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Research Article
Turkish Journal of Maritime and Marine Sciences

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Determination of Fish Diversity in The Rocky Habitat Around Mersin Boğsak Island (Northeast Mediterranean) by Visual Census Method

Mersin Boğsak Adasi (Kuzeydoğu Akdeniz) Çevresinde Yer Alan Kayalık Habitattaki Balık Çeşitliliğinin Görsel Sayım Yöntemiyle Belirlenmesi

Türk Denizcilik ve Deniz Bilimleri Dergisi
Cilt: 10 Sayı: 4 (2024) 194-205

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ABSTRACT

The study was carried out in the rocky bottom habitat of Boğsak Island in Mersin Bay in November, March and July 2022-2023, using visual census method between 0-18 m depth and the survey was conducted along a transect. In the study, the structure of the fish community of the rocky bottom habitat of Boğsak Island was determined and the seasonal availability, abundance, feeding status and distribution of native and non-native fish species were determined. Shannon-Weiner Diversity Index (H') was used to determine species diversity and multidimensional scaling analysis was used to determine the relationship between the data. A total of 29 fish species, including 28 bony fish species belonging to 15 families and 1 cartilaginous *Gymnura altavela* (Linnaeus, 1758) were identified in the study area. The richest family in terms of species diversity was Sparidae (6), followed by Mullidae (3), Serranidae (3), Tetraodontidae (2), Soleidae (2), Labridae (2) and other families with 1 species each. It was determined that 52% of the fish species found in the study area throughout the year were Atlantic, 37% were Indo-Pacific and 11% were cosmopolitan. It was determined that 41% of the fish species were invertivores, 22% were carnivores (feeding on fish and invertebrates), 18% were omnivores, 11% were planktivores, 4% were herbivores and 4% were piscivores. The most abundant species were *Torquigener flavimaculosus* in summer, *Cheilodipterus novemstriatus* in spring and *Chromis chromis* in fall. Shannon species diversity (H'=2.956), richness (d=4.804) and homogeneity (J=0.800) were highest in the fall, followed by spring and summer.

Keywords: Rocky habitat, Fish communities, Underwater observation, Mersin Bay, Türkiye.

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ÖZET

Araştırma, Mersin Körfezi Boğsak Adasık kayalık habitatında, 2022-2023 yılı Kasım, Mart ve Temmuz aylarında, 0-18 m derinlik arasında görsel sayım yöntemi kullanılarak gerçekleştirilmiş ve araştırma doğrusal bir hat boyunca yapılmıştır. Çalışmada, Boğsak Adası'nın kayalık dip habitatının balık topluluğunun yapısı belirlenmiş, yerli ve yerli olmayan balık türlerinin mevsimsel bulunabilirliği, bolluğu, beslenme durumu ve dağılımı tespit edilmiştir. Tür çeşitliliğin belirlenmesinde Shannon-Weiner Çeşitlilik İndeksi (H'), veriler arasındaki ilişkinin belirlenmesinde çok boyutlu ölçeklendirme analizi kullanılmıştır. Çalışma alanında 15 familyaya ait 28 kemikli balık türü ve 1 kıkırdaklı *Gymnura altavela* (Linnaeus, 1758) olmak üzere toplam 29 balık türü tespit edilmiştir. Tür çeşitliği açısından en zengin familya Sparidae (6) olup, bunu Mullidae (3), Serranidae (3), Tetraodontidae (2), Soleidae (2), Labridae (2) ve 1'er türle diğer familyalar takip etmektedir. Çalışma alanında yıl boyunca tespit edilen balık türlerinin %52'sinin Atlantik, %37'sinin Hint-Pasifik ve %11'inin kozmopolit olduğu belirlenmiştir. Balık türlerinin %41'inin invertivor, %22'sinin karnivor (balık ve omurgasızlarla beslenen), %18'inin omnivor, %11'inin planktivor, %4'ünün herbivor ve %4'ünün piscivor olduğu tespit edilmiştir. Yaz aylarında *Torquigener flavimaculosus*, ilkbaharda *Cheilodipterus novemstriatus* ve sonbaharda *Chromis chromis* en bol bulunan türlerdir. Shannon tür çeşitliliği (H'=2.956), zenginliği (d=4.804) ve homojenliği (J=0.800) sonbahar mevsiminde en yüksek olup ilkbahar ve yaz mevsimi bunu izlemiştir.

Anahtar sözcükler: Kayalık habitat, Balık toplulukları, Sualtı gözlemi, Mersin Körfezi, Türkiye.

1. INTRODUCTION

Habitats formed by rocky substrates are rich in biodiversity, despite the fact that coastal ecosystems are known to be the harshest ecosystems in terms of abiotic environmental impact. Rich biodiversity is possible through adaptation to harsh environmental conditions. Rocky ecosystems are more affected by tides in the mediolittoral zone, while bottom habitats are less affected. In addition to natural factors such as interspecific interactions, competition, prey-predator relationships, invasive species pressure, negative impacts of direct human activities such as uncontrolled or unintentional hunting, pollution, unintentional urbanization / development and tourism have an impact on the biodiversity of rocky ecosystems (Golani, 1999; Molnar *et al.,* 2008; Bonaviri *et al.,* 2009; Satyam and Thiruchitrambalam, 2018).

Rocky ecosystems may dominate along the littoral zone starting from the coast, but may also be surrounded regionally by dune ecosystems or reefs (Satyam and Thiruchitrambalam, 2018). This has implications for biodiversity. Every surface is a habitat for aquatic organisms. The existence of suitable habitats, especially for sessile species that can survive by clinging to a place and mobile species such as fish, seaslug and cephalopods, plays an important role in enriching biodiversity. Since each species has a different function in the ecosystem, the preservation of the ecological balance is only possible through the regular maintenance of their relationships with each other and their environment.

Rocky bottom habitats support a large number of vertebrate and invertebrate species, are rich in nutrients and oxygen, and the burrows created by the impact of rough waves between the rocks provide shelter from predation for many species (Planes *et al.,* 2000). On the other hand, the abundance and diversity of food attract predators (Hindell *et al.,* 2000, Hyndes *et al.,* 2003, Ornellas and Coutinho, 1998) and the suitable breeding environment attracts many non-native fish species (Cocheret de la Morinière *et al.,* 2002; García-Rubies and Macpherson, 1995; Lloret *et al.,* 2002; Nagelkerken and van der Velde, 2004).

Regional and seasonal changes are observed in the structure of fish communities in rocky habitats. This change is influenced by competition between species for food, shelter, reproduction and habitat, the dominance of non-native species, the structure of algal communities and anthropogenic factors (Fishelson *et al.,* 2002; Guidetti and Boero, 2004; Guidetti *et al.,* 2002; Hughes *et al.,* 2003; Kucuksezgin *et al.,* 2006; Letourneur *et al.,* 2001; Pinnegar and Polunin, 2004; De Raedemaecker *et al.,* 2010).

Depth, wave exposure, bottom slope and abundance of seagrass meadows have been considered in studies of fish communities in rocky ecosystems in the Mediterranean (Colloca *et al.,* 2003; Gust *et al.,* 2001; Kallianiotis *et al.,* 2000; Valesini *et al.,* 2004) and it has been reported that the highest fish abundance was found in seagrass meadows and shallow rocky habitats (De Raedemaecker *et al.,* 2010).

It has been emphasized that many vertebrate and invertebrate species such as pufferfish and lionfish, poisonous sea urchins (*Diadema setosum*), jellyfish (*Rhopilema nomadica*) have caused changes in the biodiversity of the northeastern Mediterranean (Öztürk and İşinibilir, 2010; Bariche *et al.,* 2013; Bilecenoğlu *et al.,* 2019; Dağhan and Demirhan, 2019). Underwater visual census (UVC) studies in the Mediterranean are generally conducted in coastal and sensitive areas (marine protected areas, marine reserves, national parks). In Turkey, on the other hand, it is noteworthy that UVC studies are limited in number and mainly conducted in artificial reef areas (Horosan, 2016).

Uncontrolled fishing activities have contributed more to this change in the biodiversity of the northeastern Mediterranean than non-native migratory pressure. Artificial reefs, designed to increase fish populations in decline, have also created new habitats, especially for species with no or limited predators. Therefore, artificial reefs may be one of the anthropogenic factors that have a negative impact on biodiversity change in the Northeast Mediterranean.

The Turkish coast in the Northeastern Levantine has natural rocky bottom habitats. One of these habitats is located around Boğsak Island in the Gulf of Mersin and the main objective of this study is to investigate the changes caused by changing biodiversity and alien species impact on this special ecosystem. The study area is a protected area away from sport, amateur and professional hunting pressure and has the ability to reflect the natural biodiversity. The aim of this study was to determine the seasonal distribution, abundance and species diversity of fish species distributed in the rocky bottom habitat of Boğsak Island and to investigate the effect of non-indigenous species on biodiversity.

2. MATERIALS AND METHODS

The research was carried out in the rocky habitat of Boğsak Island in Mersin Bay, Mersin Bay in November, March and July, representing the fall, spring and summer periods of 2022-2023, by making underwater observations between 0-18 m depths. Coordinates of the study area: 36.268621 N, 33.828460 DE. The map of the study area is shown in Figure 1, and the maximum depth of the bottom dominated by rocky substrate structure was measured to be 18 m.

Diving and visual census techniques were used in the study. To obtain the data in the study, an underwater visual census was conducted using the scanning technique over a transect in the study area. An underwater camera (GoPro 12 Black) was used for underwater video recording. A total of 24 dives of approximately 40 hours were made, with video recording continued for 10-15 minutes depending on the behavior of the fish. Dives were conducted during the day before noon.

In this visual census-based study conducted by scuba diving along a linear line, the entire area was videotaped and the species observed and their numbers were determined. Describes the methodology and application used in this study and does not identify the possible effects of the choice of methodology on the data obtained. This study was not designed to determine the possible effects of the UVC method and its application on the data obtained. Therefore, even though the time spent in the scanned area was recorded, it was not considered sufficient to standardize the method due to the large scanned area chosen.

In the study, the fish communities in the rocky habitat of Boğsak Island were determined by using video recordings made during dives with the scanning method on a single transect within the area shown in Figure 2. The recordings observed and visualized during the dives in the field were analyzed in a computer environment. Akşiray (1987), Eschmeyer (2003), Fricke *et al.,* (2007) and Bilecenoğlu *et al.,* (2014) were used for the identification of local and non-native fish species.

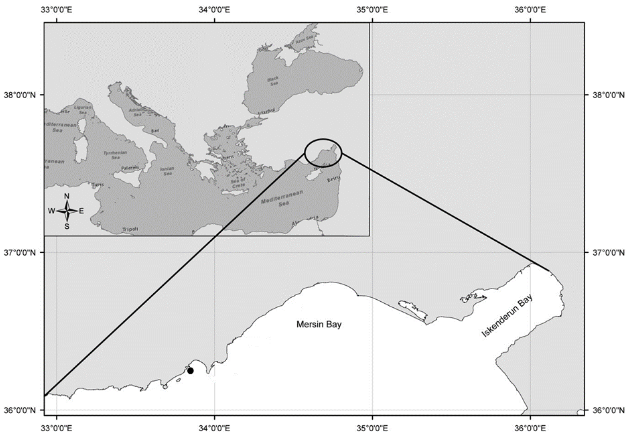

Figure 1. The area indicated by the black dot (●) is the Boğsak Island rocky bottom habitat sampling site

Figure 2. The red line shows the scanned area at the study site

The Shannon-Weiner diversity index (H'), the homogeneity and relative diversity index Evennes (J), the Dominance Index (D) and the species richness index Margalef Rhichnes (d) were used to determine species diversity (Zar, 1984). Biodiversity and statistical analyses were performed using the PAST version 4.09 package. Non-metric multidimensional scaling (N-MDS) analysis was used to visualize the relationship between the number of individuals in seasonal dives. The Euclidean formula was used to calculate the distances between coordinates in the N-MDS scaling. The stress value for N-MDS analysis was set to 0.04. To accurately represent the data, the final stress values should ideally be less than 10% and no greater than 30%. The ellipse indicates similarity between species 95% confidence intervals. Principal component analysis (PCA) was used to determine the effect of seasonal variation on species diversity and abundance.

3. RESULTS

In this study, the structure of fish communities in the rocky bottom habitat of Boğsak Island was investigated by underwater visual census technique in the fall, spring and summer. As the sea conditions were not favorable in winter, the study was planned for three seasons. The identified species were systematically classified and their distribution areas, feeding patterns and seasonal changes in the structure of the fish community were determined.

A total of 28 bony fish species belonging to 15 families and one cartilaginous fish species belonging to the family Gymnuridae were identified in a total of 24 dives conducted in November, March and June in 2022-2023 in the rocky bottom habitat of Boğsak Island. The richest family in terms of species diversity was Sparidae (6), followed by Mullidae (3), Serranidae (3), Tetraodontidae (2), Soleidae (2), Labridae (2) and other families with 1 species each.

In our study, Sparidae was found to be the richest family in terms of species diversity in the rocky bottom habitat of Boğsak Island.

Of the fish species identified throughout the year in the study area, 52% were Atlantic, 37% Indo-Pacific, and 11% cosmopolitan species.

The distribution of the fish community in the study area was determined according to their diet. Invertebrate-eating fish species constituted 41% of the community, followed by carnivores (feeding on fish and invertebrates) with 22%, omnivores with 18%, planktivores with 11%, herbivores with 4% and psychivores with 4%.

T. flavimaculosus in the summer season, *C. novemstriatus* in the spring season and *C. chromis* in the fall season were determined as the species with the highest abundance in the structure of the rocky bottom habitat fish community of Boğsak Island.

Table 1 shows the seasonal standardized

abundance of species observed during the November, March, and June dives in the study area and the average number of individuals of each species observed. As shown in Table 1, 22 fish species were observed in the fall season, 20 in the summer season and 17 in the spring season. Since the study area was determined by scanning on a transect, the results show the number of species and individuals encountered at the time of the dive. According to the similarity index scatter plot of the multidimensional scaling analysis, the number of individuals of *T. flavimaculosus*, *C. chromis* and *C. novemstriatus* showed a statistically significant difference from other species. In the N-MDS analysis, the value of the correlation coefficient was R^2=0.8772 for spring, R^2=0.5224 for summer and R^2=0.0668 for fall, with a stress value of 0.04, which was found to be significant ($P<0.01$). Principal component analysis results show that *C. novemstriatus* in March, *T. flavimaculosus* in June, and *C. chromis* in November have a statistically significant difference in abundance from other species (Figure 3). Principal component analysis has 80.03% variation in PC1 and shows similar results with N-MDS analysis, which shows seasonal separation in the abundance of fish species. The distance between taxa observed during the sampling period by visual census was determined. Ward's method algorithm was used to calculate the distance between taxa and the Euclidean similarity index was used. Although the observation results do not emphasize a sharp separation between the groups, it is seen that the groups can be separated from each other. *T. flavimaculosus*, *C. chromis* and *C. novemstriatus* appear to cluster separately from the other species observed (Figure 4).

Seasonal changes in fish community structure in the rocky bottom habitat of Boğsak Island were determined by diversity index analysis. Shannon diversity index refers to the diversity and abundance of fish species, Margalef richness (d) refers to the species richness, i.e. the number of species in the community, and evenness (J) value refers to the homogeneity of species distribution in the community. According to Shannon's diversity index (H'), the highest species diversity and evenness (J) were found in the fall (H' =2,956; J=0.801), followed by spring (H' =2.501; J=0.642) and summer (H' =2.458; J=0.531), while the Margalef D index of species richness was highest in fall (d=4.804), summer (d=3.917) and spring (d=3.632) (Table 2).

The analysis of the Shannon Diversity Index shows the seasonal separation of the fish species diversity found in the study area. It can be seen that the diversity is higher in the fall (November) season than in the spring (March) and summer (June) seasons. In terms of species richness, November was the highest followed by June and March observations. In the study, it was found that the similarity of March and June observations in terms of species richness in the fish community of Boğsak Island was high, while November observations showed a difference.

It was determined that 52% of the fish species observed in the study area were local species and 37% were Indo-Pacific species. The abundance of Indo-Pacific species clearly indicates competition with local species.

Table 1. Seasonal availability of species and number of individuals observed

Family	Species	Spring	Summer	Autumn	Origin	Feeding type (Froese and Pauly, 2024)
Gymnuridae	*Gymnura altavela*	-	1	0	Atlantic	Omnivore
(Mullidae	*Mullus barbatus*	1	1	0	Atlantic	Invertivore
	Upeneus moluccencis	13	7	3	Indo-Pasific	Invertivore
	Parupeneus forskalii	9	12	7	Indo-Pasific	Invertivore
Haemulidae	*Pomadasys stridens*	0	2	0	Indo-Pasific	Carnivore
Sparidae	*Spicara flexuosa*	3	1	3	Atlantic	Planktivore
	Spicara smaris	1	6	1	Atlantic	Planktivore
	Boops boops	2	6	1	Atlantic	Omnivore
	Diplodus vulgaris	12	14	10	Atlantic	Invertivore
	Sparus aurata	1	1	0	Atlantic	Omnivore
	Diplodus annularis	3	2	2	Atlantic	Invertivore
	Dussumeria elopsoides	0	0	2	Indo-Pasific	Carnivore
Scorpaenidae	*Pterois miles*	2	4	0	Indo-Pasific	Piscivore
Siganidae	*Siganus rivulatus*	0	0	2	Indo-Pasific	Herbivore
Serranidae	*Epinephelus aeneus*	0	0	7	Circumglobal	Invertivore
	Serranus cabrilla	10	8	6	Atlantic	Carnivore
	Serranus hepatus	2	13	8	Atlantic	Carnivore
Mugilidae	*Mugil cephalus*	0	0	1	Cosmopolitian	Carnivore
	Chelon ramada	4	0	11	Cosmopolitian	Omnivore
Apogonidae	*Cheilodipterus novemstriatus*	35	22	10	Indo-Pasific	Planktivore
Sphyraenidae	*Sphyraena chrysotaenia*	0	0	2	Indo-Pasific	Invertivore
Carangidae	*Trachurus trachurus*	0	1	6	Atlantic	Carnivore
Labridae	*Coris julis*	1	4	5	Atlantic	Invertivore
	Thalassamo pavo	9	10	8	Atlantic	Invertivore
Pomacentridae	*Chromis chromis*	20	25	18	Atlantic	Omnivore
Soleidae	*Monochisrus hispidus*	0	2	2	Atlantic	Invertivore
	Solea solea	0	0	1	Atlantic	Invertivore
Tetradontidae	*Lagocephalus sceleratus*	2	3	1	Indo-Pasific	Carnivore
	Torquigener flavimaculosus	12	68	3	Indo-Pasific	Invertivore

Table 2. Diversity Index Analysis values of fish species observed in the study area

	Month		
Index	**March**	**June**	**November**
Shannon_H	2.501	2.458	2.956
Evenness_$e^{H/S}$	0.642	0.531	0.800
Margalef	3.632	3.917	4.804

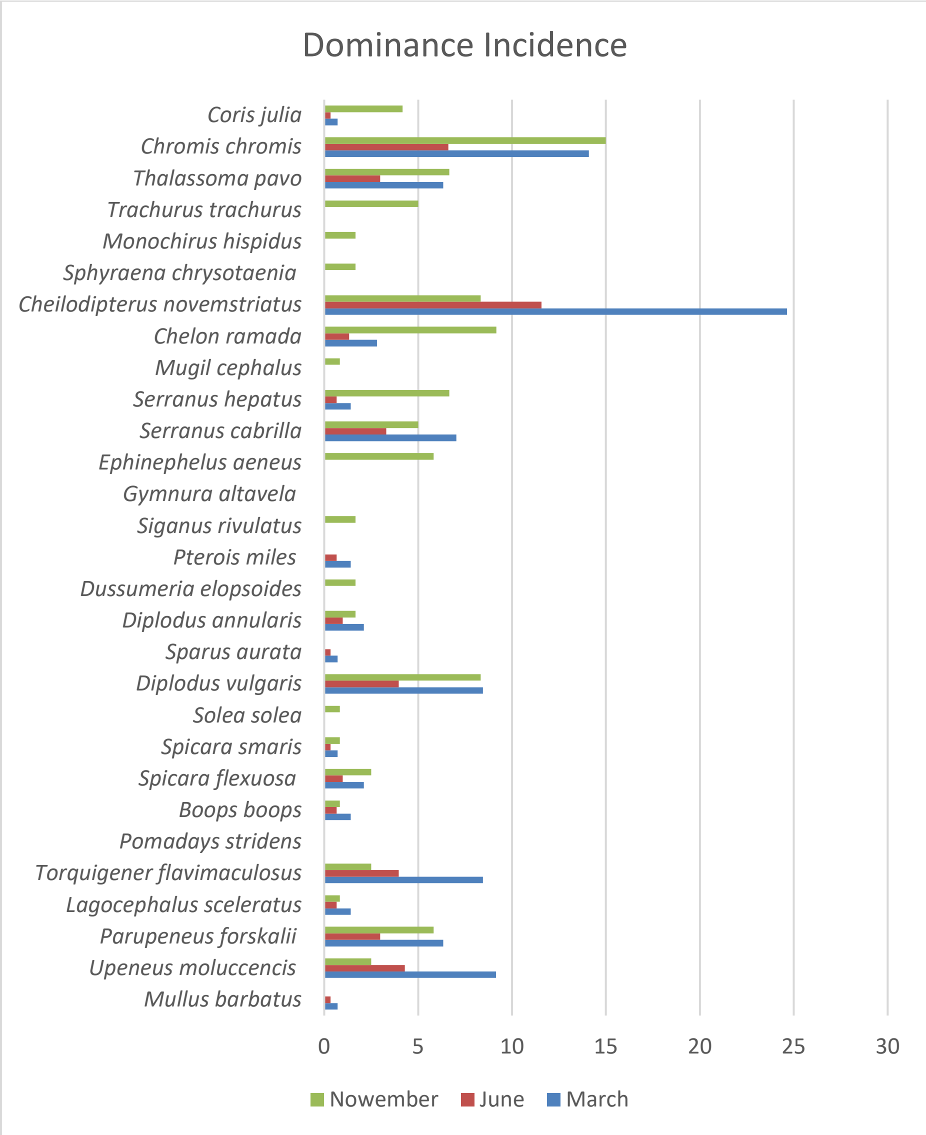

Figure 3. Dominance incidence of fish

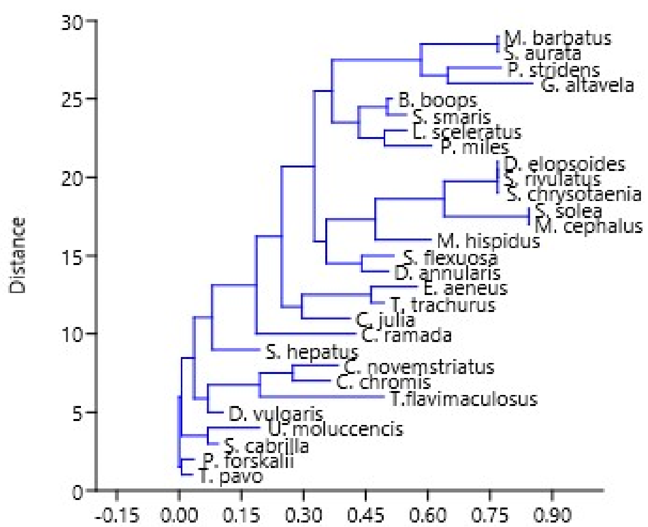

Figure 4. Dendrogram of distance between taxa with Bray-curtis similarity index

4. DISCUSSIONS

Boğsak Island a suitable habitat for many aquatic organisms including vertebrates and invertebrates. Due to its rich biodiversity, it is one of the special area that have ecological importance and should be protected.

In addition to fishes, *Spondylus gaederopus* Linnaeus, 1758, a spiny clam belonging to the phylum Mollusca, and *D. setosum* (Leske, 1778), a long spiny sea urchin belonging to the phylum Echinodermata, have been observed in the study area. *Halophila stipulaceae* (Forsskål) Ascherson, 1867, a tropical seagrass, and *Padina pavonica*, a brown algae, were also observed in the study area.

Among the findings of previous studies investigating fish communities in rocky bottom habitats in the eastern Mediterranean, the presence of 62 species belonging to 27 families between 0-32 m depths in the Lebanese rocky bottom habitat (Harmelin-Vivien *et al.,* 2005), 79 species belonging to 31 families in 3 different study sites in Israel rocky bottom habitat (Golani *et al.,* 2007); 61 fish species belonging to 21 families in 14 different rocky bottom habitats around the island of Arki in the Aegean Sea (De Raedemaecker *et al.,* 2010); 54 species belonging to 21 families in 26 islands in the rocky bottom habitat of the Cyclades Archipelago (Northeast Mediterranean) (Giakoumi and Kokkoris, 2013) were reported.

In similar studies conducted in the northwestern Mediterranean, 38 species belonging to 17 families were reported in shallow rocky reef areas in Sardinia, Italy (Pais *et al.,* 2007), and 68 species belonging to 22 families in rocky coastal habitats of Cape Portofino in the Ligurian Sea (Tunesi *et al.,* 2006).

In our country, Horosanlı (2016) investigated the distribution of fish species in *Posidonia oceanica* habitats between 0-15 m between April and September 2016 in a study conducted by underwater visual census method in Yıldız Bay in Gökçeada Underwater Park by Horosanlı (2016). A total of 64 fish species belonging to 20 families were identified in the study area.

This study was conducted seasonally in the rocky bottom habitat of Boğsak Island in the Gulf of Mersin, and the number and abundance of fish species observed cannot be compared because no similar study has been conducted in the same area. However, the number and abundance of fish species are lower compared to previous studies conducted in the eastern Mediterranean. It is thought that this difference may be due to differences in methodology. Fish abundance and richness may vary depending on depth and substrate structure, as well as anthropogenic pressures such as fishing activities, pollution, etc. Previous similar studies conducted in the Levant Basin reported that Labridae was the richest family in terms of species diversity, followed by Sparidae in second place (De Raedemaecker *et al.,* 2010; Giakoumi and Kokkoris, 2013; Sini *et al.,* 2019). In the Ligurian Sea in the western Mediterranean, the Sparidae family was reported to be richer in species diversity than the Labridae family (Tunesi *et al.,* 2006).

While *C. chromis*, *Oblado melanura*, *Spicara smaris*, *Coris julis* and *Thalassamo pavo* were reported to be the dominant fish species along the Lebanese coast (Harmelin-Vivien *et al.,* 2005), *T. pavo*, *C. julis* and *C. chromis* were similarly reported in the rocky habitats of Arki Island (De Raedemaecker *et al.,* 2010). *C. chromis*, *Boops boops*, *T. pavo*, *S. smaris*, *C. julis*, and *Diplodus vulgaris* have been reported in rocky habitats in the Aegean (Sini *et al.,* 2019). Yalgın and Türker (2023) reported that *C. chromis*, *Sparisoma cretense*, *Siganus luridus*, *Siganus rivulatus*, *T. pavo*, *S. smaris*, *C. julis*, and *D. vulgaris* were the most abundant species in terms of abundance in a visual census study conducted on the northern coast of Cyprus. In this study conducted in the rocky habitat of Boğsak Island, *T. flavimaculosus* was the most abundant species followed by *C. chromis*, *C. novemstriatus*, *D. vulgaris*, *Parupeneus forsskalii*, *T. pavo*, *Serranus cabrilla*, *U. mollucensis* and *Serranus hepatus*.

The fact that the species with the highest abundance in the rocky bottom habitat of Boğsak Island are species that entered the Mediterranean through species migration originating from the Indo-Pacific suggests that non-native species are more dominant in the study area. *C. novemstriatus* is associated with *D. setasum* and both species coexist. *C. novemstriatus* is protected from predators by the long spines of *D. setasum*. The increase in the abundance of *C.*

novemstriatus in the rocky bottom habitat of Boğsak Island can be seen as a natural consequence of the increase in the sea urchin population. The dwarf pufferfish *T. flavimaculosus* is a species of pufferfish found in the northeastern Mediterranean Sea. This species also exerts pressure on the fish communities in the rocky bottom habitat of Boğsak Island by competing for food. *C. chromis* was observed as the second most abundant species in the rocky bottom fish community of Bogsak Island. With the exception of *T. flavimaculosus* and *C. novemstriatus*, the rocky bottom fish assemblage of Bogsak Island is similar to the native dominant species reported in previous studies conducted in the Levant Basin.

Harmelin-Vivien *et al.,* (2005) reported that 13% of the fish species diversity and 19% of the abundance in the rocky habitat of the Lebanese coast were Indo-Pacific species. Golani *et al.,* (2007), in their study of rocky habitat ichthyofauna in different regions of the Israeli coast, identified 79 species, of which 29% (23 species) were local, 44% (35 species) were area dependent, and 27% (21 species) were migratory species from the Red Sea. The researchers stated that the low number of migratory species may be due to the lack of a continuous rocky habitat connecting the northern Gulf of Suez, the Suez Canal, and the southern Mediterranean coast of Israel. Yalgın and Türker (2023) determined the distribution of 72 different fish species, belonging to 26 families, in a visual monitoring study between 0 and 40 m depth along the coast of Northern Cyprus. They reported that 56 of the species were native to the Mediterranean, and 14 of them were of Indo-Pacific origin. The rocky bottom habitat of Boğsak Island, where this study was conducted, seems to have a higher value in terms of Indo-Pacific species diversity than the results of previous studies conducted on the coasts of Lebanon, Israel and Northern Cyprus. This can be explained by the abundance of nutrients and oxygen, clarity and, most importantly, interspecific competition. The high abundance of *T. flavimaculosus* and *C. novemstriatus*, which are Indo-Pacific species, in this study conducted in the rocky bottom habitat of Boğsak Island may indicate non-local species pressure on local species. Among the Indo-Pacific species, *P. forsskali* is an economically important demersal species. This species can compete for the habitat of local demersal species such as *M. barbatus* and *M. surmuletus*. *U. mollucensis*, another Indo-Pacific species of the Mullidae family, is another species that creates competition for local species, as does *P. forsskalii*.

De Raedemaecker *et al.,* (2010) reported that among the fish species distributed in the rocky habitats of the islands around Arki Island in the Aegean Sea, invertebrate-feeding fish species were dominant, followed by planktivores, carnivores, herbivorous invertebrates, and omnivores. Similarly, invertebrate-feeding fishes were found to be dominant in the fish communities of the rocky bottom habitat of Boğsak Island compared to species with other feeding types. The low number of herbivorous species may be related to the vegetation.

Previous research provides limited information on the temporal distribution of fish assemblages. De Raedemaecker *et al.,* (2010) emphasized that temporal segregation of fish assemblages can be interpreted through more comprehensive studies. Indeed, the fact that seasonal temperature differences are lower in tropical and temperate rocky coastal habitats than in cold climate habitats suggests that more sensitive studies may be needed to interpret this distinction.

Most quantitative estimates of fish abundance in rocky habitats in the Mediterranean are based on underwater visual surveys. The results of fisheries surveys may not provide accurate data for such estimates, mainly due to problems of fishing efficiency and selectivity (Katsanevakis *et al.,* 2012). In addition, underwater visual surveys are usually conducted in protected areas to monitor marine protected areas (Rius, 2007), and information on the status of fish populations in Mediterranean rocky habitats is very limited (Sala *et al.,* 2012; Guidetti *et al.,* 2014).

Of the fish species observed, 41% were species that feed only on marine invertebrates. This was followed by carnivorous and omnivorous species. There are *H. stipulacea* beds in dune habitats around the study area. Studies report that this species is consumed by the green sea turtle *Chelonia mydas* (Becking *et al.,* 2014).

In previous studies conducted to determine the fish species composition of rocky bottom habitats in the east (Golani *et al.,* 2007), west (De Raedemaecker *et al.,* 2010) and south (Harmelin-Vivien *et al.,* 2005) of the Levant Basin, the results obtained are higher than the number determined in this study. This can be explained by the variation in area, number of stations, depth, observation frequency, environmental conditions, food abundance and anthropogenic factors. In addition, David *et al.,* (2024) emphasized that the diver effect may prevent some species from being observed due to escape from the environment. In this study, the reason why some species known to be present in the study area (species belonging to families such as Blenniidae, Gobiidae, Holocentridae, Pempheridae) could not be observed may be related to the effect of factors such as diving time and diver effect.

5. CONCLUSIONS

The rocky bottom habitat is a biologically rich environment. Often found where land and sea meet, rocky bottom habitats provide a unique habitat for some special fauna. Fauna living in rocky bottom habitats are exposed to daily and seasonal changes such as currents and temperature. Common animal groups found in rocky bottom habitats include sea urchins, sponges, sea anemones, molluscs and some fish communities. Today, this habitat type and its flora and fauna, which are of great importance for the health of marine ecosystems, are threatened by climate change caused by anthropogenic activities. In addition, the fish communities of rocky bottom habitats in this part of the Mediterranean are changing due to the migration of Indo-Pacific species to the eastern Mediterranean. This study, which is the first to examine the structure of rocky habitat fish communities in the northeastern Mediterranean coast of Turkey, is expected to contribute to the literature for the Levant Basin, which shows a dynamic structure in terms of biodiversity.

AUTHORSHIP CONTRIBUTION STATEMENT

Mert ATEŞ: Sampling **Nuray ÇİFTÇİ:** Conceptualization, Methodology, Writing-Original Draft, Writing-Review and Editing, **Deniz ERGÜDEN:** Data Curation, Review and Editing, **Deniz AYAS:** Conceptualization, Visualization, Writing-Review and Editing.

CONFLICT OF INTERESTS

The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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Antimicrobial Effects of Chitosan Extracted from Crayfish Shells in Cream Formulations

Kerevit Kabuklarından Elde Edilen Kitosanın Krem Formülasyonlarındaki Antimikrobiyal Etkileri

Türk Denizcilik ve Deniz Bilimleri Dergisi

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ABSTRACT

The objective of the present study was to investigate the antimicrobial properties of a chitosan-based cream. To achieve this, the antimicrobial effects of a cream enriched with chitosan were compared with those of a control group. Chitosan, sourced from crayfish obtained frozen from Eğirdir Lake, Eğirdir, Isparta, served as the primary material. The study involved a comparison between control (F1) and treatment (F2) groups. While both cream formulations exhibited bacterial inhibition, only the F1 formulation demonstrated a significant reduction in viable microorganisms for C. albicans. The cytotoxicity assessment revealed a concentration-dependent increase in the cytotoxic effects of the samples. Notably, the F1 formulation exhibited higher toxicity to healthy cells compared to the F2 formulation. In conclusion, further investigation is necessary to understand the mechanisms underlying their cytotoxic effects and to optimize their formulations to enhance biocompatibility. Moreover, the chitosan-based cream developed in this study demonstrated notable antimicrobial efficacy against the tested bacteria.

Keywords: Chitosan, Crayfish, Antimicrobial effect, Cytotoxicity, Skin health

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ÖZET

Bu çalışmanın amacı, kitosan bazlı bir kremin antimikrobiyal özelliklerinin araştırılmasıdır. Bunun için, kitosan ilave edilmiş bir kremin antimikrobiyal etkileri kontrol grubu ile karşılaştırılmıştır. Ana materyal olarak, çalışma kapsamında Eğirdir Gölü'nden dondurulmuş bir şekilde temin edilen kerevitlerden ekstrakte edilen kitosan kullanılmıştır. Çalışma, kontrol (F1) ve muamele (F2) grupları arasında bir karşılaştırmayı içermektedir. Her iki krem formülasyonu da bakteriyel inhibisyon sergilerken, yalnızca F1 formülasyonu C. albicans için canlı mikroorganizmalarda önemli bir azalma göstermiştir. Sitotoksisite değerlendirmesi, örneklerin sitotoksik etkilerinde konsantrasyona bağlı bir artış olduğunu ortaya koymuştur. Özellikle, F1 formülasyonunun sağlıklı hücreler üzerinde F2 formülasyonuna kıyasla daha yüksek bir toksisite sergilediği gözlenmiştir. Sonuç olarak, sitotoksik etkilerinin altında yatan mekanizmaları anlamak ve formülasyonlarını biyouyumluluğu artıracak şekilde optimize etmek için daha fazla araştırma yapılması gerekmektedir. Ayrıca, bu çalışmada geliştirilen kitosan bazlı krem, test edilen bakterilere karşı dikkate değer antimikrobiyal etkinlik göstermiştir.

Anahtar sözcükler: Kitosan, Kerevit, Antimikrobiyal etki, Sitotoksisite, Deri sağlığı

1. INTRODUCTION

The rapid expansion of the global population necessitates the more efficient utilization of limited food resources. Consequently, the aquaculture industry has garnered significant attention on a global scale, aligning with the escalating population growth, and has substantially bolstered the overall production of animal protein (Gomez et al., 2019; Bohnes et al., 2020; Regueiro et al., 2021). In this context, the sector of food production, particularly animal protein production, plays an important role in nutrition and food safety (Maulu *et al.,* 2022). The seafood production sector is thought to be growing rapidly and demonstrating enhanced environmental performance compared to land-farmed animals (Troell *et al.,* 2019).

Seafood encompasses significant animal protein sources obtained through aquaculture and fishing. These products differ from land-based protein sources in that they contain essential micronutrients and fatty acids important for overall health (FAO, 2022). Additionally, shellfish and other seafood products contribute to ecologically advantageous nutrient cycles, making them environmentally beneficial systems. Therefore, they can be a crucial resource for sustainable food production in the future (Cai *et al.,* 2019).

Global seafood production recorded a growth of 69.2% from 2002 to 2022, reaching 184.6 million tons in 2022. It is reported that more than half of production is obtained from aquaculture (FAO, 2022). Shellfish account for more than 9% of total seafood production (FAO, 2022). Shellfish, especially species such as shrimp, crab, and lobster, constitute a diverse group of arthropods and play a significant role in aquaculture. Observations indicate that the shells of these crustaceans, especially lobster shells, hold potential for biological use and inspire new research topics (Chen *et al.,* 2020; Mazlum *et al.,* 2022; Mazlum and Yazıcı, 2023).

Crayfish, which play a pivotal role in freshwater ecosystems, are known to consume a diverse array of resources including other invertebrates, macrophytes, algae, and detritus. Moreover, they demonstrate cannibalistic behavior and display selective feeding habits, preferring certain invertebrates and macrophytes (Mazlum *et al.*, 2021). Crayfish, a popular freshwater organism, contributes directly to the food chain and species diversity when consumed in large quantities. Crayfish are preyed upon by numerous predators, thereby serving as a vital energy source within upper trophic levels. From an economic perspective, crayfish represent a valuable food resource (Nyström, 2002).

Freshwater crayfish, classified within the phylum Arthropoda, class Crustacea, and order Decapoda, comprise a total of over 737 species (Crandall and De Grave, 2017). Crayfish, widely consumed worldwide and recommended as a high-quality food source as part of a balanced diet, have contributed to their growth in the market, establishing them as a valuable resource for human nutrition. Crayfish shells, considered by-products produced in approximately 100.000 tons annually, vary based on species, season, and habitat characteristics (Peng *et al.*, 2016). During crayfish processing, the waste ratio typically falls between 40% and 50%, with chitin comprising 40% of these by-products (Cai *et al.*, 2017). Crayfish shells are rich in omega-3 fatty acids, pigments, 20–30% protein, 30–40% calcium carbonate, and 20–30% chitin. The commercial value and unique properties of these shells have garnered increased attention, leading researchers to explore various applications (Cai *et al.*, 2017). Crayfish exoskeletons are believed to meet emerging research criteria including biomaterial structure, diversity, sustainability, function, and cost-effectiveness (Binnewerg *et al.*, 2020; Araujo *et al.*, 2021; Nekvapil *et al.*, 2021). These properties make crayfish shells valuable in various industrial applications and attract the attention of researchers (Cai *et al.*, 2017). Chitin, a polysaccharide found in crayfish shells, is one of the most abundant natural biopolymers in nature. Previous studies have demonstrated the potential of chitin to be transformed into chitosan, a higher value-added product used in various sectors.

Due to safety and comfort during use, chitosan has replaced synthetic polymers in ophthalmic applications (Dutta et al., 2012). Lenses derived from chitosan offer benefits for ocular injuries owing to their antimicrobial and wound healing attributes (Figiela et al., 2018). Moreover, chitosan has found application as a seed coating agent aimed at pest management and enhancement of plant resilience against microorganisms (Zeng et al., 2010). It has been reported that chitosan affects the growth and germination of Ajowan (Trachyspermum ammi) seeds (Mahdavi and Rahimi, 2013).

Chitosan and its derivatives are preferred due to their plant protective properties against fungi, viruses, bacterial diseases and nematodes (Ramírez *et al.*, 2010). As a wound dressing, chitosan can promote faster regeneration of skin epithelial cells and collagen production by fibroblasts. Adding chitosan to food prevents microbial growth, unpleasant appearances, bad tastes and economic losses. Studies have shown that adding chitosan to cheese improves its microbiological quality, inhibits mold and yeast growth, and extends its shelf life (El-Diasty *et al.*, 2012). The antibacterial properties of chitosan also make it suitable for use as an edible active packaging material (Muzzarelli and Muzzarelli, 2005). Chitosan has been shown to increase fertilizer absorption, release nitrogen, and act as a bio stimulant to increase crop yields. Chitin and chitosan find applications in food, pharmaceuticals, cosmetics, textiles and various industrial sectors. Moreover, its attractive properties such as biodegradability, biocompatibility, biodegradability, and non-toxicity contribute to its importance in providing health benefits (Casadidio *et al.*, 2019).

The purpose of current study was to reveal the effects of antimicrobial properties of chitosan-based cream. For this purpose, the antimicrobial effects of chitosan added cream were compared with the control group.

2. MATERIALS AND METHODS

2.1. Chitosan source and extraction

Crayfish were obtained frozen from Eğirdir Lake, Eğirdir, Isparta (Figure 1).

Figure 1. Crayfish shell materials used in chitosan extraction

Crayfish shells were used as the primary source for chitin extraction. Chemical and biological methods are commonly used to extract chitin from these shells (Pachapur *et al.*, 2016).

The crayfish shells were first washed, dried and ground (Figure 2).

Figure 2. A view of ground crayfish (*Pontastacus leptodactylus*) shells

For chitin extraction, demineralization, and decoloration processes were applied respectively (Pal *et al*., 2014). Chitosan used in the study was obtained after deacetylation (strong alkaline treatment with 12.5 M NaOH). Before using chitosan in the cream formulation, it was prepared with glacial acetic acid at the desired concentration (Trung *et al*., 2020) (Figure 3).

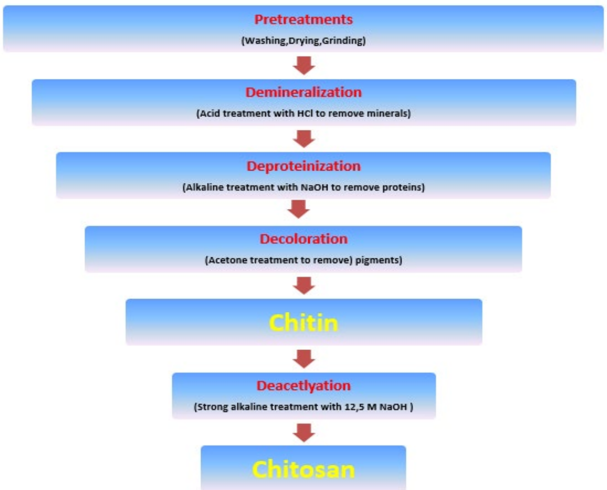

Figure 3. Schematic representation of chitin and chitosan production

2.2. Preparation of chitosan-based cream

Chitosan-based cream formulations are given in Table 1.

Table 1. Chitosan-based cream formulations

Phases	Bileşen	F1(%)	F2(%)
A	Gum	0.3	0.3
A	Glycerine	4	4
A	Distilled Water	69.2	65.7
B	Emulsifier (OLIVEM 1000)	6	6
B	Cetyl Alcohol-Cetyl Alcohol (Vegetal Derived Thickener) -Nafol50/50	2	2
B	Jojoba oil	7	7
B	Argan oil	6	6
B	Shea butter	3	3
C	Vitamin E	0.5	0.5
C	Natural Herbal Preservative (Lexgrad Natural)	1.5	-
C	Chitosan	-	5
C	Lavender Essential Oil	0.5	0.5
	Total	100	100

A, B and C represent phases of cream production

2.3. Microorganisms

In this study, microbial strains *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923 and Candida albicans ATCC 10231* were used for the challenge test. The bacterial strains were activated on Tryptic Soy Agar (TSA) (Merck, Germany) at 37°C for 24 hours while the fungal strain was activated on Sabouraud dextrose agar (SDA) at 30°C for 48 hours. Activated microorganisms were suspended in a physiological saline solution to obtain a density equal to 0.5 McFarland turbidity (approximately 1.5 X 10^8 CFU/mL).

2.4. Challenge test

The microbial control test was conducted following the procedures outlined in the European Pharmacopoeia (EP) guideline 7.0, 2010. Twenty grams of the cream samples (F1 and F2) were aseptically transferred into sterile petri dishes and subsequently inoculated separately with 0.2 mL of bacterial suspension and 0.1 mL of fungal suspension. The inoculated samples were thoroughly mixed and then incubated in the dark at 20–25°C. On days 0, 7, 14, and 28 post-inoculation, 1 g of the inoculated samples was aseptically withdrawn, diluted in physiological saline, and spread onto TSA agar plates for bacterial analysis and SDA agar plates for fungal analysis. The plates were then respectively incubated at 37°C and 28°C for bacteria and fungi. Following the incubation period, the number of viable cells in the samples was enumerated. The results were reported as log colony-forming units per milliliter (log CFU/mL). Each experiment was conducted in triplicate (Fang *et al.*, 2016).

2.5. Cytotoxicity

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay was used to analyze the cytotoxic activities of cream samples F1 and F2 against the L929 mouse fibroblast cell line (ISO 10993-5). The 96-well cell culture plates were seeded with $1x10^4$ cells per well and incubated for 24 h. The cream samples were applied to cells in eight different concentrations (1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.007813 mg/mL). The cell culture plates were incubated in the incubator for 24 hours at 37°C and 5% CO_2.The samples were applied three times at each of the concentrations tested. DMEM medium without samples was used as the negative control. Following the incubation period, 50 µL of MTT solution (1 mg/mL) was added to each well. After 2 hours of further incubation at 37°C, 100 µL of isopropanol was added to the wells, and the absorbance values were measured at 570 nm using a microplate reader to determine cell viability. The percent viability was calculated relative to the control groups, employing the following formula:

$$\text{Cell Viability} (\%) = \frac{\text{Sample OD}}{\text{Control OD}} \times 100 \quad (1)$$

The cytotoxicity studies were made in triplicate and the data were given as mean ± standard deviation (SD). A paired samples t-test was used to compare the control (F1) and treatment (F2) groups.

3. RESULTS

Figure 4 and Figure 5 show chitin and chitosan obtained from crayfish (*Pontastacus leptodactylus)* shell, respectively.

Figure 4. Chitin obtained from crayfish (*Pontastacus leptodactylus*) shell (Mazlum *et al.*, 2022)

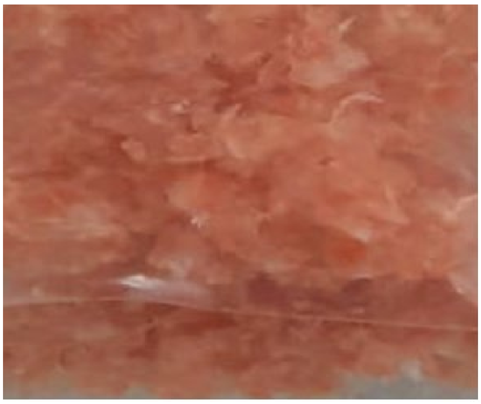

Figure 5. Chitosan obtained from crayfish (*Pontastacus leptodactylus*) shell (Mazlum *et al.*, 2022)

According to the European Pharmacopoeia (EP), "The preservative properties of the preparation are considered adequate if, under the conditions of the test, there is a significant decrease or no increase, as appropriate, in the number of microorganisms in the inoculated preparation after the specified durations and at the prescribed temperatures". The criteria for evaluation of antimicrobial activity are a minimum 3 log reduction after 7 days of inoculation for bacteria, a minimum 2 log reduction after 14 days of inoculation for fungi, and no increase in number of viable microorganisms at 28 days compared to the previous reading. Growth inhibition of tested microorganisms in cream formulations are presented in Figure 6. The log reduction in the number of viable microorganisms is given in Table 2.

Although both cream formulations met the criteria for bacterial inhibition, only the F1 formulation caused the necessary reduction of viable microorganisms for *C. albicans*.

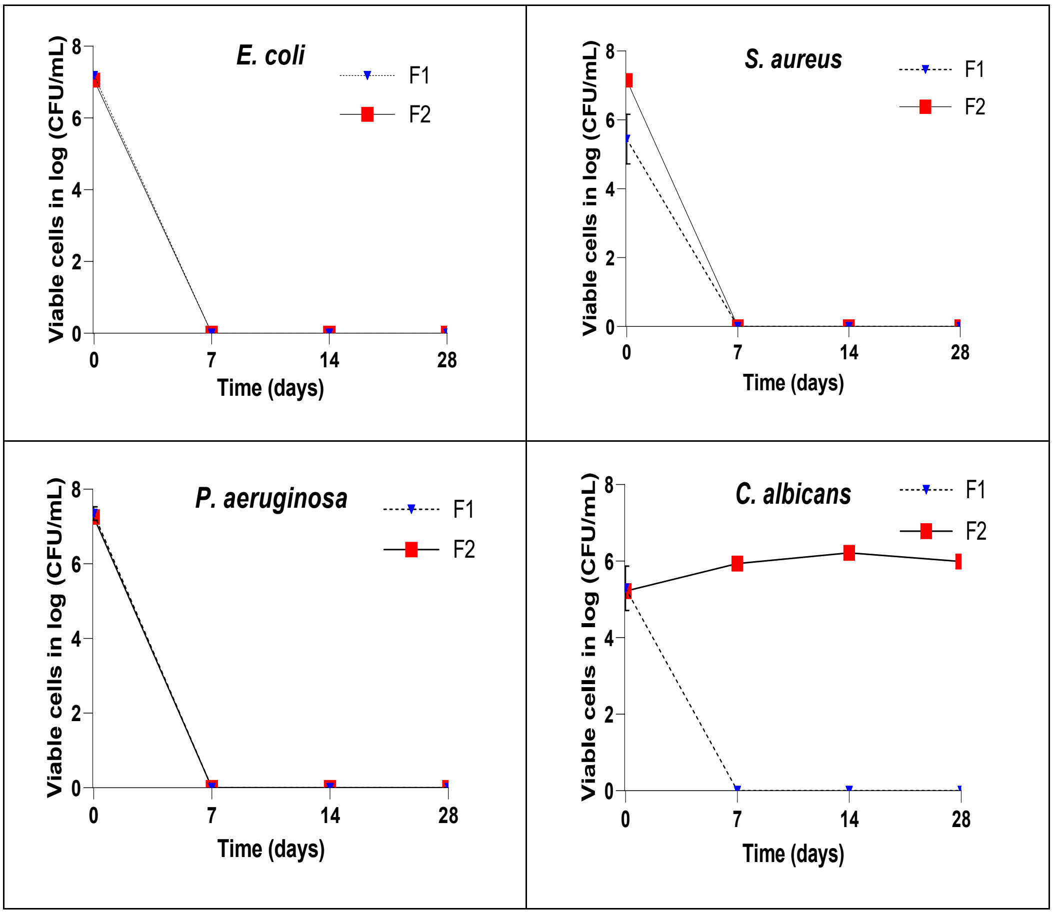

Figure 6. Growth inhibition of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 in cream formulations F1 and F2.

Table 2. The log reduction in the number of viable microorganisms

	F1			F2		
	7 d	14 d	28 d	7 d	14 d	28 d
Escherichia coli	>3	>3	>3	>3	>3	>3
Staphylococcus aureus	>3	>3	>3	>3	>3	>3
Pseudomonas aeruginosa	>3	>3	>3	>3	>3	>3
Candida albicans	>2	>2	>2	NR*	NR	NR

*Not reduction

Upon analysis of the cytotoxic activity results, it was observed that the samples exhibited increased cytotoxic effects in a concentration-dependent manner (Figure 7). The toxicity of the F1 formulation on healthy cells was found to be higher than that of the F2 formulation.

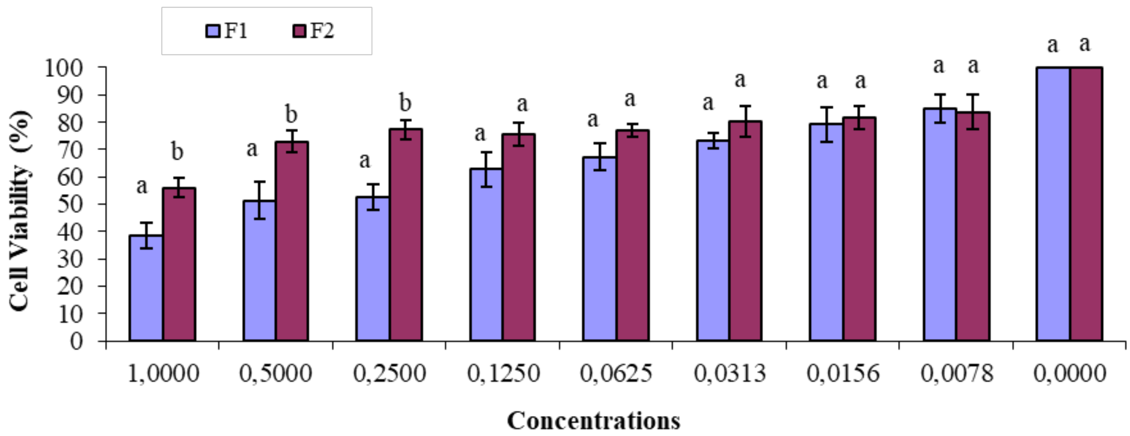

Figure 7. The cytotoxic activities of cream samples against L929 mouse fibroblast cell line

4. DISCUSSIONS

The current study aimed to investigate the antimicrobial effects of adding chitosan extracted from crayfish shells to the control group formulation. Within the scope of the study, control (F1) and treatment (F2) groups were compared. There are studies in the literature demonstrating the beneficial effects of cosmetic products containing chitosan on wound healing (Kim *et al.*, 2015; Ahmad *et al.*, 2018; Bektas *et al.*, 2020). Studies in which chitosan is added to cosmetic products in different forms such as nanoparticles are also available in the literature (Ta *et al.*, 2021; Mondéjar-López *et al.*, 2022a, b). In addition, there are also studies in which different plant extracts are added to the formulations to increase the antimicrobial effect of chitosan in the cream formulation (Wisuitiprot *et al.*, 2022; Parwati and Wikantyasning, 2023).
Pérez-Díaz *et al.* in 2016 showed that cell viability in human fibroblasts changed depending on the concentration in chitosan gel formulations to which AgNPs were added. These results support that chitosan gel formulations loaded with AgNPs show good biocompatibility. The current study supports the literature information that the chitosan-containing cream formulation has lower cytotoxicity on fibroblasts. However, different natural resources can also be utilized to improve the biocompatibility of these chitosan-containing cosmetic products (Azuma *et al.*, 2015; Movaffagh *et al.*, 2022; Bagheri *et al.*, 2022). Khattaket *et al.* (2022) compared the antimicrobial effect of the cream prepared by adding chitosan with the antimicrobial effect of the cream containing only bacitracin. Researchers found that the cream containing chitosan had similar antimicrobial effects on *E. coli*, *S. aureus*, *B. cereus* and *P. aeruginosa*. Chaiwong *et al.* (2022) evaluated the antimicrobial effect of Mangosteen extract deodorant cream to which they added carboxymethyl chitosan synthesized from high molecular weight natural chitosan.

5. CONCLUSIONS

In conclusion, the results of the study revealed that the tested cream had an antimicrobial effect on *S. aureus*, *E. coli* and *P. aeruginosa* bacteria. Chitosan-based creams have received considerable attention for their potential biomedical applications, such as wound healing and skincare. It is essential to understand the cytotoxicity of these creams to evaluate their safety and effectiveness. Further research is necessary to clarify the mechanisms underlying their cytotoxic effects and to optimize their formulations for improved biocompatibility. Although it was observed that the chitosan-based cream produced in this study had a good antimicrobial effect on the bacteria studied, it is suggested that some plant extracts can be used to increase the effect of this formulation on yeasts.

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AUTHORSHIP CONTRIBUTION STATEMENT

Yavuz MAZLUM: Conceptualization, Validation, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Software, Visualization, Supervision, Project administration. **Selin SAYIN:** Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Data Curation, Software, Visualization, Supervision, Project administration. **Betül AYDIN:** Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Software, Visualization, Supervision. **Mehmet NAZ:** Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Software, Visualization, Supervision. **Metin YAZICI:** Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Visualization, Supervision. **Şerife AKKÜÇÜK:** Methodology, Validation, Formal Analysis, Resources, Writing-Review and Editing, Data Curation, Visualization, Supervision, Project administration

CONFLICT OF INTERESTS

The author(s) declare that for this article they have no actual, potential or perceived conflict of interests.

ETHICS COMMITTEE PERMISSION

No ethics committee permissions is required for this study.

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Investigation of Microplastic Contamination in *Diadema Setosum* Obtained from a Fishing Barn

Balıkçı Barınağından Elde Edilen Diadema *Setosum*'da Mikroplastik Kontamisyonunun Araştırılması

Türk Denizcilik ve Deniz Bilimleri Dergisi

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ABSTRACT

This study is undertaken to evaluate microplastic contamination levels in *Diadema setosum specimens* obtained from a fishing barn. Microplastic (MP) pollution levels and their potential impacts on marine biota are still unknown compared to coastal and offshore environments. For this purpose, 19 individuals of *D. setosum* were collected and microplastic abundance in their gastrointestinal tract (GIT) and gonad were investigated. Mean microplastic abundance in GITs was 3.0 MPs±3.1 MPs per individual and 0.9±1.0 MPs per g wet weight. Mean microplastic abundance in the gonads was 0.3±0.6 MPs per individual and 0.08±0.2 MPs per g wet weight. Among all MPs, 45% of extracted MPs were fibers, followed by fragments (44%) and pellets (11%). Regarding size, the majority of the MPs extracted from GITs and all of the MPs extracted from gonads were small size MPs (less than 1 mm in size). FTIR analysis validated the plastic nature of suspected particles. Polyethylene (PE) (50%) and polypropylene (PP) (50%) were the most common type of polymers. These are the main polymers used in production of fishing nets; therefore, this result seems to validate the anthropogenic influence in the study area. This study contributes to the knowledge of the transfer of microplastics to the marine food web and highlights the need for protective measures.

Keywords: İskenderun Bay, Microplastics, Microplastic ingestion, Pollution, Türkiye

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ÖZET

Bu çalışma, bir balıkçı barınağından toplanan *Diadema setosum*'u örneklerindeki mikroplastik kontaminasyonunun varlığını araştırmak amacıyla yapılmıştır. Mikroplastik (MP) kirlilik seviyeleri ve bunların deniz biyotası üzerindeki potansiyel etkileri, kıyı ve açık deniz ortamlarıyla karşılaştırıldığında hala bilinmemektedir. Bu amaçla 19 *D. setosum* bireyi toplanmış ve gastrointestinal sistem (GİS) ve gonaddaki mikroplastik bolluğu araştırılmıştır. GİS'teki ortalama mikroplastik bolluğu kişi başına 3,0 MPs±3,1 MP ve ıslak ağırlığın gramı başına 0,9±1,0 MP olarak bulundu. Gonaddaki ortalama mikroplastik miktarı birey başına 0,3±0,6 MP ve yaş ağırlık başına 0,08±0,2 MP olarak bulundu. Tüm MP'ler arasında, ekstrakte edilen MP'lerin %45'inin fiber, %44'ünün fragment ve %11'inin pellet olduğu bulunmuştur. Boyutla ilgili olarak, GIT'ten çıkarılan MP'lerin çoğunluğu ve gonaddan çıkarılan MP'lerin tümü küçük boyutlu MP'lerdi (boyutu 1 mm'den küçük). FTIR analizi, şüpheli parçacıkların plastik yapısını doğruladı ve yaygın polimer türü olarak polietilen (PE) (%50) ve polipropilen (PP) (%50) bulundu. Bu polimerler balık ağlarının üretiminde kullanılan başlıca polimerlerdir, dolayısıyla bu sonuç, çalışma alanındaki antropojenik etkiyi doğrulamaktadır. Bu çalışma, mikroplastiklerin deniz ürünleri ağına aktarımı konusundaki bilgi birikimine katkıda bulunmakta ve koruyucu ölçümlerin gerekliliğini vurgulamaktadır.

Anahtar sözcükler: İskenderun Körfezi, Mikroplastik, Mikroplastik alımı, Kirlilik, Türkiye

1. INTRODUCTION

The plastic industry continues its significant growth due to the common usage of plastics in almost every area of our daily lives. Yet, low recycling rates and the absence of clear efficient management systems (Jambeck *et al*., 2015) cause the entrance of plastic waste into marine environments. Once these persistent polluters enter marine environments, they break down into smaller particles (Manzoor *et al*., 2022).

Microplastics are defined as plastic particles which are less than 5 mm in size. Even though their first appearance in the aquatic environment was back in the 1970s (Carpenter *et al*., 1972), their significant threat to marine biota was recently understood. Recent studies showed that microplastic pollution is a paramount concern even in the deepest part of the ocean (e.g., Mariana Trench (Peng *et al*., 2018).

The Mediterranean Sea is known as one of the most important hotspots of microplastic contamination since it has a semi-enclosed water circulation system with limited water flow (Everaert *et al*., 2020). The contamination level in the coastal regions is more alarming due to river discharges, anthropogenic activities such as industrial and domestic wastewater discharges, shipping, tourism, fishing industry, etc. (Suaria *et al*., 2016). Thus, marine organisms living in coastal regions are at more risk than those living in offshore waters (Compa *et al*., 2019; Lebreton and Andrady, 2019).

Sea urchins live in the rock and reef habitats of coastal environments (Nyawira and McClanahan , 2020). They are important benthic grazers and they have a crucial function in "material circulation and energy flow" in benthic environments (Dethiera *et al*., 2019; Feng *et al*., 2020). These species are commonly omnivorous grazers and detritus feeders, feeding on the algae or seagrass by scraping from hard substratum (Nyawira and McClanahan, 2020). Since seaweeds are potentially sink of microplastic particles (Gutow *et al*., 2016), these small home range species are more susceptible to microplastic contamination in their natural habitat (Murano *et a*l., 2020). Laboratory studies showed that microplastic ingestion causes oxidative stress (Richardson *et al*., 2021), decreases fertilization rate (Lyons *et al*., 2021), deforms morphology (Bertucci and Bellas, 2021), and impacts the immune system (Murano *et al*., 2023). Besides, microplastic particles act like a gate for toxic compounds (Rendell-Bhatti *et al*., 2021; Di Natale *et al*., 2022) and increase the toxicity risk of hazardous chemicals like heavy metals (Rial *et al*., 2023). It needs to be emphasized that these species are potential candidates for protection according to the

International Union for Conservation of Nature (IUCN) (Manzo and Schiavo, 2022). Therefore, microplastic contamination have become more alarming and threatening issue for sea urchins.

For more than three decades, sea urchins have been used as model organisms for ecotoxicological studies (Manzo and Schiavo, 2022). These species are used as an effective bioindicators for the monitoring of several pollutants including microplastics (Lawrence, 2001, 2013; Bayed *et a*l., 2005; Soualili *et al*., 2008; Hennicke *et al*., 2021; Huseini *et al*., 2021), since they have low mobility and good response to benthic biota (Huseini *et al*., 2021). Previous studies have demonstrated that microplastic concentration in the sea urchins' bodies is strongly correlated with the sediment (Hennicke *et al*., 2021; Huseini *et al*., 2021; Rahmawati *et al*., 2023), indicating that these organisms are more likely to reflect the pollution status of the surrounding environment into their body (Pinheiro *et al*., 2020).

In addition to their ecological role, sea urchins are used for human consumption due to their delicious gonad. Stefánsson *et al*. (2017) reported that 50000 tons of sea urchins were harvested and consumed in Japan, Chile, New Zealand, and the Philippines, globally. They also reported that their consumption become popular in European countries such as Italy, France, and Spain, and 3000-3500 tons of sea urchins are harvested from the Mediterranean Sea annually (Stefánsson *et al*., 2017).

Current literature mainly focuses on microplastic occurrence in vertible species i.e. fish. Yet, information related to microplastic occurrence in invertebrate species is scant (de Sa *et al*., 2018). Among invertebrate species, most of the studies have focused on mollusks especially the bivalvae class (Kögel *et al*., 2020), and the presence of MPs in other taxons seems to be ignored. On the other hand, information regarding microplastic presence in sea urchins is highly valuable considering their ecological role, market value, nutrition value, and endangered potential.

This study was conducted to examine the microplastic abundance in the gastrointestinal tract and gonad of *Diadema setosum* specimens collected from a fishing barn in İskenderun Bay. Sampling location has particular importance because microplastic pollution levels in fishing barns are particularly unknown compared to other coastal and offshore environments. Today, it is a well-known fact that the degradation of fishing nets/ropes in shallow marine environments like fishing barns causes the release of microplastics (Welden and Covie, 2017). Similarly, a recent study has demonstrated that a significant portion of microplastics found in the sediment of fishing barns originated from the abrasion of fishing gears (Xue *et al*., 2020). This study is the first report that evaluates the MPs presence in a sea urchin species from Türkiye.

2. MATERIALS AND METHODS

2.1 Study area and sampling

Morphologically undamaged 19 *D. setosum* specimens were collected from the İskenderun fisheries barn (36°35'34"N 36°10'33"E) in the Hatay province of Türkiye, northeastern Mediterranean Sea (Figure 1).

Field observation revealed that the study area is severely contaminated with plastics. For that reason, it is hypothesized that the study area is severely contaminated with microplastics; since, the fisheries industry is one of the major sources of microplastics (An *et al*., 2020; Xue *et al*., 2020).

Figure 1. The sampled area and samples of *D. setosum* specimens.

2.2 Sampling dissection and digestion

The methodology was adopted from Feng *et al*. (2020). Each specimen was weighed, thawed, and washed with distilled water to remove any sediment or outdoor contamination. The diameter length was measured by a vernier

caliper. Next, each specimen was dissected from the mouth of the sea urchin carefully to avoid harm to internal tissue. Then, the gastrointestinal tract and gonad of each individual were extracted, separately weighted, placed into glass beakers, and covered with aluminum foil immediately.
After the dissection process, 50 mL of H_2O_2 was added to the beakers. H_2O_2 was selected as a digestion agent due to environmental concerns. The beakers were placed on a hot plate and kept at 50 °C for 12 h until the solution was homogenized, and tissues were completely digested. Later, the remaining solution was filtered through 0.47 µm glass filters through a glass filter system at low vacuum. Then, filters were placed into sterile Petri dishes until microscopic examination.

2.3 Microscopic examination

Filters were examined for the existence of microplastic particles by Olympus SZX7 microscope. Plastic nature was pre-validated by the hot needle method (Hanke *et al*., 2013; De Witte *et al*., 2014). Identified particles were classified according to type (fiber, fragment), color (black, red, blue, white, transparent, green, brown, and yellow), and their size. Filters that have MPs larger than 1 mm were placed in glass Petri dishes and set aside for Fourier transform infrared (FTIR) analysis.

2.4 Polymer identification

Fourier transform infrared spectroscopy (FTIR) was employed to validate the plastic nature of identified MPs. At this stage, out of 62 MPs, 6 MPs suitable in size (>1 mm) were taken as subsamples and used in spectroscopic analyses. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with single reflection attenuated total reflectance (ATR) accessory. The spectrum range was arranged as 4000–400 cm-1 and a resolution was set to 4.0 cm-1 with 32 scans for each measurement. The polymer type was identified by comparing absorbance spectra to reference libraries of SHIMADZU library.

2.5 Quality assurance and control

Collected specimens were quickly placed into pre-washed (i.e. three times with prefiltered distilled water) glass jars to eliminate airborne contamination.
Extensive precautions were taken in the closed laboratories with restricted access. Windows and air conditioners were always kept closed and turned off to minimize the airflow (Bessa *et al*., 2019). Before each analysis, laboratory surfaces, dissection equipment, and glass beakers were cleaned three times with pre-filtered distilled water (Torre *et al*., 2016). Used chemicals and distilled water were filtered before use at all times. Only authorized personnel were allowed to enter the laboratories and they wore nitrile gloves and cotton aprons at all times. Filters were checked under the microscope for the presence of MPs before use. Three wet blank filters were placed in the laboratory during the dissection and microscopic examination steps. Only one fiber sample was detected in the blank samples which suggests that the results are scientifically acceptable. The dataset was corrected by extracting the contamination data.

2.6 Statistical analysis

The normality of the data was checked by Shapiro-Wilk test. Since normality was not validated, Spearman correlation analysis was used to evaluate the correlations between morphological parameters (test diameter, test height, total weight, weight of GIT and gonad) and MPs abundance in the GIT and gonad.
The Kurskal-Wallis test was employed to examine the variations in microplastic abundance between organs. Calculations were carried out using PAST program version 4.03 (Hammer *et al*., 2001).

3. RESULTS

In this study, 19 individuals of *D. setosum* were employed to investigate the impact of fishing barns on marine biota by examining the microplastic abundance in the gastrointestinal tract and gonad of sea urchin. Mean diameter, mean height and mean weight of sampled individuals were 118.3±12.2 mm, 88.2±4.4 mm,

and 50.3±19.1 g, respectively (Table A1).
Statistical analysis showed that there is no correlation between morphological parameters and microplastic abundance (i.e. MP amount in the GIT & total weight, MP amount in the gonad & total weight, MP amount in the GIT & test diameter, MP amount in the gonad & test diameter, MP amount in the GIT & test height, MP amount in the gonad & test height, MP amount in the GIT & wet weight of GIT, MP amount in the gonad and wet weight of gonad)(Table A9).
In total 62 MPs were extracted from the specimens. Among all, 57 of them were extracted from GIT and 5 of them were extracted from gonad. Mean microplastic abundance was estimated as 3.0 MPs±3.1 MPs per individual (ind) and 0.9±1.0 MPs per g wet weight in GIT. When it comes to gonads, mean microplastic abundance was found as 0.3±0.6 MPs per individual and 0.08±0.2 MPs per g wet weight. Among 19 samples, the GIT of 15 specimens and the gonad of 5 specimens were contaminated with MPs.
The results showed that both microplastic presence rate and the extracted microplastic amount are higher in GIT than in gonad tissue. Kruskal Wallis test confirmed a significant variance in sample medians of MPs extracted from GIT and gonad (H (chi^2):13,11; Hc (tie corrected):15,1; p:0.0001021)
Fiber-shaped MPs were found to be dominant in both organs. Among all MPs, 45% of extracted MPs were fiber and that were followed by fragments (44%) and pellets (11%). Extracted MPs were divided into 5 categories in terms of color black, blue, green, red color, and colorless (white and transparent MPs). Blue-colored MPs were dominant (Figure 2). Regarding size, the majority of the MPs extracted from GIT and all of the MPs extracted from gonad were small-size MPs (less than 1 mm in size) (Figure 2).

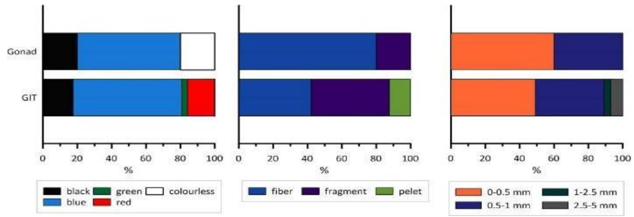

Figure 2. Characteristics of extracted microplastic particles from gastrointestinal tract (GIT) and gonad of *D. setosum*

Six of the MPs extracted from the GIT were validated by FTIR analysis. Analysis showed that all of the suspected particles were plastic in nature and polymer types were determined as 3 polyethylene MPs (50%) and 3 polypropylene MPs (50%) (Appendix Figure A1-A6).

4. DISCUSSIONS

İskenderun Bay is located inside one of the biggest plastic hotspots of the Mediterranean Sea (Papadimitriu and Allinson, 2022) and it is severely contaminated with microplastics (Gündoğdu and Çevik, 2017). Besides, contamination levels become more alarming in the coastal regions due to anthropogenic activities and intense fishing activities. Even though many studies investigate the microplastic pollution levels in the surface water and sediment of the northeastern Mediterranean Sea, there is no information regarding the microplastic pollution levels in fishing barns. Yet, fishing activities have particular importance in the formation and distribution of microplastics. In fact, they are defined as a major source of secondary microplastics (An *et al*., 2020; Xue *et al*., 2020). In this study, microplastic presence in the sea urchin *Diadema setosum* sampled from the fishing barn was investigated to highlight the danger potential of MPs pollution levels in the fishing barns on the marine biota. Besides, this study is the first report regarding microplastic accumulation in *D. setosum* from Türkiye.
In this study, among 19 examined specimens, 15 of them contained microplastic in their GIT (79%) and 5 of them contained MPs in their gonad (22%) (Table A1). The high frequency of occurrence in the GIT points out that these species are highly confronted with microplastics. High frequency of microplastic occurrence in

GIT found in this study is in line with previous reports (Table A2). Feng *et al*. (2020) investigated the microplastic presence (n=210) in the 4 different sea urchin species from the Yellow Sea and reported that 89.5% of examined specimens contained microplastic in their GIT. Other studies reported a 100% microplastic occurrence rate in the *Paracentrotus lividus* sampled from the Eastern Aegean Sea, Greece. (Hennicke *et al*., 2021), *D. setosum* sampled from Barranglompo Island, Indonesia (Savannah *et al*., 2021), *Tetrapygus niger* sampled from Peru (De-la-Torre *et al*., 2020).

Microplastic particles deposited on the sediment cover the top layer of sea gross or rocky bottom and they are eventually consumed by grazer organisms. There seems to be quite a difference in the average amount of microplastics extracted from GIT in the literature. The highest MPs abundance in the GIT of *D. setosum* was reported from Untung Jawa, Jakarta as 2175.55±584.26 MPs per ind (Huseini *et al*., 2021). Followed by a study conducted in Indonesia, which reported the mean MPs abundance as 23.70±2.99 MPs per ind (Sawalan *et al*., 2021). Results obtained in this study (3.0±3.1 MPs per ind) were significantly lower than previous reports which employed *D. setosum* as biomonitoring organisms; however, similar to those employed *P. lividus* (Raguso *et a*l., 2022; Murano *et al*., 2022), *Strongylocentrotus intermedius*, *Temnopleurus hardwickii*, *Temnopleurus reevesii*, *Hemicentrotus pulcherrimus* (Feng *et al*., 2020), *Diadema africanum* (Sevillano-González *et al*., 2022), *Tetrapygus niger* (De-la-Torre *et al*., 2020) from different parts of the ocean (Table A2). Previous reports showed that microplastic concentration in the sediment severely impacts the microplastic abundance in the GIT (Hennicke *et al*., 2021; Huseini *et al*., 2021; Rahmawati *et al*., 2023). Therefore, variations in the reported mean abundance seem to primarily result from microplastic concentration in the ambient environment rather than species type.

Only five specimens contained MPs in gonads with a mean value of 0.9±1.0 MPs per ind, which is coherent with the previous reports (Feng *et al*., 2020; Murano *et al*., 2020). Statistical analysis showed that MPs abundance in the organs was significantly different ($p<0.05$). Previous research speculated that MPs accumulated in the gonads were not transfer from the intestine (Leddy and Johnson, 2000; Feng *et al*., 2020). Gonads receive MPs found in the coelomic fluid while they fill the whole-body cavity which accumulates MPs originate from peristomal gill and tube feed (Leddy and Johnson, 2000; Feng *et al*., 2020). Our results support this idea because of their significantly smaller size and lower amount compared to GIT.

Ingested MPs size is restricted to the animal size (Jâms *et al*., 2020). Coherent to this, almost all ingested MPs extracted from the GIT were smaller than 1 mm. In addition, the size of MPs extracted from gonads were varied between 218 µm to 658 µm. Pyl *et al*. (2022) reported that MPs which are greater than 10 µm could not originate from intestinal wall of *P. lividus*. Therefore, extracted MPs could originate from ceramic fluid (Leddy and Johnson, 2000; Feng *et al*., 2020) or an alternative route that needs to be tested in the future.

Microplastic type (fiber, fragment or pellet) is an important parameter in determining exposure risk and bioavailability of MPs. Fiber-shaped MPs were considered to be the most harmful type for marine invertebrates (Wright *et al*., 2013). Fiber microplastics are commonly attributed to the fragmentation of fishing nets (Koongolla *et al*., 2020) and it is the most commonly ingested type of MPs in the marine biota of the Mediterranean Sea (Koraltan *et al*., 2022; Kılıç and Yücel, 2022; Yücel and Kılıç, 2023a, b). Similarly, both this study and previous studies conducted in sea urchins reported the dominance of fiber microplastic (Hennicke *et al*., 2021; Sawalman *et al*., 2021; Sevillano-González *et al*., 2022; De-la-Torre *et al*., 2020; Raguso *et al*., 2022; Murano *et al*., 2022; Huseini *et al*., 2021; Feng *et al*., 2020). Therefore, sea urchins seem to have a higher ingestion preference for fiber microplastics compared to other types. Apart from this, sea urchins generate secondary microplastics while grazing on plastic surfaces (Porter *et al*., 2019). So, it is possible that urchins fed on newly generated fiber or fragment-type microplastics while grazing on the contaminated seaweed of fishing nets on the sea bottom. This condition is significantly important for fishing barns. Because, in a high microplastic-containing region like fishing barns, they accelerate the biodegradation of macroplastics (Boudouresque and Verlaque, 2007; De-la-Torre *et al*., 2020). While rasping on the surface of the plastic materials by calcionic teeth, they leave scratchers and generate secondary microplastics (Boudouresque and Verlaque, 2007; De-la-Torre *et al*., 2020). That in turn increases the ecotoxicological risk of microplastics for themselves and other marine animals.

Blue was the most dominant color in the extracted MPs, which overlaps with the previous reports in sea urchins (Hennicke *et al*., 2021; Sawalman *et al*., 2021; Sevillano-González *et al*., 2022; De-la-Torre *et al*., 2020; Murano *et al*., 2022; Huseini *et al*., 2021; Feng *et al*., 2020), fish (Güven *et al*., 2017; Giani *et al*., 2019). Previous reports demonstrate that *P. lividus* might select blue-colored particles due to their similarity to the main food source. Besides, the study area is filled with the remaining blue-colored fishing nets, which seems to increase the ingestion rate of MPs.

Microplastic abundance in the fishing area is linked to the fishing effort (Wright *et al*., 2021), and higher MPs abundance is observed in the regions where fisheries activities are dominant (Xue *et al*., 2017). Today, the majority of fishing gears are produced from synthetic polymers such as polyethylene and polypropylene (Nelms *et al*., 2021). In this study, half of the examined MPs were PE, and the remaining were PP. Since the polymer is an important factor that is used to link between contamination source and ecotoxicity of animals, the results presumably imply that the majority of the extracted MPs were derive from fishing activities.

It is a well-known fact that microplastics entering the food chain might cause adverse effects on the upper trophic levels including humans (Hennicke *et al*., 2021). Since sea urchins are a traditional food source for highly populated countries such as Japan, China, and Mediterranean European countries (Stefánsson *et al*., 2017). Therefore, microplastics found in sea urchins might create a pathway for microplastics to the human body. Species that are eaten with gastrointestinal tracts and gills such as small-size fish and bivalves may pose a greater risk to human health (Smith *et al*., 2018; Baechle *et al*., 2020; Hennicke *et al*., 2021). Previous studies in the İskenderun Bay reported 2.9±2.7 MPs per ind in fish *Mullus barbatus*, 3.4±2.7 MPs per ind in fish *Saurida undosquamis* (Kılıç and Yücel, 2022), 0.26±0.5 MPs per ind in patella *Patella caerulea* (Yücel and Kılıç, 2023a), 0.2±0.5 MPs/ind in mussel *Brachidontes pharaonis* (Yücel and Kılıç, 2023b). Compared to other species like fish, the edible parts of this species contained fewer MPs. Thus, it is reasonable to assume that consumption of the gonads of *D. setosum* is safe in terms of microplastic accumulation. In other words, this species poses a considerably low to moderate threat to human health. More detailed studies are required to evaluate the potential hazards of consumption of microplastic-containing aquatic products.

5. CONCLUSIONS

This study is conducted to investigate the microplastic contamination levels in sea urchin *D. setosum* since sea urchin species are a good reflector of microplastic pollution in the ambient environment. Results validate the omnipresence of microplastics since the majority of the specimens contained microplastics in the bodies. Polypropylene and polyethylene were the main types of polymers extracted from the GIT which might reveal the impact of the fishing industry since these are the main types of polymers used in fishing nets. As far as we know, this is the first focusing on the marine animals habituated in a fishing barn in Turkiye; therefore, the results demonstrate the importance of the fisheries industry in the formation and distribution of microplastics. There is still little known in the transfer of microplastics throughout food web, yet studies like this will provide insight to the microplastic contamination in different trophic levels and different environments.

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Ece KILIÇ: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft.
Erkan UĞURLU: Validation, Formal Analysis, Resources, Writing-Review and Editing, Data Curation, Software, Visualization, Writing-Review and Editing.

CONFLICT OF INTERESTS

The author(s) declare that for this article they have no actual, potential or perceived conflict of interests.

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Appendix I

Table A1. Morphological statistics i.e. test diameter, test height, GIT weight, gonad weight (mean ± standard deviation) and microplastic abundance and frequency of occurrence (%) in the examined organs

Test diameter (mm)	Test height (mm)	GIT weight (g ww)	Gonad weight (g ww)	# of MPs	# of fiber MPs	# of fragment MPs	# of pelet MPs	MPs per ind	MPs per g ww	FO (%)
118.3±12.2	88.2±4.4	0.37+0.16	5.25+3.5	GIT						
				57	24	26	7	3±3.1	0.8±1.0	79
				Gonad						
				5	4	1	0	0.3±0.6	0.08±0.2	22

Appendix II

Table A2. Recent literature indicating mean microplastic abundance in organs as well as predominant type of microplastic in similar species

Species	Location	GIT MPs per ind	GIT MPs per g ww	Gonad MPs per ind	Gonad MPs per g ww	Common type	Common color	References
Diadema setosum	İskenderun Bay, Türkiye	3.0 MPs± 3.1	0.9±1.0	0.3±0.6	0.08±0.2	Fiber	Blue	This study
Paracentrotus lividus	Greece	26	1.9			Fiber	Blue	Hennicke et al., 2021
Diadema setosum	Barranglompo Island, Indonosia	23.70±2.99	-			Fiber	Blue	Sawalman et al., 2021
Diadema africanum	Canary Island, Spain	9.2 ± 3.0				Fiber	Blue	Sevillano-González et al., 2022
Diadema africanum	El Porís	10.0 ± 4.5				Fiber	Blue	Sevillano-González et al., 2022
Tetrapygus niger	Peru	3.22 ± 0.49				Fiber	Blue	De-la-Torre et al., 2020
Paracentrotus lividus	Sardinia, Italy	1.0 ± 0.3				Fiber	Grey	Raguso et al., 2022
Diadema setosum	Untung Jawa, Jakarta	2,175.55 ± 584.26				Fiber	-	Huseini et al., 2021
	Tidung, Jakarta	1,786.66 ± 451.17				Fiber	-	
Paracentrotus lividus	Southern Italy,	1.2		0.83		Fiber	Black and blue	Murano et al., 2022
4 different species	Yellow Sea, China	3.04		0.82		Fiber	Blue, gray	Feng et al., 2020

Appendix III

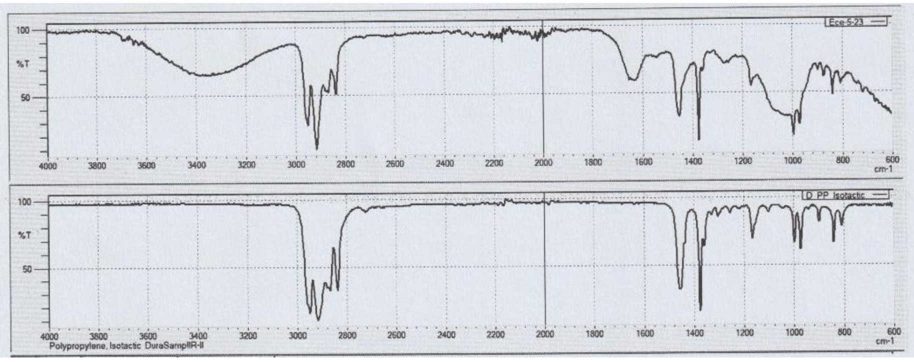

Figure A1. FTIR spectra of microplastic particle 1 (*Polypropylene)*

Appendix IV

Microplastic Particle 2:

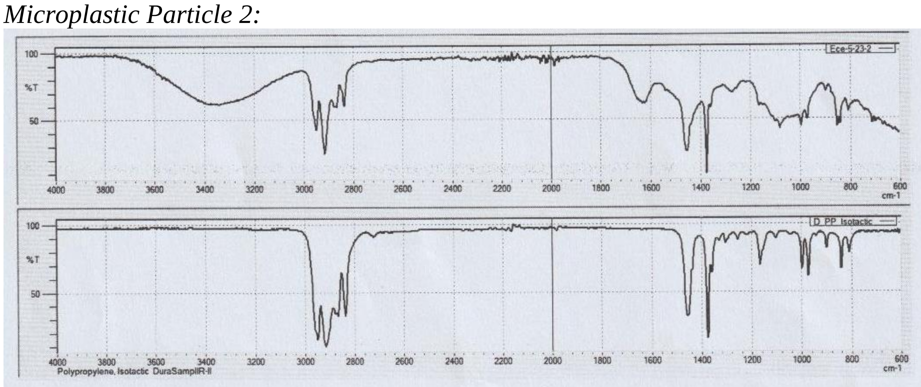

Figure A2. FTIR spectra of microplastic particle 2 (*Polypropylene)*

Appendix V

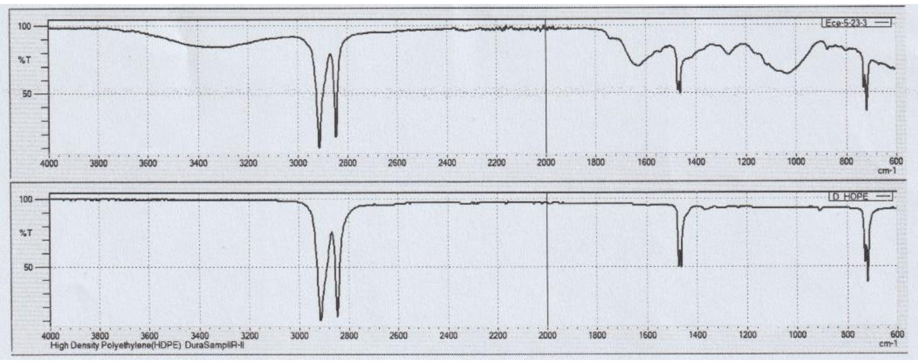

Figure A3. FTIR spectra of microplastic particle 3 (*Polyethylene)*

Appendix VI

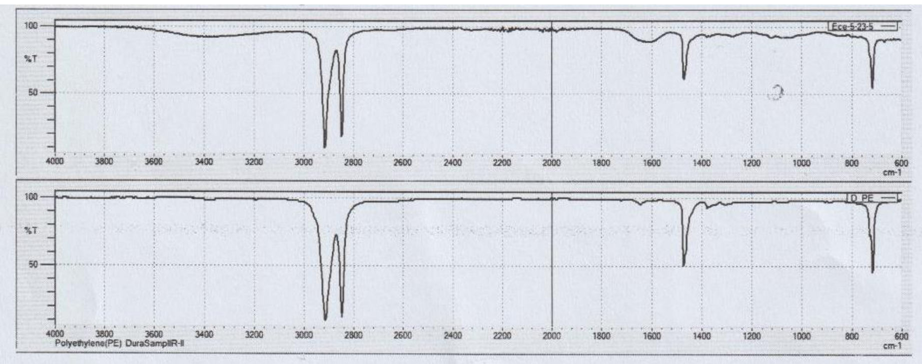

Figure A4. FTIR spectra of microplastic particle 4 (*Polyethylene)*

Appendix VII

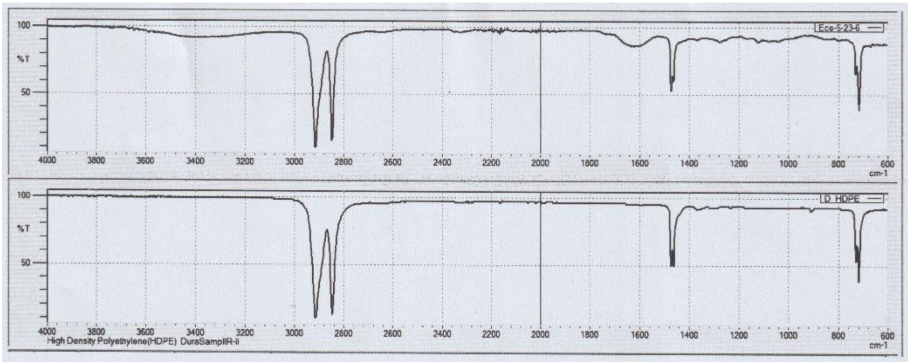

Figure A5. FTIR spectra of microplastic particle 5 (*Polyethylene)*

Appendix VIII

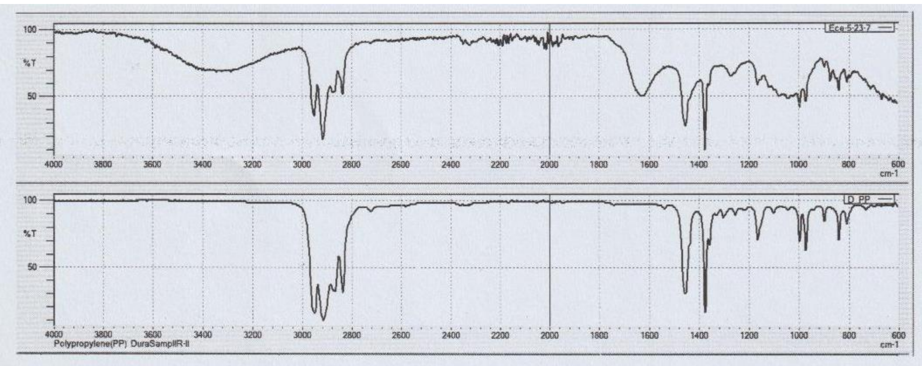

Figure A6. FTIR spectra of microplastic particle 6 (*Polypropylene)*

Appendix IX

Table A9. Results of correlation analysis (Below column shows statistical value, while upper column show p value)

	Test diameter	Test height	Weight	GIT weight	Gonad weight	MP in GIT	MP in Gonad
Test diameter		1,30E-07	6,63E-05	0,054845	0,00018	0,14943	0,9042
Test height	0,90215		0,000104	0,13054	0,000317	0,61684	0,66162
weight	0,78596	0,77315		0,1659	0,000744	0,1793	0,75556
GIT weight	0,44728	0,35956	0,33128		0,097013	0,57951	0,36812
Gonad weight	0,75614	0,73717	0,70526	0,39192		0,15055	0,96001
MP in GIT	-0,34388	-0,12268	-0,32166	-0,13574	-0,34299		0,72934
MP in Gonad	-0,02962	0,10741	0,076512	-0,21881	0,012341	-0,085	

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Codend Selectivity of Demersal Trawl with Square Mesh Panel (SMP) for Blunt-snouted mullet (*Mullus ponticus* Essipov, 1927) in the Southern Black Sea Shores of Türkiye

Türkiye'nin Güney Karadeniz Kıyılarında Barbunya (*Mullus ponticus* Essipov, 1927) Balığı için Kare Gözlü Panel Kullanılan Dip Trolünün Torba Seçiciliği

Türk Denizcilik ve Deniz Bilimleri Dergisi

Cilt: 10 Sayı: 4 (2024) 232-244

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ABSTRACT

The study was conducted on the Southern Black Sea shores of the Mediterranean Basin and selectivity parameters of the trawl codend (36 mm, 40 mm diamond and 40 mm square mesh panel), were estimated in blunt-snouted mullet (*Mullus ponticus*, Essipov, 1927) fishing. End of the 12 valid hauls, selectivity parameters for 36 mm diamond (D), 40 mm diamond (D) and 40 mm **S**quare **M**esh **P**anel (SMP) were estimated selection length of 50 % (L50) 11.56 cm, 12.53 cm and 13.56 cm; selection range (SR) 4.88 cm, 5.02 cm and 3.91; selection factor (SF) 0.29, 0.31 and 0.34 respectively. 50 % selectivity length of 36 mm codend was smaller than the legal **M**inimum **L**anding **S**ize (MLS=13 cm), 40 mm codend was more nearly MLS but it's not enough and 40 mm Square Mesh Panel (SMP) on the codend has to use in the bottom trawl fisheries. Catch of small size (juvenile) blunt-snouted mullet will decrease by square mesh panel method must consider in the demersal trawl fishery of Black Sea.

Keywords: Trawl fishery, Selectivity, Square mesh panel, Blunt-snouted mullet, Black Sea

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ÖZET

Akdeniz Havzası'nın Güney Karadeniz kıyılarında yürütülen çalışmada, Karadeniz barbunya balığı (*Mullus ponticus,* Essipov, 1927) avcılığında kullanılan dip trol ağı torbasının (36 mm, 40 mm baklava gözlü ve 40 mm kare gözlü panel) seçicilik parametreleri tahmin edilmiştir. Araştırma süresince 12 geçerli ağ çekimi sonunda, 36 mm baklava gözlü (D), 40 mm baklava gözlü (D) ve 40 mm kare gözlü ağ panel (SMP) için seçicilik parametreleri, sırasıyla %50 seçicilik boyu (L_{50}) 11,56 cm, 12,53 cm ve 13,56 cm olarak tahmin edilmiştir. Seçicilik aralığı (SR) 4,88 cm, 5,02 cm ve 3,91; seçicilik faktörü (SF) sırasıyla 0,29, 0,31 ve 0,34 olarak hesaplanmıştır. 36 mm' lik torbanın %50 seçicilik boyunun, balığın yasal **M**inimum **A**vlama **B**oyu (MAB) olan 13 cm daha küçük olduğu belirlenmiştir. 40 mm' lik torbanın MAB' na daha yakın olduğu ancak yeterli olmadığı ve Karadeniz dip trolü balıkçılığında torba üzerinde 40 mm' lik kare gözlü ağ panel kullanılmasının MAB açısından gerekli olduğu saptanmıştır. Dip trol torbasında kare gözlü ağ panel kullanılması ile küçük boydaki (genç) barbunya balığının avının azalacağı dikkate alınmalıdır.

Anahtar sözcükler: Trol balıkçılığı, Seçicilik, Kare gözlü ağ panel, Barbunya, Karadeniz

1. INTRODUCTION

Today, one of the important problems facing the global fishing sector is overfishing and overexploitation of aquatic fish stocks. Especially with industrial fishing (trawls and purse seine), a lot of fish are caught in the oceans and seas, and a significant part of this catch consists of juvenile fish that cannot reproduce at least once. For this reason, selectivity in fishing gear emerges as an important parameter of fisheries with an ecosystem approach.

Ecosystem approach fisheries management has been accepted over the years in order to protect biodiversity in aquatic ecosystems. and ensure sustainable fisheries (Mytilineou *et al*., 2022). The worldwide has been adopted the Ecosystem Approach to Fisheries (EAF) and more specifically in Europe within the Common Fisheries Policy and has been included in fisheries policy and management (FAO, 2003; COM, 2008).

A specific regulation for the management of Mediterranean fisheries was approved by the European Union, emphasizing the importance of the Mediterranean basin for the Common Fishery Policy (CFP). In this regulation, a set of measures were adopted to improve the sustainable exploitation of Mediterranean stocks. In particular, minimum conservation reference sizes were defined a minimum legal mesh size in the trawl net cod-end of 40 mm square mesh or 50 mm diamond mesh in 2006 (Vitale *et al*., 2018; Bonanomi *et al*., 2020). In particular, increasing the mesh size was not sufficient to increase the selectivity of towing nets (Madsen and Holst, 2002; Kaykaç, 2007; Özbilgin *et al*., 2012; Çiçek, 2015; Özvarol and Bolat, 2017). The interest has improved recently in the potential of other modifications to the design of fishing gear to progress selectivity for escape of more small size fishes from the net (Tokaç, 2010; Özbilgin, *et al*., 2011; Özdemir *et al*., 2012; Özdemir, 2014; Eryaşar and Özbilgin, 2015; Demirci *et al*., 2017).

Square-Mesh Panels (SMPs) are among the simplest technological measures that can be applied to bottom trawls when codend size selection alone does not prevent retention of undersized individuals. SMPs are used in many different fisheries over the world and are now mandatory in several EU fisheries (Suuronen and Sardà, 2007; Bonanomi *et al*., 2020).

Demersal trawl fisheries in Türkiye seas is carried out in grounds having multispecies fishery (Özbilgin, 2005; Erdem *et al*., 2007; Kaykaç, 2018; Ceylan *et al*., 2014; Öğreden and Yağlıoğlu, 2017). Red mullet (*Mullus barbatus*) is one of the most abundant ground fish in Black Sea (Yıldız and Karakulak, 2016; Ceylan and Şahin, 2019; Yılmaz *et al*., 2019; Özdemir *et al*., 2021; Samsun, 2022) and small sizes of this fish can be reflected, frequently discarded species in trawl catch composition. Because of its cheap market price and legal regulation just big size fish are landed and a large ratio of the yield is discarded into the sea in the operations.

The minimum landing size (MLS) for red mullet (*Mullus barbatus*, L. 1758) is 13 cm (total length) according to Turkish Fisheries Regulations (BSGM, 2020). However, fishermen said to species is striped red mullet (*Mullus surmuletus* L., 1758) entered in trawl nets of the Black Sea and MLS for this species is 11 cm (total length). Besides, red mullet species in the Black Sea was *Mullus barbatus ponticus*, Essipov 1927 notified by most scientists (Hureau, 1986; Samsun, 1990; Genç, 2000; Keskin and Can, 2009; Vasilijeva, 2012; Erdem, 2018; Özdemir *et al*., 2021; Erdoğan-Sağlam, 2023) but the name of this species was updated as *Mullus ponticus* (Froese and Pauly, 2024). Minimum landing size (MLS) for fishes of Mullidae family is not defined in the Turkish Fisheries Regulations (TFR). There is confusion for members of mullidae family in the Black Sea.

Nowadays, low selectivity in the codend of towing nets has been an essential matter in large and small scale fisheries of Turkish as in the all seas and oceans of the world. Turkish fisheries regulations allow trawlers to use polyethylene (PE) codend the legal mesh size is 40 mm diamond in the coasts of the Black Sea (BSGM, 2020). Furthermore, it is also known that some trawl fishermen unlawfully use small mesh size codend more than 40 mm codends, as well as increase catch amount.

This study investigates the cod-end selectivity of a diamond mesh (36 mm and 40 mm) and with square mesh window panel (40 mm) for blunt-snouted mullet caught with demersal trawl on the shores of the southern Black Sea.

2. MATERIALS AND METHODS

The sea experiments were conducted on board commercial fishing boats western shores of Sinop in the Black Sea (Figure 1), between October and March months in 2006-2007 fishing season.

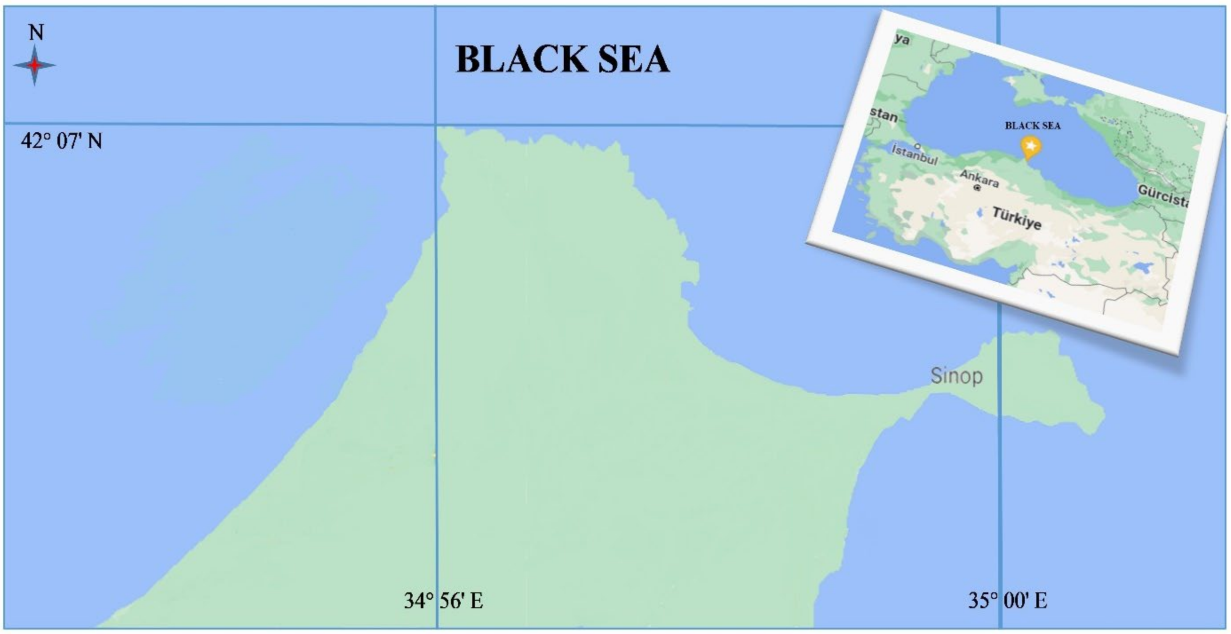

Figure 1. Map of study area

36 mm (D), 40 mm (D and SMP) were tested on the codend of the trawl net and a total of 12 valid hauls were achieved. The depth of the fishery field varied between 60 and 120 meters. The towing speed of trawl net ranged between 2.5-3 miles/hour and towing time was 1.5 hour in the whole trials.

The traditional, 800 meshes around the mouth, commercially used demersal trawl (Figure 2). Sea experiments were carried out to examine the impact of 36 mm diamond (36 D), 40 mm diamond (40 D) and 40 mm square mesh panel (40 SMP) size PE netting diamond mesh codend. A commercially used trawl codend that was 300 meshes around the circumference and approximately 7.20 m in stretched length was constructed. The small mesh covered codend was used in the sea trials (Pope *et al*., 1975; Stewart and Robertson, 1985; Sparre and Venema, 1998). The process of measuring selectivity trawl codend was based on the encircled covered codend practice (Wileman *et al*., 1996).

The covered codend technique is the most commonly used for estimating the codend selectivity of towed fishing gears such as demersal trawl, midwater trawl, beam trawl and dredge. The positive aspect of the practice is that the codend selectivity can be estimated directly because the fish escaping will be captured in the cover codend (Madsen and Holt, 2002).

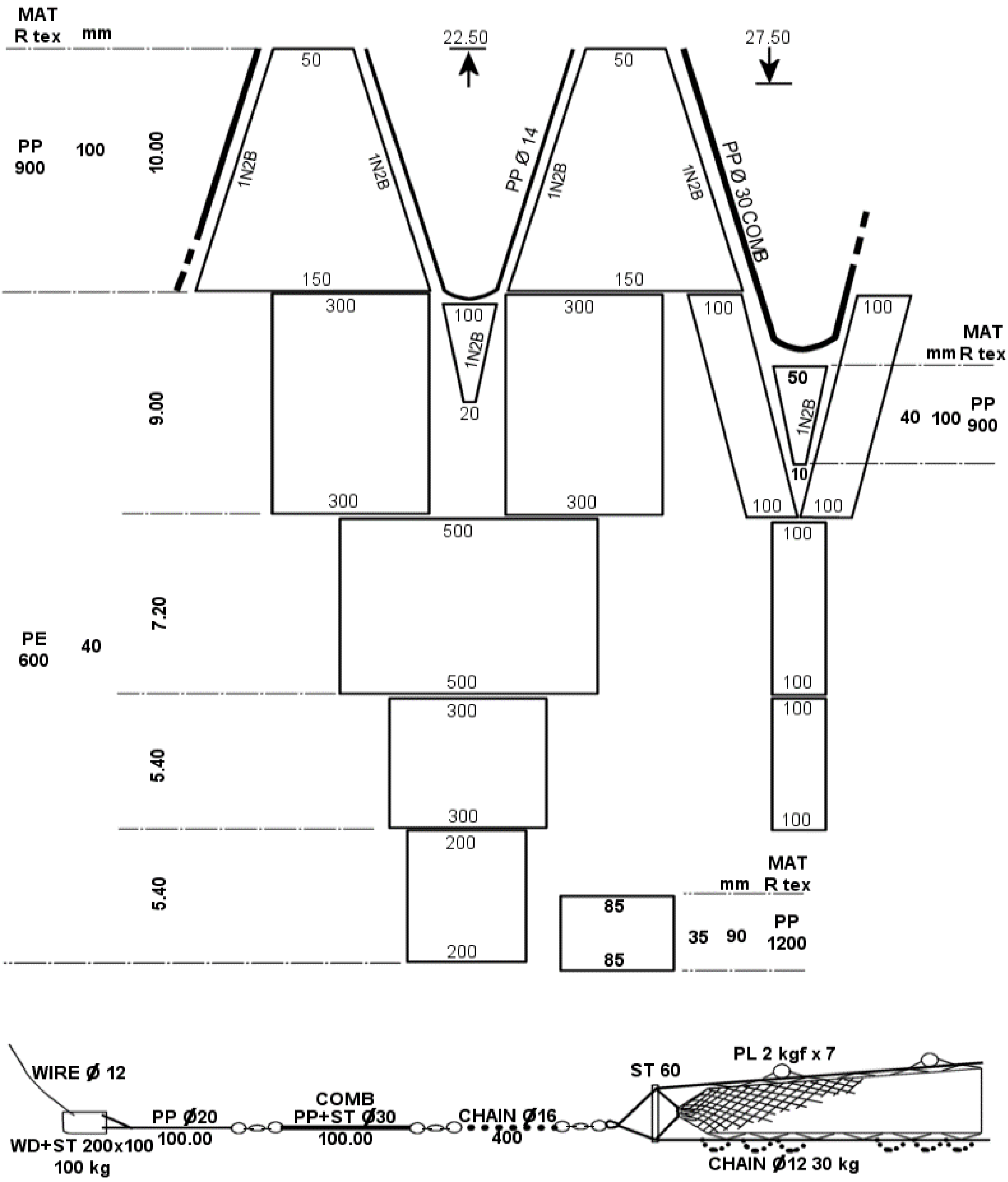

Figure 2. Technical plan of commercial demersal trawl used in the Black Sea coasts

The cover was 10 m in length and was made of multifilament PA (polyamide) diamond mesh netting of 20 mm mesh size. Two circles in 1.5 m radius assisted it (Figure 3). After each haul, the catches in codend and cover were sorted separately according to red mullet. Then total lengths of red mullet were measured to 1 mm.

Selectivity parameters were estimated for pooled data utilizing of an MS-Excel program file (Tokai, 1997), which is run by the 'SOLVER' tool. Data was analyzed by using logistic equation with the maximum likelihood method (Wileman *et al*., 1996).

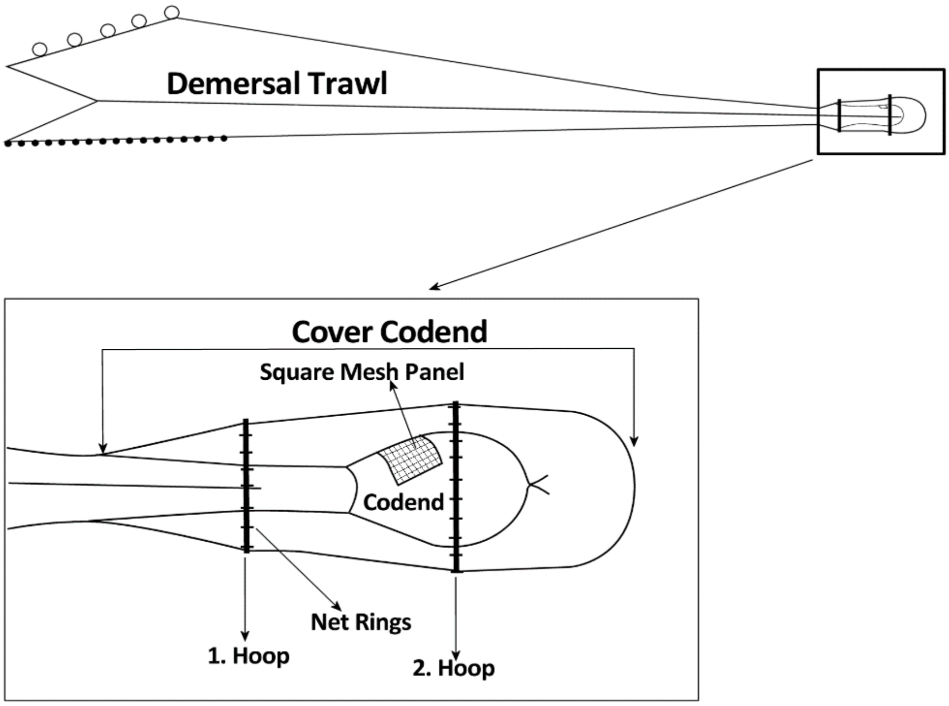

Figure 3. Trawl codend with the inserted covered hoops cover

The selectivity of codends was determined from the relationship between the probability ***p*** of a fish entering the codend and fish length ***l***. This relationship is described by the logistic function (Fryer, 1991):

The selectivity of codends was determined from the relationship between the probability ***p*** of a fish entering the codend and fish length ***l***. This relationship is described by the logistic function (Fryer, 1991):

$$p(l) = \frac{\exp(v_1 + v_2 l)}{1 + (\exp(v_1 + v_2 l))} \quad (1)$$

Where the parameters; v_1 and v_2 are the intercept and slope of the linear logistic function.

$$Ln\left(\frac{p}{1-p}\right) = v_1 + v_2 l \quad (2)$$

As a result, the values of L_{50}, L_{25}, L_{75}, Selection range (SR), Selection factor (SF) can be estimated from the expressions

$$L_{50} = \frac{-v_1}{v_2} \quad (3)$$

$$L_{25} = \frac{(-\text{Ln}\,(3) - v_1)}{v_2} \quad (4)$$

$$L_{75} = \frac{(\text{Ln}\,(3) - v_1)}{v_2} \quad (5)$$

$$SR = L_{75} - L_{25} \quad (6)$$

$$SF=\frac{L_{50}}{(\text{mesh size})} \qquad (7)$$

3. RESULTS

A total catch of 1.176 tons was captured in the cover and codend during 1080 minutes of trawl operation in 12 accepted hauls. In total, 402.074 kg, 466.754 kg and 307.172 kg red mullet was captured in the 36 mm (D), 40 mm (D) and 40 mm (SMP) with codend, respectively. A high percentage (39.69 %) of the fishes were caught in the 40 mm (D) codend. These percentages were 34.19 % and 26.12 in the 36 mm (D) and 40 mm (SMP) with codend, respectively.

A total numbers 1974 red mullet were caught 904 in 36 mm (D) codend and 1070 in the cover. 50% selectivity sizes (L50), selectivity range (SR), selectivity factor (SF) and regression parameters for pooled data of 36 mm (D) codend were shown in Table 1.

Table 1. Selectivity parameters for 36 mm (D) codend

v_1	-5.2086
v_2	0.4504
Standard error of v_1	0.240
Standard error of v_2	0.002
L_{25}	9.12
L_{50}	11.56
L_{75}	14.00
Selection range	4.88
Selection factor	0.32
Codend	904
Cover	1070

Figure 4 indicates the data points and the logistic selectivity curves get from pooled data for red mullet in 36 mm (D) codend. The figure also shows normalized size frequency distributions, calculated as percentage of fish in each size class, for 36 mm (D) codend, at 36 mm (D) codend and cover codend totals of 904 and 1070 red mullet were captured, respectively. Total length ranged from 6.3 to 19.5 cm with the codend, the large plurality between 9.5 and 14.5 cm. Total length ranged from 4.9 cm to 14.4 cm in the cover codend. Size frequency distributions at 36 mm showed great tops of 11.5 cm in the codend and 9.5 cm in the cover codend that captured the codend and got away.

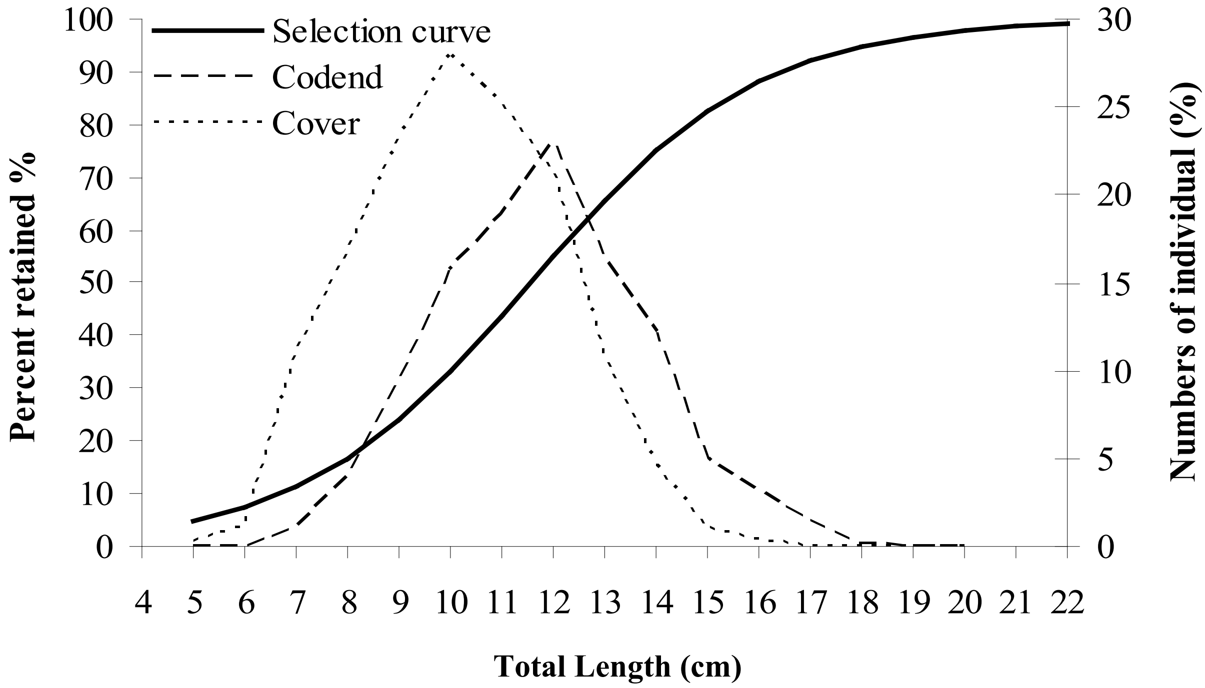

Figure 4. Selection curve for 36 mm mesh size (D) and size frequency distributions of red mullet

A total of numbers 2291 red mullet caught were 996 in 40 mm (D) codend and 1295 in the cover. 50% selectivity sizes (L50), selectivity range (SR), selectivity factor (SF) and regression parameters for pooled data of 40 mm codend were shown in Table 2.

Table 2. Selectivity parameters for 40 mm (D) codend

v_1	-8.2413
v_2	0.6834
Standard error of v_1	0.120
Standard error of v_2	0.008
L_{25}	10.01
L_{50}	12.06
L_{75}	15.04
Selection range	5.03
Selection factor	0.30
Codend	996
Cover	1295

Figure 5 also shows the data points and the logistic selectivity curves get from pooled data for red mullet in 40 mm (D) codend. The figure also shows normalized size frequency distributions, calculated as ratio of fish in each size class, for 40 mm (D) codend. At 40 mm (D) codend and cover codend totals of 996 and 1295 red mullet were captured, respectively. Total length ranged from 6.9 to 18.1 cm with the codend, the large plurality between 10.5 and 16 cm. Total length ranged from 4.9 cm to 16 cm in the cover codend. Size frequency distributions at 40 mm (D) indicated great tops of 12.5 cm in the codend and 11 cm in the cover codend.

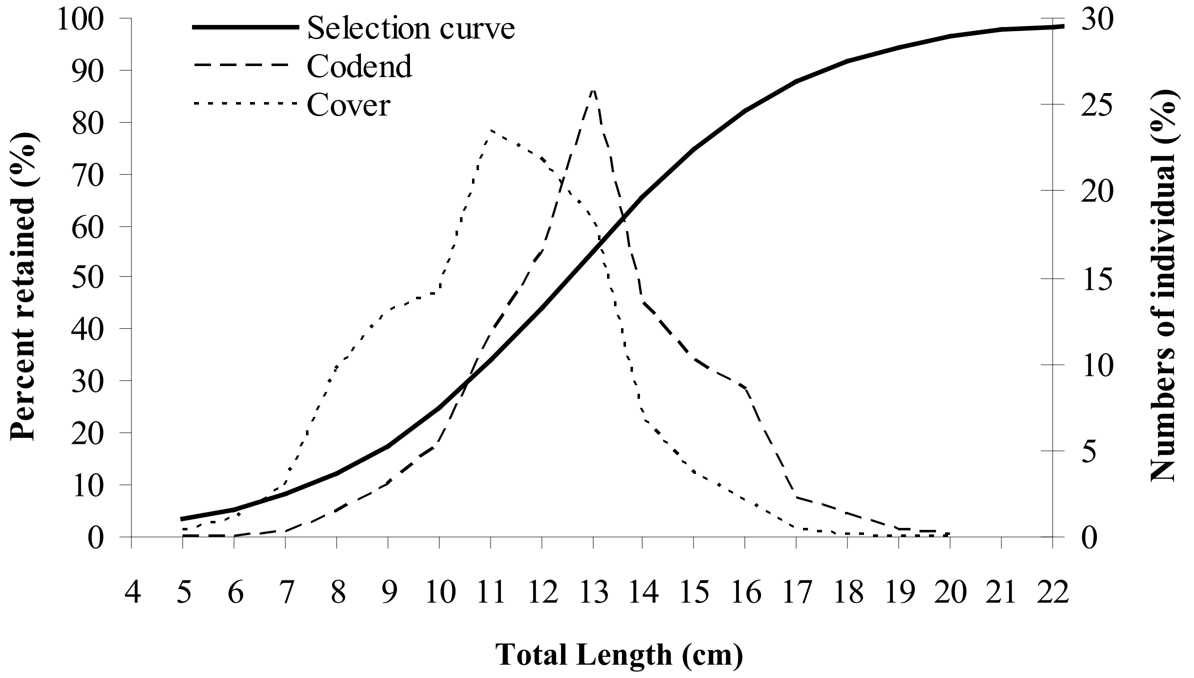

Figure 5. Selection curve for 40 mm mesh size (D) and size frequency distributions of red mullet that captured the codend and got away.

A total of numbers 1508 of red mullet caught were 652 in 40 mm (SMP) with codend and 856 in the cover. Table 3 shows 50% selectivity sizes (L50), selectivity range (SR), selectivity factor (SF) and regression parameters for pooled data of 40 mm square mesh panel with codend.

Table 3. Selectivity parameters for 40 mm (SMP) codend

v_1	-7.6325
v_2	0.5627
Standard error of v_1	0.140
Standard error of v_2	0.005
L_{25}	11.61
L_{50}	13.56
L_{75}	15.52
Selection range	3.91
Selection factor	0.34
Codend	652
Cover	856

Figure 6 also shows the data points and the logistic selectivity curves get from pooled data for red mullet in the 40 mm (SMP) codend. The figures also show normalized size frequency distributions, calculated as percentage of fish in each size class, for 40 mm (SMP) codend. At 40 mm (D) codend and cover codend totals of 996 and 1295 red mullet were caught, respectively. Total length ranged from 6.9 to 18.1 cm with the codend, the large plurality between 10.5 and 16 cm. Total length ranged from 4.9 cm to 16 cm in the cover codend. Size-frequency distributions at 40 mm (D) demonstrated major peaks of 12.5 cm in the codend and 11 cm in the cover codend.

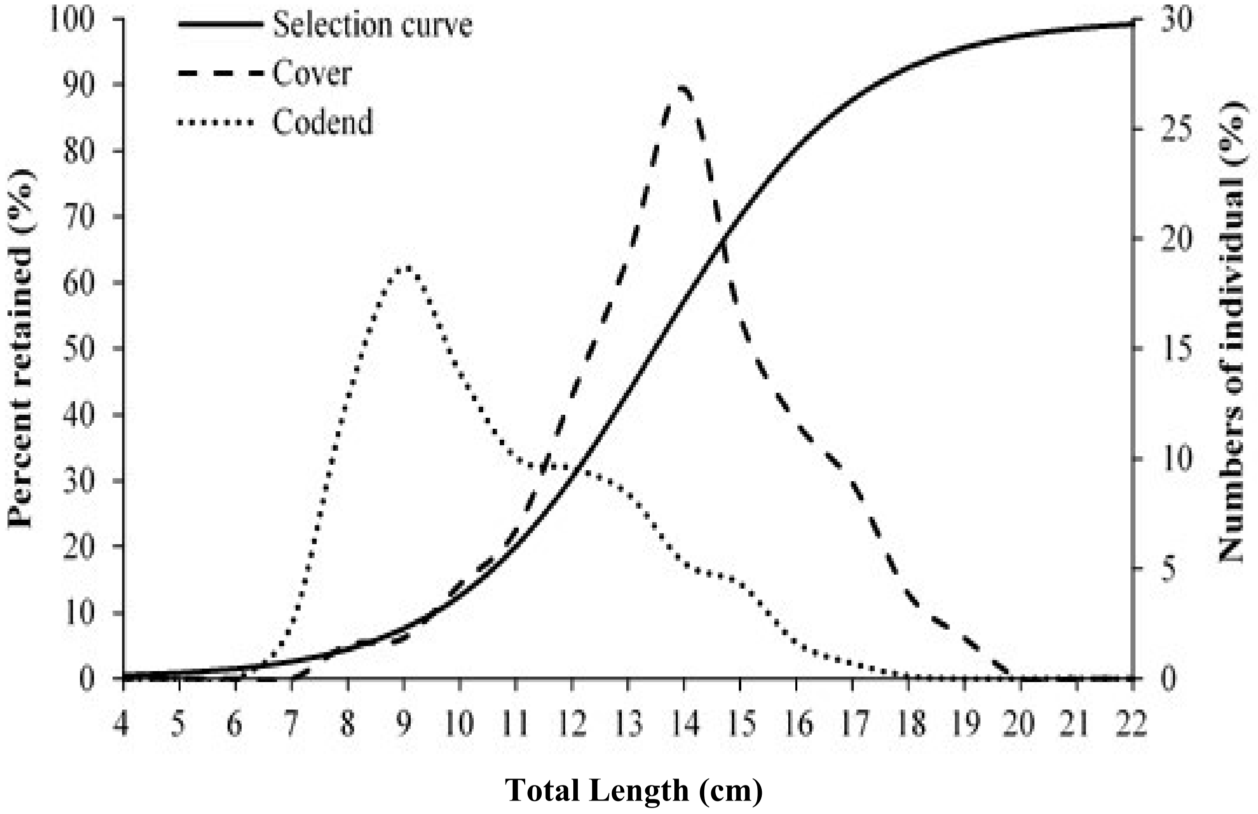

Figure 6. Selection curve for 40 mm (SMP) and size frequency distributions of red mullet that captured the codend and got away.

4. DISCUSSIONS

Sea experiments indicate that a significant in selectivity for blunt-snouted mullet (*Mullus ponticus)* can be used the codend with 40 mm (SMP) instead of 36 mm (D) and 40 mm (D) mesh codend in the Black Sea demersal trawling. The estimated L_{50} for red mullet with the 40 mm (SMP) is higher (13.52 cm) than the presently minimum landing size (MLS = 13 cm) and significantly higher than that of 36 mm (D) and 40 mm (D) mesh codend (L_{50} = 11.56 cm and 12.06 cm). Surely, the 40 mm (SMP) with codend would symbolize a marked progression in the fishing model of the economical main fishes of the Black Sea.

It is able that the first maturity size of red mullet in the Black Sea is indicated by various scientists to be between 10.0 and 12.5 cm (Vassilopoulou, 1987; Genç, 2000; Genç, 2001; İşmen *et al*., 2000; Erdem, 2018). Therefore, an essentially higher mesh size than 40 mm (D) codend would be necessary if the spawn at least once principle was to be satisfied for this species. For example, 42 mm (D), 44 (D) mm, 40 mm (S) or 40 mm (SMP).

Furthermore, it is required to catch rate of this species (*Mullus barbatus barbatus*, L. 1758, *Mullus surmuletus* L., 1758 and *Mullus barbatus ponticus,* Essipov 1927) entered the trawl and species fixing of red mullet captured with demersal trawl in the Turkish seas. Size of first maturity of these three species shows difference such as 16-18 cm for *Mullus surmuletus,* 10.5-12.5 cm for *Mullus barbatus ponticus* and 13.3 cm for *Mullus barbatus barbatus* (Vassilopoulou, 1987; Dorel, 1986; Metin, 2005; Erdem, 2018; Erdoğan-Sağlam, 2022).

As a result, codend mesh size must be more than present legal mesh size (40 mm D) of trawl codend, take into consideration other fish species in the Black Sea. However, 40 mm square mesh panel (SMP) on the codend could practised in the bottom trawl fisheries. In this case capture of small size red mullet will decrease by square mesh panel method is considering. (Metin *et al*., 2005; Dereli and Aydın, 2016). However, has been done to estimate the selectivity of a square mesh panel (SMP) and square mesh codend (SMC) in the extremely variant multi-species terms dominant in the Black Sea demersal trawl fishery (Özdemir *et al.,* 2012; Özdemir *et al.,* 2014; Kaykaç *et al*., 2018; Ceylan and Şahin, 2019; Zengin *et al.,* 2019).

Moreover, we confirm that a square mesh codend would not be effective for all commercial fishes, such as many higher bodied species and some flatfish (Petrakis and Stergiou, 1997). This situation shows the trouble of progress size selection of fishes in highly multispecies fisheries. The same mesh size is not appropriate for all fishes; it will every time be too large for some fishes and too small for others. To make feasible a more efficient decrease of none-target species and ideal size selection for target species, species selectivity should be advanced in suitability with size selectivity (Valdemarsen and Suuronen, 2003; Fonseca *et al.,* 2005).

5. CONCLUSIONS

In consequence, the exploitation rate (E) of red mullet fish caught with bottom trawl nets in the Black Sea was estimated E: 0.47 (Aksu *et al*., 2011), E: 0.54, (Samsun, 2017), E: 0.59 (Kasapoğlu, 2018), E: 0.83 (Özdemir *et al*., 2021), E: 0.73, (Samsun, 2022) respectively. These studies also show that there is an increasing fishing pressure on the species over the years. It once again reminds us of the use of fishing gear with improved selectivity for the sustainability of the species with maximum yield.

The present study demonstrates that a 40 mm (SMP) with codend would support the development of the overfishing and overall exploitation modal in the Black Sea multi-species trawl fisheries. Moreover, many experiments on trawl codend selectivity in the Black Sea demersal trawl fisheries have the same results (Genç*,* 2002; Özdemir and Erdem, 2008; Özdemir *et al*., 2012; Kaykaç *et al*., 2018; Zengin *et al.,* 2019; Ceylan and Şahin, 2019).

However, it is considerable to understand that any rising to selectivity in fleet of the Black Sea fisheries would rising the mean age at first catch for the great plurality of trading significant fishes even if a definite optimal is not reached for all fishes. Supposing that most of the escaped fishes from trawl codend stay alive, this is probably to be favorable for the fish stocks.

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AUTHORSHIP CONTRIBUTION STATEMENT

Süleyman ÖZDEMİR: Designed the study and interpreted data. Performed the sea experiments, collecting of data and laboratory work. Validation, formal analysis, writing-original draft, writing-review and editing, data curation, software, visualization, supervision.

Yakup ERDEM: Methodology, validation, formal analysis, data curation, interpreted data, software, visualization, writing-review and editing.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ETHICS COMMITTEE PERMISSION

No ethics committee permissions is required for this study.

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First record of *Bursatella leachii* de Blainville 1817 (Mollusca; Gastropoda; Aplysiidae) in Black Sea

Bursatella leachii de Blainville 1817'nin (Mollusca; Gastropoda; Aplysiidae) Karadeniz'deki ilk kaydı

Türk Denizcilik ve Deniz Bilimleri Dergisi

Cilt: 10 Sayı: 4 (2024) 245-253

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ABSTRACT

This study reports the presence of the ragged sea hare *Bursatella leachii* de Blainville 1817 in the Black Sea. A total of 484 specimens of this species were observed during scuba diving and snorkeling at depths ranging from 1.0 to 4.0 meters in September 2023. The samples were observed on the sandy-muddy bottom of Kumbaba Beach and the sandy bottom of Uzunkum Beach in Şile district (Istanbul province). The density of individuals per square meter of the seabed was calculated and the highest number of individuals was detected on the Kumbaba Beach near the mouth of the Türknil River. Considering the location of discovery, it is logical to assume that the introduction of this species into the Black Sea may have occurred naturally. This hypothesis can be supported for two main reasons; a) Şile beaches are a few kilometers away from the Bosphorus Channel, b) the report of *B. leachii* closest to Şile district dates back to January 2020, when the species was observed on the seabed on the southern coast of the Dardanelles. Long-term monitoring and more in-depth studies should be conducted in the region to give certainty to these hypotheses.

Keywords: *Bursatella leachii*, Black Sea, Türkiye, Alien invasive species, Non-Indigenous species.

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ÖZET

Bu çalışma, Karadeniz'de dağınık deniz tavşanı *Bursatella leachii* de Blainville 1817'nin varlığını rapor etmektedir. Eylül 2023 ayı içerisinde 1,0 ila 4,0 m arasında değişen derinliklerde tüplü dalış (SCUBA) ve şnorkel dalışı ile toplam 484 örnek gözlemlenmiştir. Örnekler, Şile ilçesi (İstanbul ili) Kumbaba plajının kumlu-çamurlu tabanında ve Uzunkum plajının kumlu tabanında gözlemlenmiştir. Deniz tabanındaki bireylerin metrekaredeki yoğunluğu hesaplanmış ve en yüksek birey sayısı Türknil nehrinin ağzına yakın Kumbaba sahilinde tespit edilmiştir. Türün tespit edildiği bölge göz önüne alındığında, bu türün Karadeniz'e girişinin doğal yollarla meydana gelmiş olabileceği düşünülmektedir. Bu hipotez iki ana nedenden dolayı desteklenebilir; a) Şile sahilleri Boğaziçi Kanalı'ndan birkaç kilometre uzaktadır, b) Şile ilçesine en yakın *B. leachii*'nin raporu, türün Çanakkale Boğazı'nın güney kıyısındaki deniz yatağında gözlemlendiği Ocak 2020'ye dayanmaktadır (Çanakkale Boğazı). Bu hipotezlere kesinlik kazandırmak için bölgede uzun süreli izleme ve daha derinlemesine çalışmalar yapılmalıdır.

Anahtar sözcükler: *Bursatella leachii*, Karadeniz, Türkiye, İstilacı tür, Yabancı tür

1. INTRODUCTION

For over a century the biota of the Mediterranean Sea and, more recently, that of the Black Sea have been subject to continuous and profound changes caused by the introduction of alien exotic species.
The presence of Non-Indigenous Species in the Black Sea has become increasingly high in recent years to the point that this basin has become home to a large number of alien species that are naturalizing in it. In the same way as what happened for the Mediterranean, also for the Black Sea there are essentially three main transport routes: a) maritime transport; b) introduction, intentional or unintentional, by humans activities; c) Mediterraneanisation, the process through which many species overcome the ecological barriers, represented by the Turkish Straits, and penetrate the Black Sea (Öztürk, 2021; Aydın and Sözer, 2016).
Molluscs are one of the phyla with the highest number of species in the world; it is estimated that they may constitute approximately 25% of the entire marine fauna of the seas and among them there are some of the most invasive species with the greatest ecological impact (Molnar *et al.*, 2008).
In the entire Mediterranean basin, it is estimated that there are over 200 species of alien mollusca (Sabelli and Taviani, 2014).
The sea hare *Bursatella leachii* de Blainville, 1817 (Gastropoda; Heterobranchia; Aplysiidae) is a circumtropical species widely distributed in the Atlantic and Indo-Pacific oceans, including the Red Sea (Bebbington, 1969; Marcus, 1972; Burn, 2006; Bazzicalupo *et al.*, 2020).
The first report of this mollusk in the Mediterranean dates back to the first half of the 1900s and was made along the coasts of Israel (O'Donoghue and White, 1940). Since then, *B. leachii* has spread throughout the Mediterranean and is currently considered the most widespread exotic taxon in this basin (Zenetos *et al.*, 2016; Crocetta *et al.*, 2017; Selfati *et al.*, 2017; Travaglini and Crocetta, 2019). The numerous reports of *B. leachii* confirm the presence of this sea hare in at least 21 of the 23 coastal countries of the Mediterranean (Selfati *et al.*, 2017; Monnier *et al.*, 2024).
B. leachii is a typical species of marine areas from warm temperate to tropical, especially in calm and shallow environments, although there are sporadic reports of specimens observed at over 20 meters of depth (O'Donoghue, 1929; Eales, 1970; Barash and Danin, 1971).
This species is commonly observed in estuaries, coastal lagoons and coastal waters of sheltered areas; sometimes it can also establish itself in lagoon environments (e.g. ponds used for shrimp cultivation) and in port waters (Lowe and Turner, 1976; Arkronrat *et al.*, 2016; Behera *et al.*, 2020; Parera *et al.*, 2020).
B. leachii is a detritivorous and herbivorous benthic mollusc that usually grazes on the surface layers of sandy and muddy seabeds as well as the

muddy layers that cover the surfaces of seabed rocks (Rudman, 1998; Clarke, 2006; Kazak and Cavas, 2007; Antit *et al.*, 2011; Otero *et al.*, 2013; Sethi *et al.*, 2015; Gimènez-Casalduero *et al.*, 2016; Ballesteros *et al.*, 2022). These eating habits have meant that this sea hare has developed a varied diet with a prevalence of cyanobacteria, Rhodophycea, Chlorophycea and Pheophyceae (Paige, 1988; Clarke, 2006).

It is therefore a particularly useful species for the biological control of toxic blooms of marine cyanobacteria (Capper *et al.*, 2005, 2006; Capper and Paul, 2008).

B. leachii has diurnal habits and is usually active at dawn, when large gatherings of individuals form, while isolated individuals are found during sunset hours (Ramos *et al.*, 1995).

Being a simultaneous hermaphroditic species with cross-fertilization, it has a rapid life cycle and reproduces several times a year (Kaplan, 1988; Otero *et al.*, 2013). It follows that *B. leachii* populations present large demographic fluctuations with periods of large aggregations of individuals which disperse after a few days. These large assemblages form when the optimal ecological and meteorological conditions for fertilization occur (Lowe and Turner, 1976; Zenetos *et al.*, 2004; Clarke, 2006; Crocetta *et al.*, 2013; Gonzàlez-Wangüemert *et al.*, 2014). In these cases, the population density can reach values of over 50 ind/m^{-2}.

The present study reports the first record of the non-indigenous ragged sea hare *B. leachii* (Figure 1) along the coastline of Black Sea, namely in the Turkish coasts, and provides an update of the known distribution of *B. leachii* in the Mediterranean Basin compared to what has been reported from previous work of Rizgalla and Crocetta (2020) and Monnier *et al.* (2024).

2. MATERIALS AND METHODS

The presence of *B. leachii* (372 individuals) was recorded for first time on September 10th, 2023 in the soft bottoms of the Black Sea, in Kumbaba Beach (41.173341° N; 29.569386° E), a beach in the Şile District (Istanbul province), about 40 kilometers east of the Bosphorus. A few days later, on September 15th 2023, 112 sea hare specimens were recorded at Uzunkum Beach (41.171767° N; 29.634561° E), also in the Şile district (Figure 2).

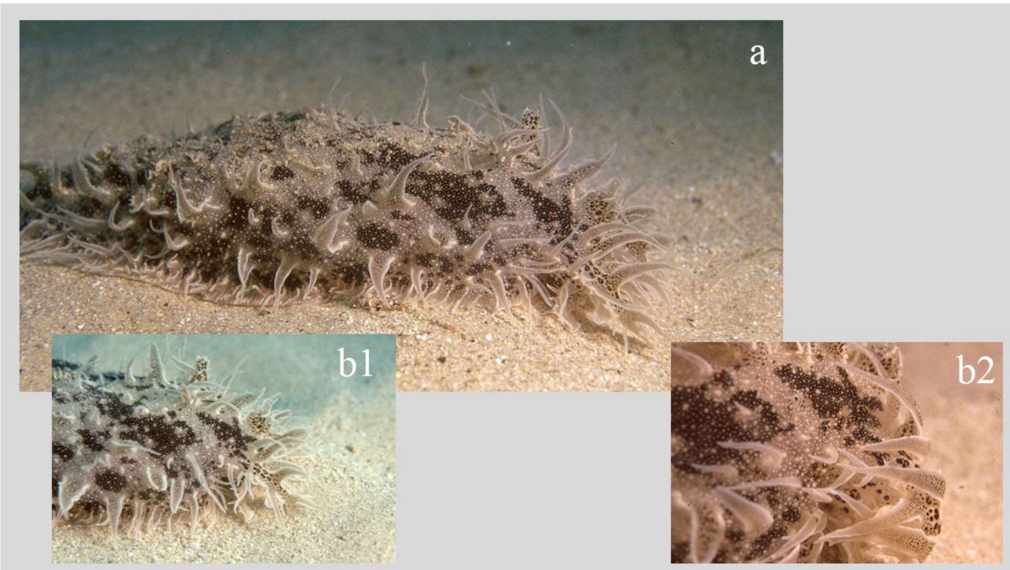

Figure 1. *Bursatella leachii* de Blainville 1827 in the Turkish Black Sea bottoms; "a": specimen on sandy mud bottom; "b1": anterior portion of a specimen; "b2" magnification of the cephalic region of a specimen.

Figure 2. Location of study area in the Black Sea where *B. leachii* specimens were observed during the present study; "a": Kumbaba Beach; "b": Uzunkum beach near Şile district.

2.1. Site descriptions

Kumbaba Beach is the west part of a long sand coast east of Şile approximately 3 kilometers long and limited to the east by the port of Şile and to the west by a very small rocky promontory; not far from it the Türknil River flows into it (41.173341° N; 29.569386° E) (Figure 2a). The seabed of this site is characterized by fine sand mixed with mud that accumulates due to the seasonal contributions that the nearby river pours into the sea during flood periods. In the seabed of this area a meadow of *Zostera noltei* Hornemann develops and on the few rocks that emerge from the mud from the bottom various algal species develop with a good abundance of the *Rhodophyta Gracilaria verrucosa* (Huds.) Papenf. and the Heterokontophyta *Dictyota dichotoma* (Huds.) Lamour.
Uzunkum beach, located east of the town of Şile, is a one kilometer long beach enclosed between two small rocky promontories (41.171767° N; 29.634561° E) (Figure 2b). The seabed of the bay is almost completely sandy and in the deepest part is characterized by sand mixed with mud with numerous rocky outcrops. In these seabeds the most evident vegetation is characterized by the seagrass *Zostera noltei*. The bottom rocks are covered by an algal population characterized by the Heterokontophytes *Gongolaria barbata* (Stackhouse), *Padina pavonica* (Linnaeus) Thivy, 1960 and *Dictyota dichotoma* (Linnaeus) Thivy and by the Rhodophyta *Gracilaria verrucosa*. Along this beach there are no river mouths and the only contributions of terrigenous material occur during the most intense meteorological events.

2.2. Sampling methods

The observations were made in September 2023 (on the 10th and 15th) at depths ranging from 1 to 4 meters by two research divers using scuba and snorkeling. The observations on the individuals of *B. leachii* were carried out with the *visual census* technique, counting all the specimens present along a transect parallel to the coastline, 300 meters long and 5 meters wide on average. Thus, obtaining a sampling surface of 1500 square meters. To obtain more precise counts of the specimens in the investigated area, the entire transect was divided into sectors 100 meters long and 5 meters wide; in each of these sectors the specimens were counted. This way of operating allowed us to obtain a more realistic estimate of the number of *B. leachii* specimens present in the study area.
The dives were always carried out in the central hours of the day, between 12:00 and 15:00, in each of the sampling sites. This allowed us to compute the average number of specimens found on each of the beaches we surveyed (Table 1).

Furthermore, 15 specimens of *B. leachii* were randomly collected for morphometric measurements from each of the two sampling sites. For each specimen collected, length, width and weight were recorded (Table 2). All captured specimens were measured in situ and promptly released into the sea.

3. RESULTS AND DISCUSSIONS

The present study describes the presence of the *B. leachii* species in the coastal marine waters of the Black Sea.

Prior to this report, specimens of *B. leachii* had been reported in the Çanakkale Strait in the Turkish Straits system, just south of the Sea of Marmara (Özalp *et al.*, 2021). This is a habitat whose marine life and ecology are a mix of what can be observed in the Mediterranean and Black Seas (Culha and Sahin 2018).

During our study we observed a total of 484 specimens; 372 of which were found on the seabed of Kumbaba Beach and 112 on Uzunkum Beach. In our opinion, the substantial variation in number of specimens recorded on the bottom of the two beaches cannot be attributed to the days of delay between observations at the two study sites, or to other meteorological or water-dynamic conditions. We believe that, however, that the current large numerical difference between the number of *B. leachii* individuals observed at Kumbaba Beach (372) and Uzunkum Beach (112) is due to the difference in the type of seabed habitat and the availability of nutrients between the two sites. The Kumbaba Beach site, in fact, is located right near the mouth of the Türknil River and the terrigenous contributions that the river pours into the sea have created the ideal habitat for these Opisthobranchs which, being mainly herbivores and detrivores, find in the bottom sediments great availability of food. On the other hand, in the Uzunkum Beach site there aren't significant coastal terrigenous contributions as the bay is closed between two promontories and has no outlets of rivers or streams, even if of a seasonal nature. Therefore, in this bay the only source of organic material available for *B. leachii* is represented by the presence of marine plants (seaweeds and/or seagrasses).

During the observations, isolated specimens of *B. leachii* were always found in portions of the seabed covered by *Z. noltei* meadows, with sandy-muddy sediments between depths of approximately 1.0 – 4.0 m, or near rocks of the bottom covered with algae.

281 specimens, more than half of the total, were found on Kumbaba Beach, in the portion of the seabed closest to the mouth of the Türknil River. This shows that these animals prefer seabeds rich in debris for the ease of finding food.

Nevertheless, the data relating to the abundance of individuals per m^{-2} appears to be very low compared to what is reported in the literature. In the Şile coastal bottoms, in fact, an average density per square meter of 0.248 ind/m^{-2} in Kumbaba and 0.074 ind/m^{-2} in Uzunkum was detected (Table 1).

These average density values of ind/m^{-2} are far from what was found in the nearby Çanakkale Strait, in the Turkish Straits system, by Özalp *et al.* (2021) who report an average of 5 ind/m^{-2}. Indeed, other authors report extremely higher ind/m^{-2} values; for example, Ballesteros *et al.* (2022) report 5-10 ind/m^{-2}, Selfati *et al.* (2017) 50 ind/m^{-2}, Behera et al (2020) 150-200 ind/m^{-2} and even Rudloe (1971) report over 600 ind/m^{-2} (Table 3).

From the data collected, it is clear that the density of ind/m^{-2} is not comparable to what is observed in other seas (Table 3). However, considering the number of specimens found, it is possible to state that the presence of this species is not an occasional event for the Black Sea.

Table 1. Number of specimen of *B. leachii* per site and per sector

Area of sampling	**Kumbaba Beach**			**Uzunkum Beach**		
Sector of sampling	Sector 1	Sector 2	Sector 3	Sector 1	Sector 2	Sector 3
N specimens	91	145	136	37	35	40
Mean per sector	124			37.333		
N° of ind/m^{-2}	0.248			0.074		
Total	372			112		

Table 2. Morphometric characteristic of *Bursatella leachii* collected in Black Sea

Site	N specimen	Body length (mm)	Body width (mm)	Body weight (g)
Kumbaba Beach	1	87	39	40
	2	75	37	39
	3	82	41	39
	4	84	38	40
	5	72	34	30
	6	86	33	42
	7	69	29	39
	8	97	46	53
	9	94	79	55
	10	112	50	64
	11	81	39	47
	12	89	41	45
	13	81	37	41
	14	98	41	48
	15	103	39	50
Mean ±		**87.333**	**41.533**	**44.8**
St. dev.		**11.812**	**11.513**	**8.325**
Uzunkum Beach	1	70	39	27
	2	69	37	26
	3	74	41	22
	4	80	38	31
	5	56	34	19
	6	76	33	22
	7	59	29	18
	8	88	46	33
	9	85	79	30
	10	101	50	46
	11	79	39	34
	12	85	41	40
	13	74	37	33
	14	88	41	34
	15	93	39	38
Mean ±		**78.467**	**41.53**	**30.2**
St. dev.		**12.188**	**11.513**	**7.965**

Table 3. Number of ind/m^{-2} detected in present study and in other studies

Location	**Şile District** Black Sea	**Çanakkale Strait** Turkish Straits	**Balearic Islands** Mediterranean	**Morocco** Mediterranean	**Bay of Bengal** Indian Ocean	**Florida** Gulf of Mexico
References	Present study	Özalp *et al.*, 2021	Ballesteros *et al.*, 2022	Selfati *et al.*, 2017	Behera *et al.*, 2020	Rudloe *et al.*, 1971
N° of ind/m^{-2}	0.16 ind/m^{-2}	5 ind/m^{-2}	5 – 10 ind/m^{-2}	50 ind/m^{-2}	150 - 200 ind/m^{-2}	> 600 ind/m^{-2}

We believe, in fact, that it is possible to hypothesize that *B. leachii* may have undertaken a phase of colonization of a new range and, for this reason, the expansion of this species in the Black Sea should be studied with adequate monitoring programs. In fact, an excessive presence of specimens of *B. leachii* could impact activities related to small-scale local fishing with gill nets.

In consideration of the ecological characteristics of *B. leachii*, which prefers estuarine and/or low salinity environments, and taking into account that the Black Sea has a lower salinity than the Mediterranean Sea, we believe that an ever-increasing number of Non-Indigenous organisms with broad ecological value can colonize the system of the Turkish Straits and, having overcome the natural "bottleneck" constituted by the Bosphorus, it will spread with ever greater success in the waters of the Black Sea (Özalp *et al.*, 2021).

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AUTHORSHIP CONTRIBUTION STATEMENT

Vincenzo DI MARTINO: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Supervision, Underwater Sampling. **Bessy STANCANELLI:** Methodology, Validation, Resources, Writing-Review and Editing, Data Curation, Underwater Sampling

CONFLICT OF INTERESTS

The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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No ethics committee permissions is required for this study.

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