzed using LC-MS/MS (Kanapathipillai et al., 2005). A characteristic peak was observed at a retention time (RT) of 0.35 min, consistent with the ectoine standard (Sigma-Aldrich, Cat. No. 81619, Lot No. BCBF0987V), confirming the presence of ectoine in the samples (Figure 6). The MS/MS fragmentation pattern showed typical ectoine fragment ions at m/z 142.8 and 96.8, further validating compound identity. Peak intensity, represented by signal height and area, was used

for quantitative analysis. For cultures grown at 15% (w/v) NaCl, the lyophilized biomass weighed 10.6 ± 0.1 mg and was extracted into 1310 μ L. A 3 μ L aliquot was analyzed by LC-MS/MS. The peak area was determined as 1.541.841. The total intracellular ectoine content was calculated as 0.517 mg (mean $\pm$ SD, $n=3$, SD=0) per 10.6 mg dry cell weight. The volumetric ectoine production was 0.395 g/L (mean $\pm$ SD, $n=3$, SD=0) and the culture reached the stationary phase at 48.5 h, total dry cell weight was 1.77 g/L DCW corresponding to an ectoine yield of 22.3% (w/w) based on dry cell weight.

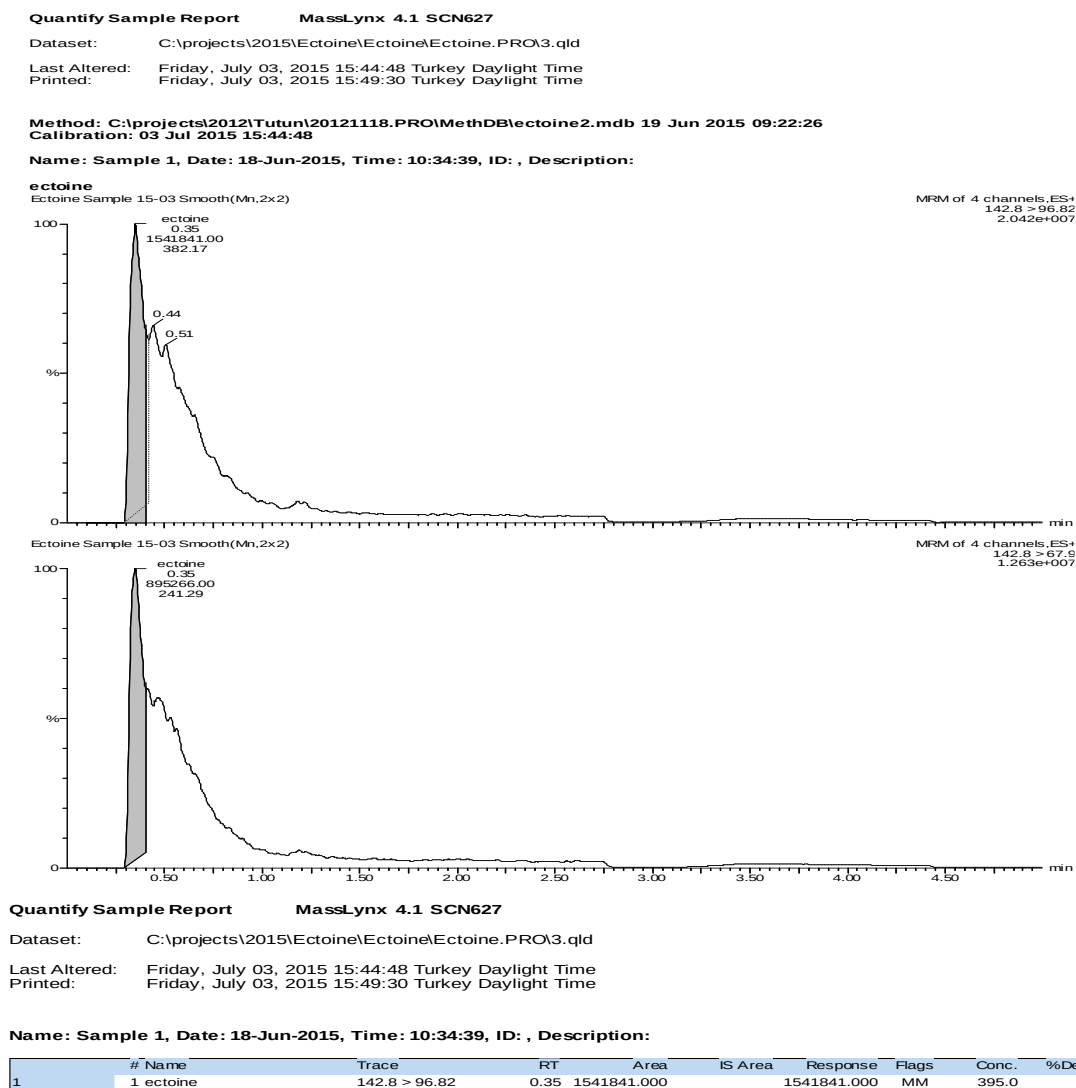


Figure 6. LC-MS/MS results of ectoine extraction.

Discussion

This study presents a study-specific design of degenerate primer sets targeting the conserved *ectB-ectC* gene region involved in ectoine biosynthesis retrieved from NCBI. The primers were developed in-house based on comparative sequence analysis of homologous genes retrieved from public databases (NCBI) and were specifically optimized for PCR-based screening of ectoine-producing strains. Unlike previously reported approaches that primarily rely on phenotypic screening or single-gene amplification strategies, the present work integrates conserved region-based primer design with *in silico* validation and experimental PCR screening. This combined strategy enhances the reliability of detecting ectoine biosynthetic potential in environmental isolates.

The newly designed degenerate primers targeting the *ectB-ectC* gene region enabled rapid and precise identification of ectoine-producing isolates, providing a significant advantage over traditional culture-based screening methods. Conventional approaches are often

labor-intensive, time-consuming, and may fail to detect strains with low or condition-dependent metabolite production (Zanjirband et al., 2008). In contrast, the integration of molecular screening with an LC-MS/MS analytical method establishes a workflow that not only detects the genetic potential for ectoine biosynthesis but also confirms actual metabolite accumulation through analytical confirmation, thereby reducing false-positive results and accelerating strain discovery (Zhang et al., 2009; Liu et al., 2021).

This study focused on the representative characterization of a selected ectoine-producing strain. Although multiple isolates were initially screened for the presence of the *ectB-ectC* gene region, only one positive strain, *Halomonas* sp. CS-5, was selected for detailed downstream analysis. This selection was based on its confirmation as an ectoine producer and its suitability as a representative model within a well-established ectoine-producing genus. The use of a single representative strain enabled the establishment of a proof-of-concept workflow

for ectoine detection and LC-MS/MS validation. The remaining positive isolates will be considered in future studies to further expand strain-level comparisons and production potential.

Halomonas sp. CS-5 cultivated in a medium containing 15% NaCl reached an optical density (OD₅₂₀) of 3.03 ± 0.68 . The total dry cell weight was 1.77 g/L, and ectoine production at 48.5 h was 0.395 g/L (mean $\pm$ SD, n=3, SD=0). The ectoine production observed in the present study is comparable to values reported under unoptimized cultivation conditions for *Halomonas smyrnensis* IIM VA-6. In that study, ectoine production reached 0.54 g/L under non-optimized conditions. Ectoine production increased substantially to 6.5 g/L after 72 h of cultivation by following optimization of the cultivation parameters, including 2.5% monosodium glutamate, 5% (w/v) NaCl, 10.0 g/L lactose at pH 5.0 and 250 rpm. Therefore, the ectoine concentration obtained in this study falls in the range reported for unoptimized culture conditions and indicates the potential for further enhancement through process optimization (Radha et al., 2026).

Using a high-cell-density fermentation combined with a bacterial milking process, *Halomonas elongata* produced approximately 7.4 g/L ectoine through repeated osmotic shock and recovery cycles (Sauer and Galinski, 1998). High-level ectoine production (85 g/L within 52 h) was achieved by *Halomonas bluephagenesis* in a 7 L open, unsterile bioreactor through ectABC operon overexpression, enhanced precursor supply, improved transport systems, and medium optimization, without the use of plasmids, antibiotics, or inducers (Hu et al., 2024). Xu et al., (2023) reported that the ectoine-related gene region from *Halomonas hydrothermalis* was cloned into *E. coli* BCT08 to enhance precursor overproduction, particularly aspartate, pyruvate, oxaloacetate, and glutamate, resulting in 36.58 g/L ectoine production within 30 h of fermentation. Ectoine titer was increased by sugar feeding rate to 131.8 g/L after 96 h of cultivation which is one of the highest reported production levels up to date (Xu et al., 2023).

Although the ectoine yield obtained in this study is lower than values reported for genetically engineered or process-optimized systems, it should be noted that *Halomonas* sp. CS-5 is a naturally isolated strain, without any genetic modification or fermentation optimization. Therefore, the results obtained here represent a realistic baseline for natural ectoine production and further emphasize the potential of this strain for improvement through targeted metabolic or process optimization strategies (Chen et al., 2018).

Although the ectoine yield obtained in this study reflects the performance of a naturally isolated strain without genetic or process optimization, it also highlights the potential for further improvement. The relatively moderate production level suggests that cultivation conditions and metabolic capacity could be limiting factors under the current setup. Therefore, process optimization strategies such as fed-batch cultivation, controlled osmotic

upshift, and optimization of carbon and nitrogen sources may substantially enhance ectoine production. In addition, metabolic engineering approaches, including overexpression of the *ectABC* operon and reinforcement of precursor pathways, have been successfully applied in other microbial systems to achieve higher ectoine yields (Schwibbert et al., 2011; Xu et al., 2023), and could similarly be considered for *Halomonas* sp. CS-5 in future studies.

The integration of degenerate primer-based molecular screening with LC-MS/MS provides a potentially effective preliminary workflow for identifying ectoine-producing strains. Compared with conventional screening methods that rely primarily on phenotypic assays and cultivation, this combined approach enables rapid detection of biosynthetic potential at the genetic level, followed by precise analytical confirmation of metabolite accumulation. Such a strategy reduces the time and labor required for strain discovery and facilitates the identification of high-value microbial candidates from complex environmental samples. Furthermore, this workflow is readily adaptable to other compatible solutes and industrially relevant metabolites, offering broad applicability in microbial biotechnology and bioprospecting (Chen et al., 2018).

The current study has several limitations. Ectoine production was evaluated at a single time point and under a limited range of salinity conditions. Future work should include detailed time-course analyses to better understand the dynamics of ectoine biosynthesis and to assess potential trade-offs between growth and metabolite accumulation. Additionally, exploring a broader range of salinity levels and medium compositions would help more precisely define optimal production parameters. Beyond process optimization, combinatorial strategies integrating genetic engineering with adaptive laboratory evolution could further enhance the production potential of naturally isolated strains (Chen et al., 2018).

Conclusion

In conclusion, this study presents an in silico primer design targeting the conserved *ectB-ectC* gene region retrieved from NCBI. Newly designed degenerate primers were developed and applied in PCR-based screening to investigate the presence of ectoine biosynthesis-related genes in environmental isolates. PCR screening revealed the presence of the *ectB-ectC* gene region in *Kocuria* sp. CT-6, *Oceanobacillus* sp. HT-9, and *Halomonas* sp. CS-5. Ectoine production was subsequently assessed by LC-MS/MS analysis. Using this combined approach, *Halomonas* sp. CS-5 was identified as a candidate strain for further analysis. The results demonstrate that the proposed workflow may support the selection and screening of potential ectoine-producing microorganisms from environmental samples.

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Conflicts of Interest

The authors declare that there are no conflicts of interest or competing interests.

Author Contributions

All authors contributed to the idea and design of the study. Material preparation and investigation were carried out by Ebru Tekin. Writing and editing were performed by Ebru Tekin, and all authors have read and approved the final manuscript.

Ethics Approval

No specific ethical approval was necessary for the study.

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Author Contributions

Author contributions should be stated in the manuscript. Author contributions should be included in the manuscript by the authors after the referee evaluation process of the article is completed and accepted by the editor.

For examples;

- D. Nak, E. Kuruoglu and Y. Nak, planned and designed the research. Z. M. Ekici, D. Koca, T. Avcılar, M. E. Sahin and A. H. Shahzad provided help in the clinic process. M. O. Ozyigit and Z. Avcı Kupeli made histopathological examinations. All authors discussed the results and contributed to the final manuscript.
- D. Çayan and E. Unur conceived the ideas of the study and writing manuscript; D. Çayan, M. Nisari, D. Patat and E. Dağlı performed data collection and analysis; H. Akalın performed gene expression stages.

Ethics Approval

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